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From Cell Biology to Tissue Engineering

Characterization of the early pathology of cochlear stereocilia in four inbred mouse strains with progressive hearing loss

Xiang Liu[1,3], Yi Xie[1,2], Shanshan Huang[1], Ang Xu[1], Mengmeng Zhao[1,2], Xiaoxia Kang[1], Aiwei Yan[1], Ping Li[1], Changzhu Jin[1,3] and Fengchan Han[1,2]

[1]Key Laboratory for Genetic Hearing Disorders in Shandong, [2]Department of Biochemistry and Molecular Biology and [3]Department of Human Anatomy and Histology and Embryology, Binzhou Medical University, Yantai, Shandong, P.R. China

Summary. Objective. Inbred strains of mice offer promising models for understanding the genetic basis of age-related hearing loss (AHL). NOD/LtJ, A/J, DBA/2J and C57BL/6J mice are classical models of age-related hearing loss and exhibit early onset of pathology of AHL. This study was carried out to characterize the early pathology of cochlear stereocilia in the four mouse strains with age-related hearing loss.

Methods. The structural features of stereocilia in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice were observed by scanning electron microscopy (SEM) at age of 2, 4, 6 or 8, and 10 or 12 weeks. Meanwhile, auditory-evoked brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) amplitudes of the mice were measured at various intervals (3, 4, 6, 8, 10 and 12 weeks of age).

Results. The ABR thresholds in NOD/LtJ, A/J and DBA/2J mice increased with age from 3 to 12 weeks. DPOAE amplitudes in NOD/LtJ, A/J, DBA/2J mice were very low at 4 weeks and became negative at 8 weeks at f2 frequency of 17 672 Hz. In addition to the progressive hearing loss, the four mouse strains displayed early onset (at 2 weeks of age) and progressive degeneration of stereocilia in hair cells.

Conclusion. Early degeneration of stereocilia contributes to the functional impairment of hair cells and hearing loss in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice.

Key words: Age-related hearing loss, Mouse model, Hair cells, Stereocilia, Scanning electron microscope

Offprint requests to: Dr. Fengchan Han or Changzhu Jin, Key Laboratory for Genetic Hearing Disorders in Shandong, Binzhou Medical University, 346 Guanhai Road, Yantai 264003, Shandong, P.R. China. e-mail: hanfengchan@gmail.com or Jincz63@hotmail.com

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Introduction

Age-related hearing loss (AHL), one of the most common perceptive diseases among the elderly population, causes both communication disorders and psychological problems (Gates and Mills, 2005; Yamasoba et al., 2007, 2013). In addition to environmental and social factors, genetic aspects are involved in pathogenesis in about 50%-60% of people with age-related hearing loss (Gates and Mills, 2005; Liu and Yan, 2007; Angeli et al., 2012). Therefore, it is of great significance to study the mechanism of genetic variance that leads to progressive hearing loss. As mice and humans share similar genetic components, anatomic structures, and pathological characteristics, mouse models play a crucial role in understanding the pathogenesis associated with these genes (Noben-Trauth and Johnson, 2009; Angeli et al., 2012; Fujinami et al., 2012). Inbred strains of mice offer promising models for understanding the genetic basis of human presbycusis or age-related hearing loss (AHL) (Johnson et al., 2000). NOD/LtJ, A/J, DBA/2J and C57BL/6J mice are classical models of age-related hearing loss (Zheng et al., 1999). They share the *ahl* allele (Noben-Trauth and Johnson,

2009) and exhibit early onset of pathology of AHL (Fetoni et al., 2011).

NOD/LtJ mouse strain exhibits very early onset hearing loss, showing 30 dB threshold elevations at 3 weeks of age, which progresses rapidly to near complete deafness by 9 weeks of age (Zheng et al., 1999; Noben-Trauth and Johnson, 2009). Mice of the A/J strain exhibit an early-onset progressive hearing loss that was first reported in 1982 (Henry, 1982). They exhibit elevated ABR thresholds by 25 days of age, and hearing loss progresses to near deafness by 3 months of age (Zheng et al., 2009; Yang et al., 2015). DBA/2J mouse strain also develops early-onset hearing loss (Zheng et al., 1999; Yang et al., 2015). This hearing loss is profound but not quite as pronounced as in NOD/LtJ mouse; hearing thresholds at 3 weeks of age are elevated by 15-20 dB and reach near deafness levels by 14 weeks (Zheng et al., 1999). The C57BL/6J (B6) mouse strain is the most widely used mouse model for the study of aging and age-associated diseases. It is well known that hearing loss occurs at about 9 to 12 months of age (Johnson et al., 1997, 2000; Han et al., 2012).

Schuknecht's seminal work on the pathology of AHL, beginning in the 1950s, proposed four pathological subtypes in presbycusis: sensory, involving hair cell loss; strial (or metabolic), involving degeneration of the stria vascularis and reduction in endocochlear potential; neural, involving loss of spiral ganglion neurons, and mechanical, involving stiffening of the basement membrane (Schuknecht and Gacek, 1993). Previous studies have shown that hair cell loss is involved in the hearing loss of all 4 mouse strains (Han et al., 2012, 2015; Yang et al., 2015; Sang et al., 2017). As we all know, cochlear hair cells for mammals are not renewable. It is important to prevent or delay the loss of cochlear hair cells to maintain the normal function of the hair cells. The cochlear stereocilia are at the top of the hair cells and are essential to the perception of sound and motion (Sekerkova et al., 2011). In this study, early alterations of the stereocilia in the cochleae in four inbred mouse strains with age-related hearing loss were characterized by scanning electron microscopy (SEM). We found that degeneration of cochlear stereocilia at the early stage may be responsible for the early hearing loss of these mouse strains.

Materials and methods

Animals

Experimental mice (NOD/LtJ, A/J, DBA/2J and C57BL/6J mice) were bred in a specific pathogen-free animal facility at Binzhou Medical University. The animal studies were conducted in accordance with the principles set forth in the Guide for the Care and Use of Laboratory Animals of Binzhou Medical University and were approved by that university's Institutional Animal Use and Care Committee (protocol 14-0514). A total of 56 mice with age from 2 to 24 weeks were included in this study.

Measurement of DPOAE amplitudes and ABR thresholds

The mice were anesthetized with 2% tribromoethanol (0.2 mL per 10 g of body weight) and then placed on a heating pad to maintain a temperature of 37°C. All operations were carried out in a soundproof and electromagnetic shielding room. A computer-aided evoked potential system (Intelligent Hearing Systems, Miami, FL, USA) was used to test the mice for auditory-evoked brainstem response (ABR) thresholds as described previously (Zheng et al., 1999). The ABR thresholds in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice at various intervals (3, 4, 6, 8, 10 weeks of age) were obtained for stimuli of click by reducing the sound pressure level (SPL) to identify the lowest level at which an ABR pattern could be recognized. The IHSS Smart EP 3.30 and USB ez Software (Intelligent Hearing Systems, Miami, FL, USA) were used to measure the distortion product oto-acoustic emission (DPOAE), which is the response generated when the cochlea is stimulated simultaneously by two pure tone frequencies (f1 and f2). For frequencies ranging from 2 to16 kHz, an Etymotic ER2 Stimulator (Etymotic Research, Inc., Elk Grove Village, IL,USA) was used and for frequencies ranging from 16 to 30 kHz, a HIS high-frequency transducer was used. Stimulus response signals were sampled at a rate of 128 kHz using a 16-bit D/A converter; L1 and L2 amplitudes were set to the same level. Frequencies were acquired with an F2:F1 ratio of 1.22. The stimuli were presented starting from the lowest frequencies tested and increasing to the highest frequencies tested. (Han et al., 2012, 2013).

Scanning electron microscopy

Scanning electron microscopy (SEM) was carried out following the methods described previously (Shin et al., 2010; Men et al., 2015; Liu et al., 2018). The mice in the four mouse strains were observed at age of 2, 4, 6, 8, 10 and 12 weeks (n=4 in each group). Briefly, the inner ears of the mice were dissected outside of the skull, fixed in 2.5% glutaraldehyde phosphate buffer (0.1 M PBS) at 4°C overnight, decalcified, and then washed 3 times in 0.1 M PBS (10 minutes each time). The organs of Corti were exposed after the overlying bones and membranes were carefully cut off. The mouse cochleae were processed in 1% osmium tetroxide acid (post-fixation) for 40 minutes, dehydrated with gradient alcohol (50%, 70%, 80%, 95%, and 100%), and dried to a critical point with liquid CO_2. The samples were then mounted onto round nails made of pure copper and sputter-coated to produce a gold coat of 10-15 nm. Finally, the samples were examined at 10 kV with an EVO MA 15/LS scanning electron microscope (Carl

Zeiss, Oberkochen Germany).

Statistical analysis

ANOVA (SPSS 16 Software) was used to analyse the ABR thresholds and EPOAE amplitudes. P<0.05 was considered to be significant.

Results

Early onset of progressive hearing loss in the four mouse strains

To evaluate hearing loss in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice, ABR thresholds at click were

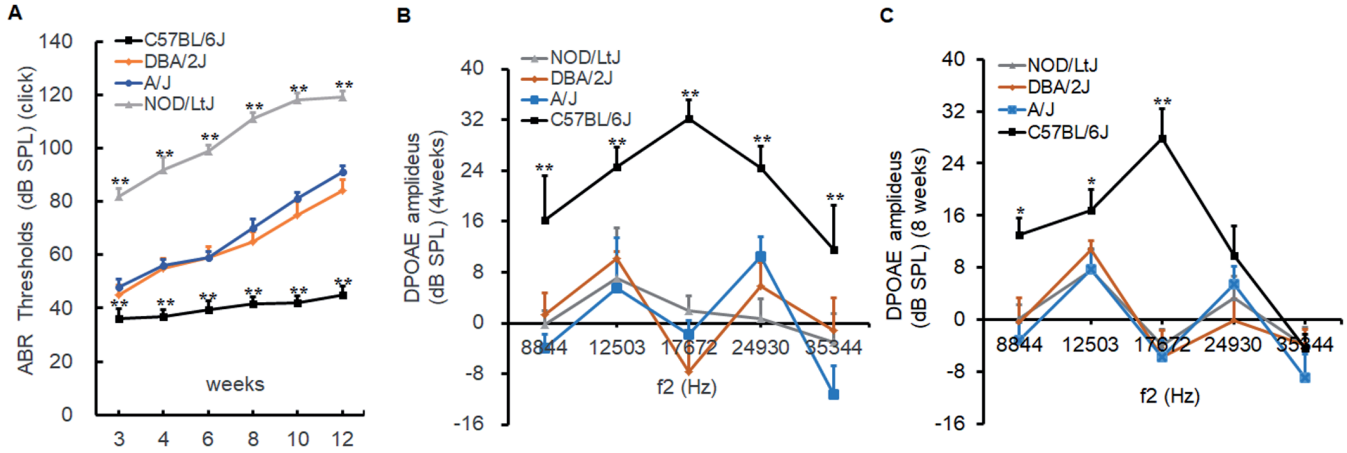

Fig. 1. ABR thresholds and DPOAE amplitudes in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice. **A.** ABR thresholds tested in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice from 3 to 12 weeks of age at stimulus frequencies of click. ABR thresholds are presented by mean and standard errors. **B, C.** The DPOAE amplitudes measured in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice at f2 frequencies from 8844 Hz to 35 344 Hz at 4 and 8 weeks. DPOAE amplitudes are presented by mean and standard errors. Asterisks on the top of a line indicate the significance of the ABR thresholds or DPOAE amplitudes in the corresponding strain (NOD/LtJ or C57BL/6J) compared with any one of the other strains at the same of age. * P<0.05; **P<0.01.

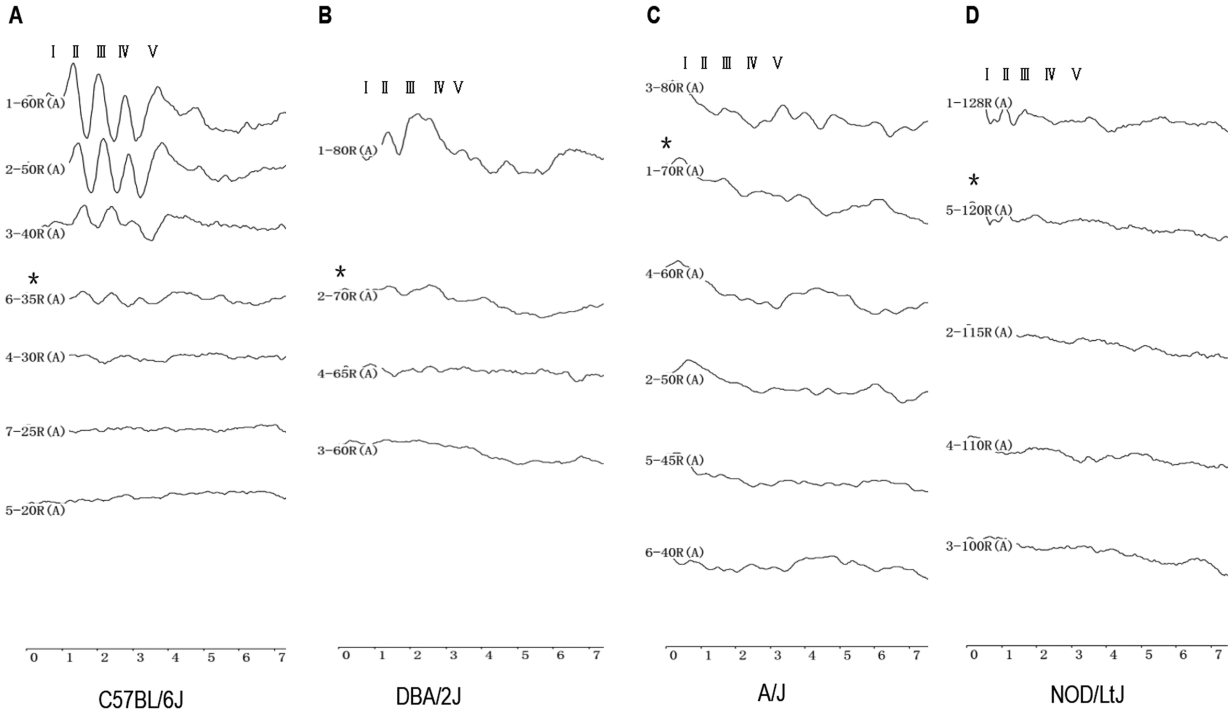

Fig. 2. ABR thresholds in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice. **A-D.** The typical illustrations of ABR tested in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice at 8 weeks at click. ABR thresholds are indicated by a black asterisk.

measured in a time-course manner. The ABR thresholds in NOD/LtJ, A/J and DBA/2J mice increased with age from 3 to 12 weeks. However, the ABR thresholds in the C57BL/6J mice did not change with age until after 9 months of age (Han et al., 2012). Unlike the C57BL/6J mice, the ABR thresholds in NOD/LtJ mice had already risen to 80 dB SPL at 3 weeks, which indicated an early onset of most severe hearing impairment. At 8 weeks, ABR thresholds in NOD/LtJ mice were about 120 dB SPL which indicated deafness for the mice. The hearing loss in A/J mice was similar to that in DBA/2J mice. Hearing impairment was progressive in the 12 weeks period of observation (Fig. 1A). Compared with C57BL/6J mice, ABR thresholds in A/J and DBA/2J mice were elevated by 35-40 dB SPL by 8 weeks (Figs. 1A, 2).

Functional impairment of outer hair cells in NOD/LtJ, A/J and DBA/2J mice

As a secondary screen of mice with known hearing impairment, DPOAE may provide additional information about outer hair cell (OHC) function (Noben-Trauth and Johnson, 2009). Therefore, DPOAE amplitudes were measured in four inbred mice at age of 4 and 8 weeks at f2 frequencies from 8844 to 35 344 Hz (Fig. 1B-C). Typically, DPOAE amplitudes in C57BL/6J mouse littermates at 4 and 8 weeks showed an inverted V curve, with the height corresponding to an f2 frequency of 17 672 Hz. However, the DPOAE amplitudes in NOD/LtJ, A/J, DBA/2J mice at age of 4 weeks were lower than those of the C57BL/6J mice at f2 frequencies from 8844 to 35 344 Hz (Fig. 1B). DPOAE amplitudes in NOD/LtJ, A/J, DBA/2J mice became negative at age of 8 weeks at f2 frequency of 17 672 Hz (Fig. 1C). These results indicate an early progressive functional impairment of OHCs in NOD/LtJ, A/J and DBA/2J mice.

Progressive degeneration of hair cell stereocilia in cochleae of the four mouse strains

Alterations of the stereocilia in cochlear basal turns in the four mouse strains were observed using SEM (Figs. 3-6). C57BL/6J mice showed almost normal appearance of OHC stereocilia in the basal turns of cochleae at 2 and 24 weeks (Fig. 3A,C). The alignment of OHCs and the characteristic array of stereocilia, which is inverted V-shaped in OHCs, were maintained normally (Fig. 3A,C). However, C57BL/6J mice

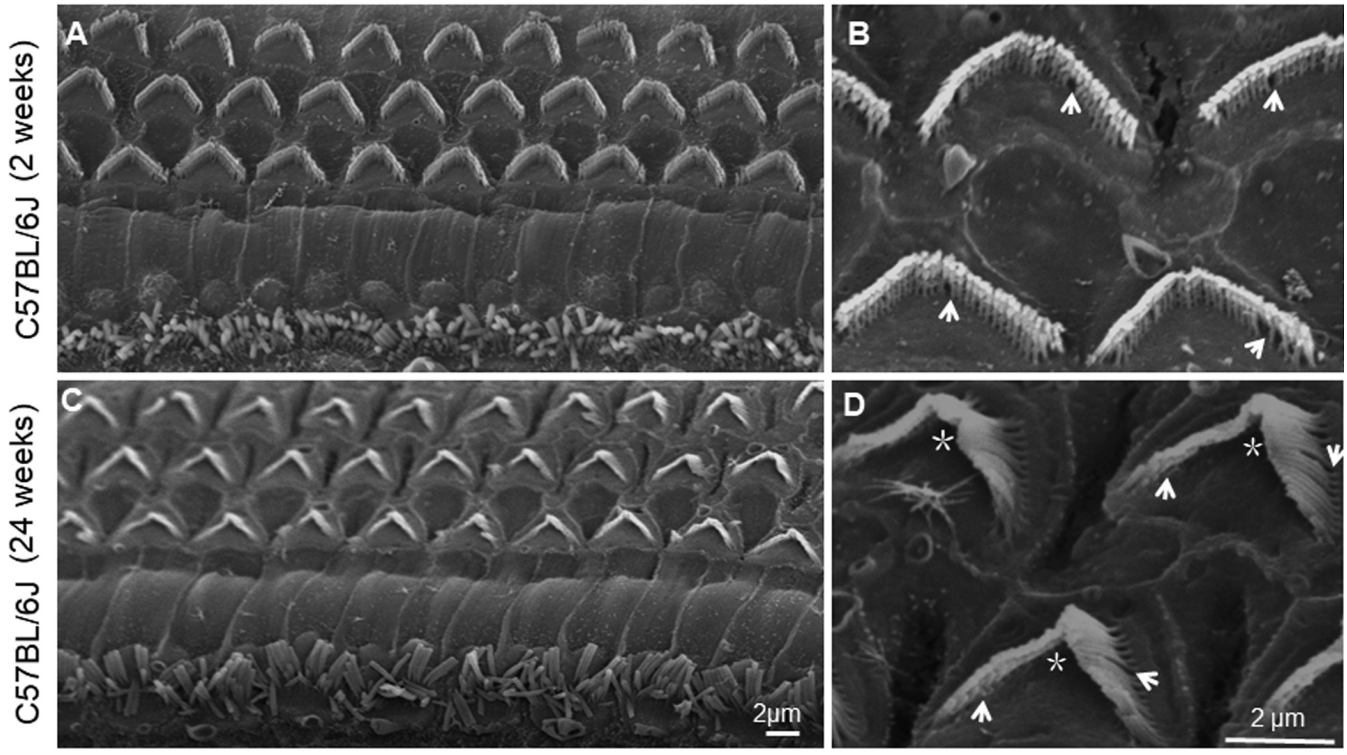

Fig. 3. Images of SEM to show the stereocilia of cochlear hair cells in C57BL/6J mice. C57BL/6J mice show almost normal appearance of stereocilia in OHCs in the basal turns of cochleae at 2 weeks **(A)** and 24 weeks **(C)** under 2 K times magnification. However, defects (or gaps) of stereocilia occurred in C57BL/6J mice at 2 weeks and 24 weeks under 10 K times magnification as indicated by arrows **(B, D)**. The fusion of stereocilia was evident at 24 weeks as indicated by asterisks **(D).** Scale bars: 2 μm.

showed defects of stereocilia at 2 weeks and fusions and bending of stereocilia at 24 weeks under 10 K times magnification (Fig. 3B,D). NOD/LtJ mice showed loss of inverted V-shaped OHC bundles at 2 weeks (Fig. 4A). Defects of single bundle of stereocilia in the residual OHCs in NOD/LtJ mice were visible at 2 weeks under 10 K times magnification (Fig. 4B). With the increase of age, NOD/LtJ mice showed more severe defects and fusions of inverted V-shape OHC bundles at 4 weeks (Fig. 5A,B) and 6 weeks (Fig. 6A,B). At 12 weeks of age, most stereocilia of OHCs in NOD/LtJ mice were lost (Table 1). The stereocilia in A/J mice were already irregular at 2 weeks (Fig. 4C,D), disrupted

Table 1. Percentages of stereociliary loss in basal turns of cochleae of the 4 mouse strains.

	2 weeks	4 weeks	8 weeks	12 weeks
C57BL/6J	0±0	0±0	0±0	0±0
DBA/2J	0±0	1.11±1.52	46.93±4.77	55.92±8.22
A/J	0±0	10.77±2.57	47.07±6.18	66.57±7.48
NOD/LtJ	11.38±6.40	44.34±4.66	63.94±9.51	89.84±4.77

n=4 for each strain at each time point.

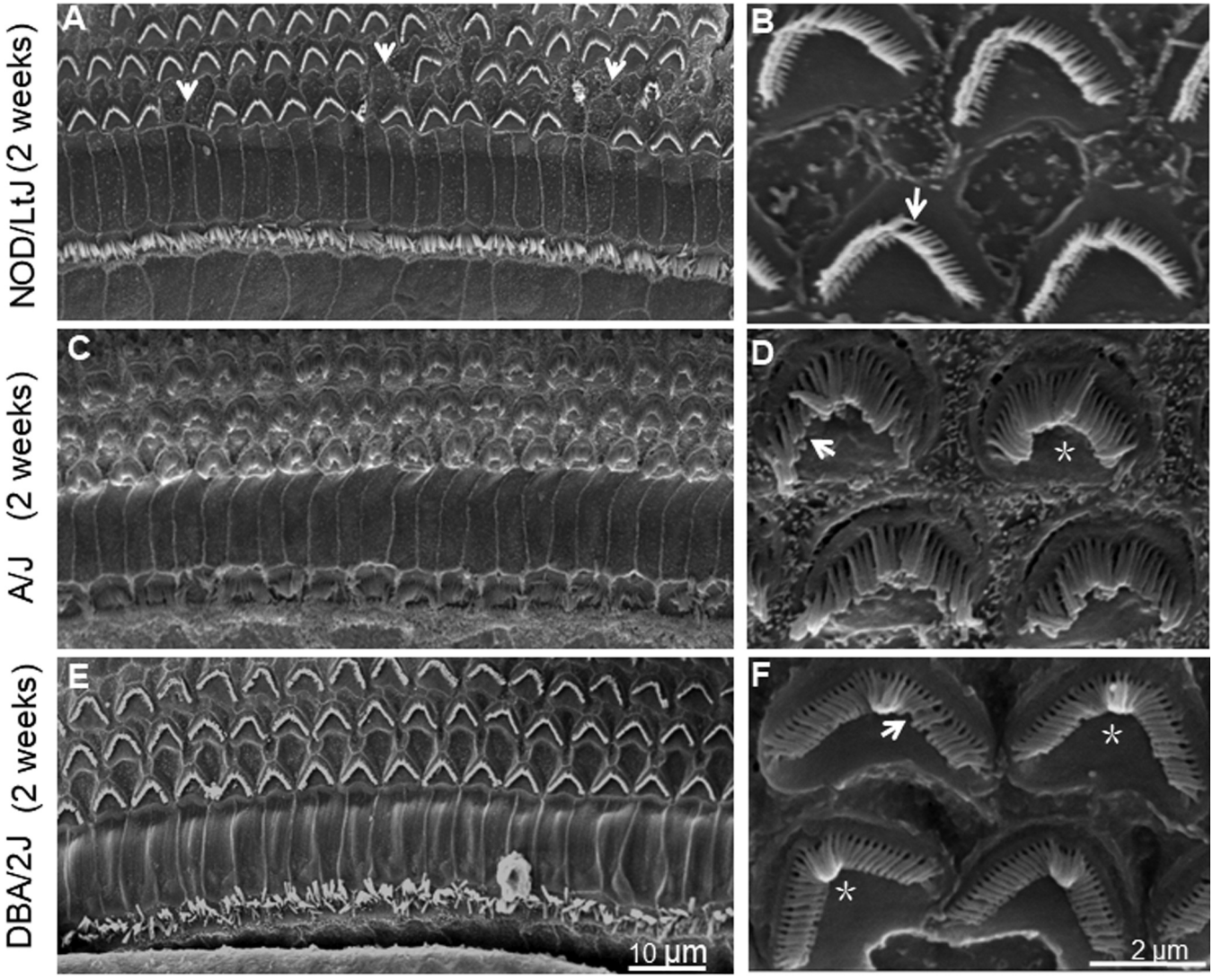

Fig. 4. Images of hair cell stereocilia by SEM in the basal turns of cochleae of NOD/LtJ, A/J and DBA/2J mice at 2 weeks of age. **A, B.** Hair cell stereocilia in the cochleae of NOD/LtJ mice. **C, D.** Hair cell stereocilia of cochleae in A/J mice. **E, F.** Hair cell stereocilia of cochleae in DBA/2J mice. Loss of entire stereocilia in an OHC in NOD/LtJ mice occurred at 2 weeks of age, as indicated by arrows **(A)**. Defects of stereocilia bundles are indicated by arrows and fusion or bending of stereocilia is indicated by asterisks **(B, D, F)**. Scale bars: A, C, E, 10 µm; B, D, F, 2 µm.

at age of 4 and 8 weeks (Figs. 5C,D, 6C,D), and lost for more than half at 12 weeks (Table 1). Particularly, the stereocilia of OHCs in A/J mice displayed abnormal U-shaped images under 10 K times magnification (Figs. 4C,D, 5C,D). DBA/2J mice showed bending of OHC stereocilia or single OHC bundle defects in the basal turns of cochleae at 2 weeks (Fig. 4E,F). Moreover, DBA/2J mice displayed multiple deformations such as

Table 2. Summary of hearing loss and stereociliary malformations in the 4 mouse strains.

Strain	Defective genes	Age of HL in our studies (click)	Age of HL in previous studies	Frequencies lost in previous studies	Previous analysis by SEM	Age of stereociliary malformations by SEM in this study
NOD/LtJ	Cdh23+unknown	3-12 weeks	3-9 weeks	all frequencies	unknown	2-12 weeks
A/J	Cdh23+Cs	3-12 weeks	3-12 weeks	all equally	unknown	2-12 weeks
DBA/2J	Cdh23+Fscn2+QTL(Chr5)	3-12 weeks	3-14 weeks	all equally	yes	2-12 weeks
C57BL6/J	Cdh23	36-102 weeks (Han et al, 2012)	24-100 weeks	higher ones first	yes	2-24 weeks

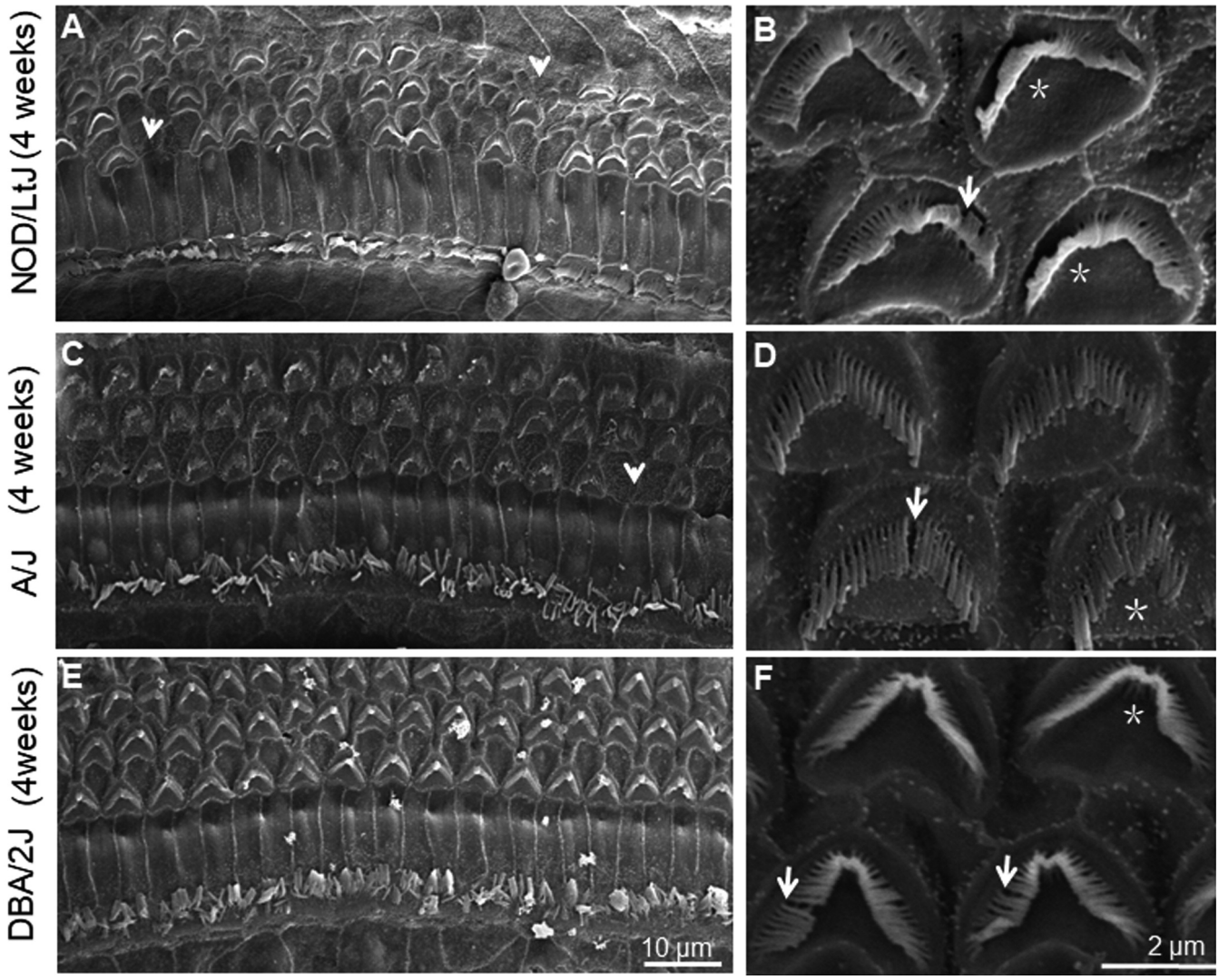

Fig. 5. Images of hair cell stereocilia by SEM in the basal turns of cochleae of NOD/LtJ, A/J and DBA/2J mice at age of 4 weeks. **A, B.** Hair cell stereocilia of cochleae in NOD/LtJ mice. **C, D.** Hair cell stereocilia of cochleae in A/J mice. **E, F.** Hair cell stereocilia of cochleae in DBA/2J mice. Loss of stereocilia in NOD/LtJ and A/J mice at 4 weeks of age is indicated by arrows **(A, C)**. Defects of stereocilia bundles are indicated by arrows and fusion or bending of stereocilia is indicated by asterisks **(B, D, F).** Scale bars: A, C, E, 10 µm; B, D, F, 2 µm.

fusions, bulging or bending of inverted V-shape OHC bundles at 4 weeks (Fig. 5E,F). Defects or disruption of stereocilia in DBA/2J mice were remarkable at 8 weeks (Fig. 6E,F), and about 56% of the stereocilia were lost at 12 weeks (Table 1). Degeneration of inner hair cell (IHC) was not so severe as that of OHC at the same time (Fig. 6A,C,E).

Discussion

NOD/LtJ, A/J, DBA/2J and C57BL/6J mice showed early onset progressive degeneration of cochlear stereocilia

NOD/LtJ, A/J, DBA/2J and C57BL/6J are mouse models of genetic hearing loss. In addition to the earlier

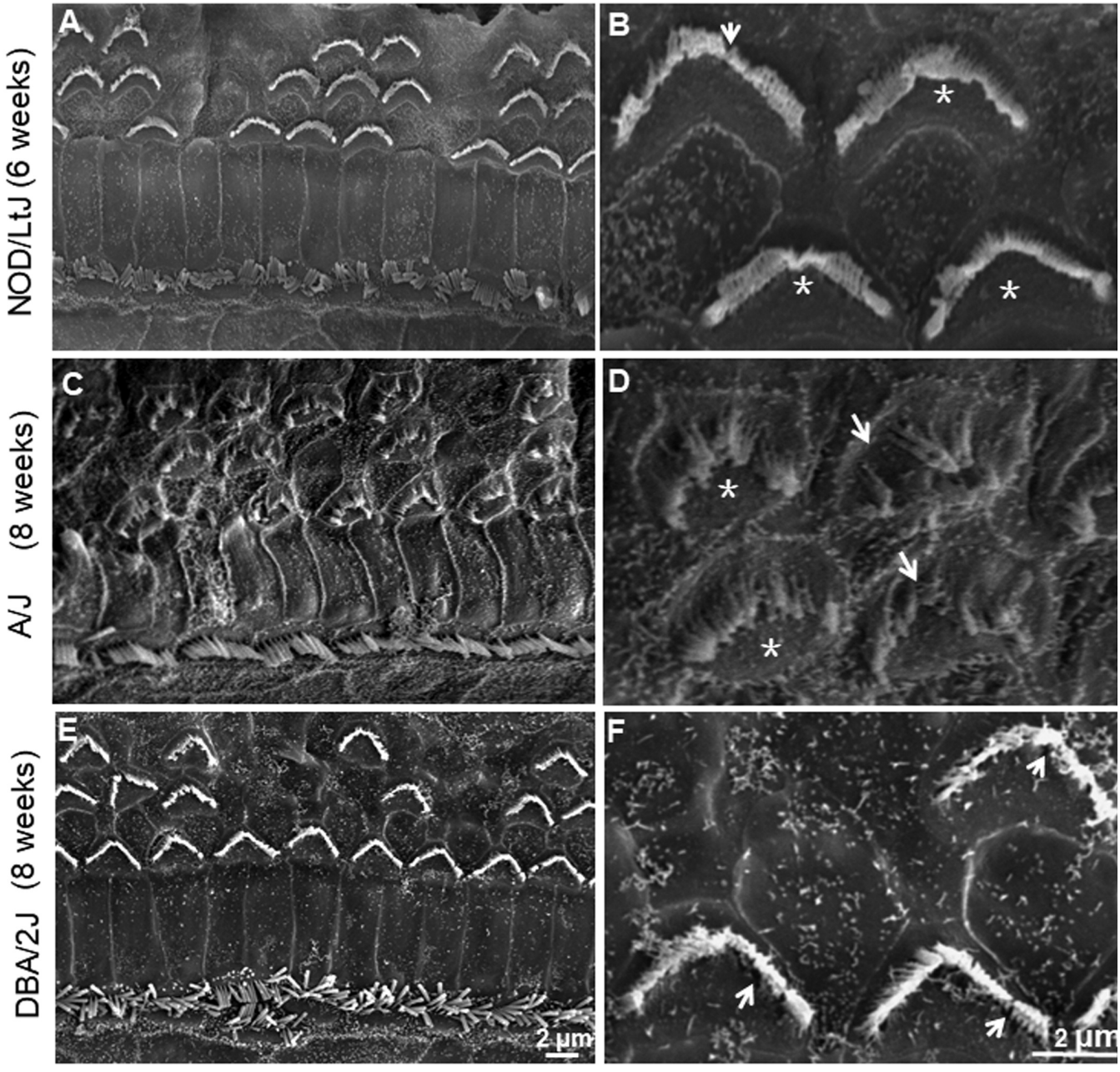

Fig. 6. Images of hair cell stereocilia by SEM in the basal turns of cochleae of NOD/LtJ, A/J and DBA/2J mice at age of 6 or 8 weeks. **A, B.** Hair cell stereocilia of cochleae in NOD/LtJ mice. **C, D.** Hair cell stereocilia of cochleae in A/J mice. **E, F.** Hair cell stereocilia of cochleae in DBA/2J mice. Loss of stereocilia in NOD/LtJ, A/J and DBA/2J mice at 6 or 8 weeks of age was remarkable **(A, C, E).** Defects of stereocilia bundles are indicated by arrows and fusion or bending of stereocilia is indicated by asterisks **(B, D, F).** Scale bars: 2 µm.

report of Zheng's group (Zheng et al., 1999), the features of hearing loss in these strains have also been described recently (Han et al., 2012, 2015; Yang et al., 2015; Sang et al., 2017; Liu et al., 2018). All the mouse strains developed progressive degeneration of OHCs and progressive hearing loss. Though hearing loss occurs primarily for high stimulus frequencies (OHC loss starts at the basal turns), ABR thresholds at low stimulus frequencies (click) were also elevated in NOD/LtJ, A/J, DBA/2J at early stage (3-16 weeks). In this study, the NOD/LtJ, A/J, and DBA/2J mice showed elevation of ABR thresholds at click from 3 to 12 weeks of age, compared with the thresholds of C57BL/6J mice. There were reports that C57BL/6J mice presented hearing loss at about 9 to 12 months of age (Han et al., 2012), and that a significant hearing loss for high-frequencies occurred at 6 months of age (Fetoni et al., 2011). Overall, ABR thresholds (at click) in the 4 mouse strains were basically in accordance with the previous reports. The function impairment of OHCs was also confirmed by DPOAE at 4 and 8 weeks of age.

Previous studies conclude that the NOD/LtJ, A/J, DBA/2J and C57BL/6J mice exhibit early onset of pathology in the cochleae. The early, intermediate and late onset of pathology of AHL is respectively referred to <12 months, 13-23 months and >24 months of age (Fetoni et al., 2011). In the present study, the early pathology of the OHC stereocilia in the basal turns of the cochleae in the 4 mouse strains was further observed under SEM. At 2 weeks of age, malformation or loss of the OHC stereocilia occurred, even in C57BL/6J mice, which has not previously been reported; at 4 weeks of age, degeneration of the bundles of stereocilia was evident in NOD/LtJ, A/J, and DBA/2J mice; at 6-8 weeks of age, disruption or loss of stereocilia was remarkable in NOD/LtJ, A/J, and DBA/2J mice; at 12 weeks of age, most stereocilia were lost for NOD/LtJ, A/J, and DBA/2J mice. In a previous study, the C57BL/6J mice were used as the "normal controls" for SEM (Liu et al., 2018). It was reported that, at 1 month and 3 months of age for DBA/2J mice, most OHC bundles showed signs of degeneration and some were missing (Shin et al., 2010). Our present study showed that degeneration of stereocilia bundles occurred at as early as 2 weeks of age in the 4 mouse strains, though diverse forms of degeneration were observed. It is well known that the cochlear stereocilia are essential to the perception of sound and motion (Sekerkova et al., 2011). Early degeneration of OHC stereocilia may play an important role in the early onset of hearing loss in these mouse strains.

Degeneration of stereocilia may be caused primarily by gene mutations in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice

The *ahl* allele is a major predisposing factor to hearing loss not only in C57BL/6J strain but also in strains such as A/J, DBA/2J and NOD/LtJ (Noben-Trauth and Johnson, 2009). It is a mutation in cadherin23 gene (Cdh23), resulting in skip of exon 7 during RNA splicing. The Cadherin23 (CDH23) is a component of the tip link in hair cell stereocilia and the corresponding mutant CDH23 (from *ahl*) may weaken the stiffness of the cell stereocilia (Kazmierczak et al., 2007; Noben-Trauth and Johnson, 2009). Meanwhile, the misfolded CDH23 protein would be retained in the endoplasmic reticulum, causing a constitutive shortage of functional cadherin 23 and ER stress (Hu et al., 2016). The above factors may contribute to the early degeneration of stereocilia in C57BL/6J. With the reduction of stiffness of the stereocilia, fusion or bending of the stereocilia is evident at 24 weeks of age.

A/J mice also share the *ahl* allele and have another AHL locus (named *ahl4*) in chromosome 10 (Zheng et al., 2009). The *ahl4* locus, which could explain about 40% of the ABR threshold variation in these mice, was identified as a mutation in the gene of *Critrate synthetase* (Cs) (Johnson et al., 2012), which was an essential enzyme and the first rate-limiting enzyme in the tricarboxylic acid cycle (TCA). Reduction of Cs gene causes excess reactive oxygen species (ROS) production and cell apoptosis *in vitro* (Cai et al., 2017). A single nucleotide insertion in the tRNA-Arg gene (*mt-Tr*) may also contribute to the phenotypic effect (Johnson et al., 2001). This same mtDNA variant was later shown to cause an increase of ROS production in cell lines (Moreno-Loshuertos et al., 2006). Excess ROS production caused by this mtDNA variant could exacerbate the stressful effects of the *Cdh23* variant and increase the rate of hair cell degeneration. Probably, pathological alterations of stereocilia in A/J mice were caused by interaction of the mutations in *Cdh23, Cs* and *mt-Tr* gene.

Apart from *ahl*, a locus named *ahl8* on Chromosome 11 was identified as the main contributor to the early onset of hearing loss in DBA/2J mice (Johnson et al., 2008). The *ahl8*-causative gene was laterly identified as Fascin2 (Fscn2), which encoded an actin crosslinking protein FSCN2 (Shin et al., 2010). FSCN2 is abundant in stereocilia and plays a critical role in stabilizing stereocilia after development (Shin et al., 2010) . In addition to the mutations in *ahl* and *ahl8*, a quantitative trait locus on chromosome 5 may also be responsible for the severe hearing loss in DBA/2J mice (Johnson et al., 2015; Suzuki et al., 2015). DBA/2J mice showed fusions and lodging of inverted V-shaped OHC bundles at 2 and 4 weeks in this study. Early degeneration of stereocilia in DBA/2J mice may mainly result from mutations in *Cdh23*, *Fscn2* and other gene(s).

Inbred strains of NOD/LtJ mice are susceptible to early-onset AHL. The major contributor to the difference in hearing loss compared with C57BL/6J mice is the *ahl2* locus on mouse chromosome 5 in NOD/LtJ mice (Johnson and Zheng, 2002). The *ahl2* locus exacerbates the effects of the ahl locus, resulting in earlier onset and rapider progression of hearing loss (Noben-Trauth and Johnson, 2009). NOD/LtJ mice showed loss of inverted

V-shape OHC bundles at 2 weeks and became more severe at 4 weeks of age. Interaction of *ahl* and *ahl2* contributed to the results.

It should be mentioned that the effects of *ahl2, ahl4,* and *ahl8* locus on hearing loss in the backcrossed mice were manifested only in mice with *ahl*/*ahl* genotype (Noben-Trauth and Johnson, 2009). That means impairment of tip links is essential for the effects of *ahl2, ahl4,* and *ahl8*. Interaction of *ahl* and *ahl2, ahl4, ahl8* or other factors determines the cochlear pathology or the severity of hearing loss in these mice. Genetic defects in these mouse strains are the main factors of cochlear pathology, leading to progressive hearing loss. A summary of the hearing loss and stereociliary malformations in the 4 mouse strains are listed in Table 2.

Multiple factors contribute the early onset hearing loss in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice

AHL is associated with an age-dependent loss of sensory hair cells, spiral ganglion neurons and stria vascularis cells in the inner ears (Gates and Mills, 2005; Yamasoba et al., 2013). Previous studies have shown that degeneration of organ of Corti and afferent neurons are involved in the hearing loss of C57BL/6J mice (Johnson et al., 1997). Hearing loss in DBA/2J mice is paralleled by degeneration of the organ of Corti and spiral ganglia (Willott et al., 2005; Johnson et al., 2008; Yang et al., 2015). Results also demonstrated that A/J mice displayed all the three features of degeneration of the sensory hair cells, spiral ganglion neurons and stria vascularis cells (Han et al., 2015). The NOD/LtJ mice show a typical metabolic hearing loss with strial capillary degeneration and subsequent strial atrophy (Ohlemiller et al., 2008; Fetoni et al., 2011). In this study, only the stereocilia defects of OHC in the 4 mouse strains were observed. In addition to the earlier degeneration of stereocilia, other factors such as spiral ganglion neurons and strial atrophy should also be taken into account for the hearing loss.

In summary, NOD/LtJ, A/J, DBA/2J and C57BL/6J mice showed early onset progressive degeneration of stereocilia, as well as progressive hearing loss. Our results indicate that early degeneration of stereocilia is an important factor for the functional impairment in hair cells and progressive hearing loss in NOD/LtJ, A/J, DBA/2J and C57BL/6J mice.

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References

Angeli S., Lin X. and Liu X.Z. (2012). Genetics of hearing and deafness. Anat. Rec. (Hoboken) 295, 1812-1829.

Cai Q., Zhao M., Liu X., Wang X., Nie Y., Li P., Liu T., Ge R. and Han F. (2017). Reduced expression of citrate synthase leads to excessive superoxide formation and cell apoptosis. Biochem. Biophys. Res. Commun. 485, 388-394.

Fetoni A.R., Picciotti P.M., Paludetti G. and Troiani D. (2011). Pathogenesis of presbycusis in animal models: a review. Exp. Gerontol. 46, 413-425.

Fujinami Y., Mutai H., Mizutari K., Nakagawa S. and Matsunaga T. (2012). A novel animal model of hearing loss caused by acute endoplasmic reticulum stress in the cochlea. J. Pharmacol. Sci. 118, 363-372.

Gates G.A. and Mills J.H. (2005). Presbycusis. Lancet 366, 1111-1120.

Han F., Yu H., Tian C., Chen H.E., Benedict-Alderfer C., Zheng Y., Wang Q., Han X. and Zheng Q.Y. (2012). A new mouse mutant of the Cdh23 gene with early-onset hearing loss facilitates evaluation of otoprotection drugs. Pharmacogenomics J. 12, 30-44.

Han F., Yu H., Zheng T., Ma X., Zhao X., Li P., Le L., Su Y. and Zheng Q.Y. (2013). Otoprotective effects of erythropoietin on Cdh23erl/erl mice. Neuroscience 237, 1-6.

Han X., Ge R., Xie G., Li P., Zhao X., Gao L., Zhang H., Wang O., Huang F. and Han F. (2015). Caspase-mediated apoptosis in the cochleae contributes to the early onset of hearing loss in A/J mice. ASN. Neuro. 7, 1759091415573985.

Henry K.R. (1982). Age-related auditory loss and genetics: an electrocochleographic comparison of six inbred strains of mice. J. Gerontol. 37, 275-282.

Hu J., Li B., Apisa L., Yu H., Entenman S., Xu M., Stepanyan R., Guan B.J., Muller U., Hatzoglou M. and Zheng Q.Y. (2016). ER stress inhibitor attenuates hearing loss and hair cell death in Cdh23erl/erl mutant mice. Cell Death Dis. 7, e2485.

Johnson K.R. and Zheng Q.Y. (2002). Ahl2, a second locus affecting age-related hearing loss in mice. Genomics 80, 461-464.

Johnson K.R., Erway L.C., Cook S.A., Willott J.F. and Zheng Q.Y. (1997). A major gene affecting age-related hearing loss in C57BL/6J mice. Hear. Res. 114, 83-92.

Johnson K.R., Zheng Q.Y. and Erway L.C. (2000). A major gene affecting age-related hearing loss is common to at least ten inbred strains of mice. Genomics 70, 171-180.

Johnson K.R., Zheng Q.Y., Bykhovskaya Y., Spirina O. and Fischel-Ghodsian N. (2001). A nuclear-mitochondrial DNA interaction affecting hearing impairment in mice. Nat. Genet. 27, 191-194.

Johnson K.R., Longo-Guess C., Gagnon L.H., Yu H. and Zheng Q.Y. (2008). A locus on distal chromosome 11 (ahl8) and its interaction with Cdh23 ahl underlie the early onset, age-related hearing loss of DBA/2J mice. Genomics 92, 219-225.

Johnson K.R., Gagnon L.H., Longo-Guess C. and Kane K.L. (2012). Association of a citrate synthase missense mutation with age-related hearing loss in A/J mice. Neurobiol. Aging 33, 1720-1729.

Johnson K.R., Longo-Guess C.M. and Gagnon L.H. (2015). A QTL on Chr 5 modifies hearing loss associated with the fascin-2 variant of DBA/2J mice. Mamm. Genome 26, 338-347.

Kazmierczak P., Sakaguchi H., Tokita J., Wilson-Kubalek E.M., Milligan R.A., Muller U. and Kachar B. (2007). Cadherin 23 and protocadherin 15 interact to form tip-link filaments in sensory hair cells. Nature 449, 87-91.

Liu X.Z. and Yan D. (2007). Ageing and hearing loss. J. Pathol. 211, 188-197.

Liu X., Zhao M., Xie Y., Li P., Wang O., Zhou B., Yang L., Nie Y., Cheng L., Song X., Jin C., and Han F. (2018). Null mutation of the fascin2 Gene by TALEN leading to progressive hearing loss and retinal degeneration in C57BL/6J mice. G3 (Bethesda) 8, 3221-3230.

Men Y., Zhang A., Li H., Zhang T., Jin Y., Zhang J. and Gao J. (2015). LKB1 is required for the development and maintenance of stereocilia in inner ear hair cells in mice. PLoS One 10, e0135841.

Moreno-Loshuertos R., Acin-Perez R., Fernandez-Silva P., Movilla N., Perez-Martos A., Rodriguez de Cordoba S., Gallardo M.E. and Enriquez J.A. (2006). Differences in reactive oxygen species production explain the phenotypes associated with common mouse mitochondrial DNA variants. Nat. Genet. 38, 1261-1268.

Noben-Trauth K. and Johnson K.R. (2009). Inheritance patterns of progressive hearing loss in laboratory strains of mice. Brain Res. 1277, 42-51.

Ohlemiller K.K., Rice M.E. and Gagnon P.M. (2008). Strial microvascular pathology and age-associated endocochlear potential decline in NOD congenic mice. Hear. Res. 244, 85-97.

Sang L., Zheng T., Min L., Zhang X., Ma X., Entenman S., Su Y. and Zheng Q. (2017). Otoprotective effects of ethosuximide in NOD/LtJ mice with age-related hearing loss. Int. J. Mol. Med. 40, 146-154.

Schuknecht H.F. and Gacek M.R. (1993). Cochlear pathology in presbycusis. Ann. Otol. Rhinol. Laryngol. 102, 1-16.

Sekerkova G., Richter C.P. and Bartles J.R. (2011). Roles of the espin actin-bundling proteins in the morphogenesis and stabilization of hair cell stereocilia revealed in CBA/CaJ congenic jerker mice. PLoS Genet. 7, e1002032.

Shin J.B., Longo-Guess C.M., Gagnon L.H., Saylor K.W., Dumont R.A., Spinelli K.J., Pagana J.M., Wilmarth P.A., David L.L., Gillespie P.G. and Johnson K.R. (2010). The R109H variant of fascin-2, a developmentally regulated actin crosslinker in hair-cell stereocilia, underlies early-onset hearing loss of DBA/2J mice. J. Neurosci. 30, 9683-9694.

Suzuki S., Ishikawa M., Ueda T., Ohshiba Y., Miyasaka Y., Okumura K., Yokohama M., Taya C., Matsuoka K. and Kikkawa Y. (2015). Quantitative trait loci on chromosome 5 for susceptibility to frequency-specific effects on hearing in DBA/2J mice. Exp. Anim. 64, 241-251.

Willott J.F., Bross L.S. and McFadden S. (2005). Ameliorative effects of exposing DBA/2J mice to an augmented acoustic environment on histological changes in the cochlea and anteroventral cochlear nucleus. J. Assoc. Res. Otolaryngol. 6, 234-243.

Yamasoba T., Lin F.R., Someya S., Kashio A., Sakamoto T. and Kondo K. (2013). Current concepts in age-related hearing loss: epidemiology and mechanistic pathways. Hear. Res. 303, 30-38.

Yamasoba T., Someya S., Yamada C., Weindruch R., Prolla T.A. and Tanokura M. (2007) Role of mitochondrial dysfunction and mitochondrial DNA mutations in age-related hearing loss. Hear. Res. 226, 185-193.

Yang L., Zhang H., Han X., Zhao X., Hu F., Li P., Xie G., Gao L., Cheng L., Song X. and Han F. (2015). Attenuation of hearing loss in DBA/2J mice by anti-apoptotic treatment. Hear. Res. 327, 109-116.

Zheng Q.Y., Ding D., Yu H., Salvi R.J. and Johnson K.R. (2009). A locus on distal chromosome 10 (ahl4) affecting age-related hearing loss in A/J mice. Neurobiol. Aging 30, 1693-1705.

Zheng Q.Y., Johnson K.R. and Erway L.C. (1999). Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear. Res. 130, 94-107.

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