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# Effects of Genetic Background on Susceptibility and the Acceleration of Hearing Loss in Mice

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**Abstract**

Acquired hearing loss, which includes age-related hearing loss and noise-induced hearing loss, is a common hearing impairment and shows phenotypic variability. One reason for phenotypic variability is influence of genetic background. The modifiers underlying genetic background are modulated and advance the hearing phenotypes through gene-gene interactions with other etiological genetic factors. Moreover, the modifiers play a role in the susceptibility of environmental hearing risk factors, namely, the strength and weakness of environmental susceptibility often modulate and advance hearing phenotypes via gene-environment interactions. The complicated gene-gene and gene-environment interactions make genetic analysis of acquired hearing loss difficult. In particular, the effects of environmental factors cannot be completely excluded or controlled. Although genome-wide approaches to identify genetic modifiers have proven challenging in humans, the responsible genes and mutations are widely unknown. In this chapter, we suggest that mouse models are useful for studying genetic background effects for acquired hearing loss. The genetic analysis of mouse models identified the genetic modifiers. We review the genetic research in mouse models for acquired hearing loss to identify and confirm the modifiers by both forward and reverse genetics approaches.

**Keywords:** genetic background effects, mouse model, quantitative trait loci (QTL), genetic modifiers, genetic interaction, epistasis, genome editing

## 1. Introduction

Hearing loss is the most common sensory disorder, which affects approximately 0.1–0.2% of newborns [1]. A genetic etiology of congenital hearing loss accounts for an estimate of at least 50–60% of hearing loss cases, whereas the remaining 40–50% develops from a nongenetic etiology, such as effects of risk factors for neonates and birth conditions [1–3]. To date, many

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mutations responsible for hearing loss have been recently identified in approximately 100 human genes [3, 4]. However, most mutations are primarily associated with congenital and severe hearing loss developed at newborn and childhood stages caused by a single gene. The identification of the more common "acquired hearing loss," such as age-related hearing loss (ARHL) and noise-induced hearing loss (NIHL) is currently understudied.

Acquired common hearing loss is a complex multifactorial disease influenced by genetic backgrounds and environments. In ARHL, an accurate estimation of the genetic etiology has not been reported. However, it is estimated that ARHL develops through the effects of genetic modifier(s) because the onset time and severity of hearing loss vary greatly among individuals [5, 6]. Moreover, there is a significant heritability of hearing phenotypes [6]. The heritability ranges from low to high [6–10], suggesting that multiple genetic modifiers and environmental factors contribute to the onset and severity of hearing loss. The documented risk factors of ARHL and acquired hearing loss are noise, smoking, alcohol consumption, diet and reduced exercise, complication of other diseases, and uses of ototoxic drugs [6, 11]. It is known that one major risk factor is exposure to loud noise, accounting for approximately 16% of the population worldwide [12]. Genetic factors from a genetic background might also play an important role in the susceptibility of NIHL [13]. However, the identification of genetic background effects in ARHL and NIHL is difficult in humans because of lower heritability of this phenotype and the influence of environmental risk factors mentioned above. In addition, genetic differences among individuals disturb genetic analysis.

To investigate genetic background effects associated with hearing loss, we propose that mouse models have several advantages to overcome weaknesses in the genetic analysis of ARHL and NIHL in humans. Mice can be controlled to avoid environmental risk factors. The techniques for evaluation of hearing, such as measurements of the auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE), have been established [14–18]. Based on the techniques, it is known that there is phenotypic heterogeneity of ARHL and NIHL caused by genetic background effects [3, 6, 11]; therefore, the genetic background effects can be analyzed using the quantitative trait loci (QTL) analysis and genome-wide association study (GWAS) of experimental populations produced by mating susceptible and resistant strains of ARHL and NIHL. This chapter applies the advantages of a genetic analysis by using mouse models to study the genetic background effects of hearing loss.

## 2. What is a genetic background effect?

First, we will explain the basic mechanisms of background effect in phenotypic modification to readers in a way that they will understand the importance of this chapter.

### 2.1. Gene-gene interaction

A complex disease is one that lacks a one-to-one correspondence of mutation and phenotype [19, 20], namely, the expression of phenotype is influenced by genetic modifier(s) underlying the genetic background. **Figure 1A** shows a simple model of the genetic background

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effect. In most cases, the genetic background effects are revealed by the expression of different phenotypes caused by the same genetic mutation in different genetic backgrounds. Let us assume that a gene associated with hearing loss was mutated by gene targeting in mice (strain R). We performed the hearing test of knock-out (KO) mice using measurements of ABR. The KO mouse exhibited latency peak responses for peaks I–V as well as the wild-type mouse; however, the amplitudes of all the peaks were weak and delayed. Next, we performed the gene targeting of the same gene in mice of different genetic backgrounds (strain S). The mouse had no discernable ABR waveform, indicating that the hearing loss of the mouse became more severe due to variation in the genetic background. These results suggest the presence of a genetic modifier, which affects the phenotype developed by the mutation of the causative gene, with respect to the genetic background of strain R. Moreover, it is assumed that the modifier interacts with the causative gene in the acceleration of hearing loss. The genetic interaction could be separated into nonadditive and additive effects to

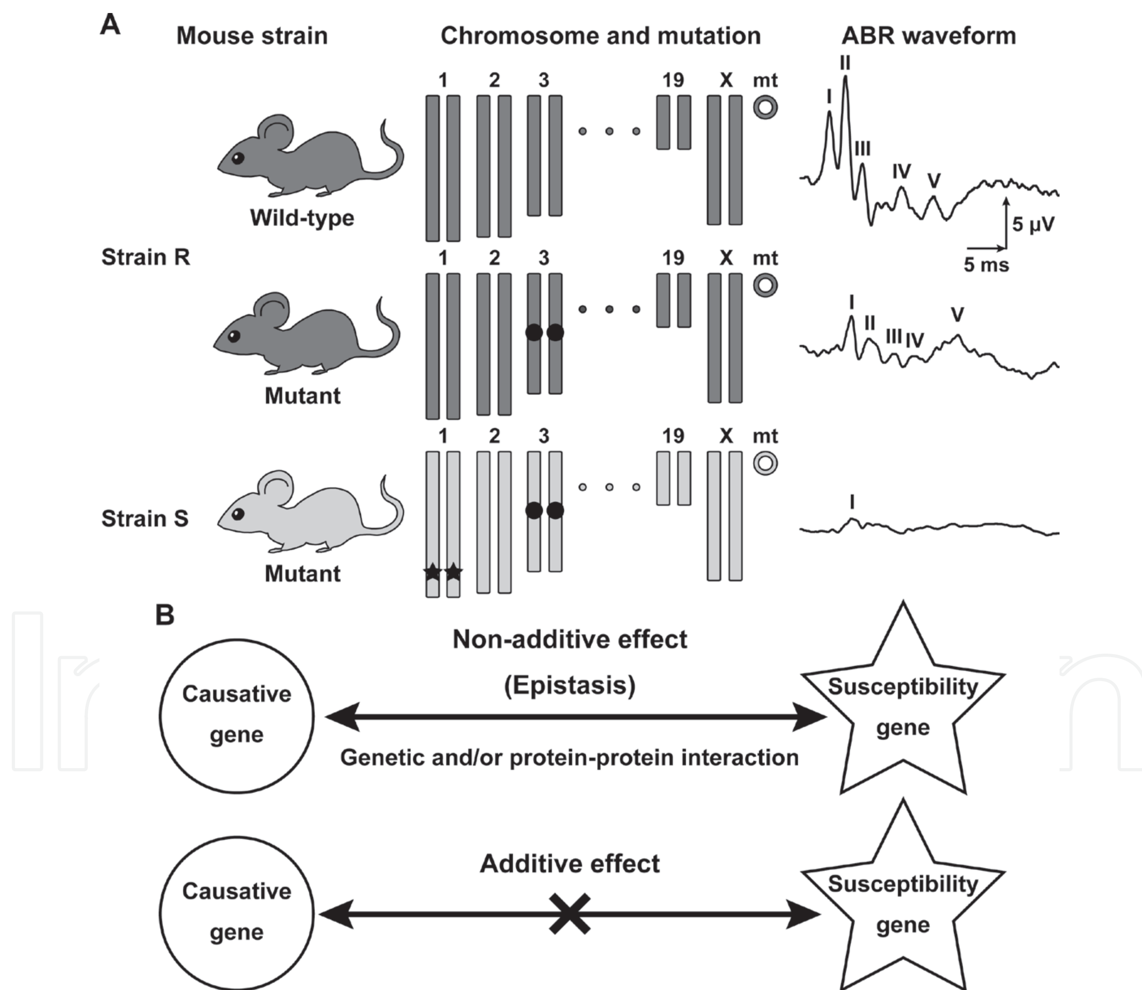


**Figure 1.** Simple models for genetic background effect in phenotypic modification. (A) Schematic representation of the genetic background effect in hearing of mice. Illustrations show the differences of auditory brainstem response (ABR) waveforms by the effects of the susceptible allele (black stars) in the hearing loss mutant. Black circles indicate mutation associated with hearing loss. The locations of ABR peaks I–V are indicated with ranges (μV) of the negative wave apex and latency (ms). (B) Definition of additive (top) and nonadditive (bottom) interaction in phenotypic modification.

evaluate whether the gene-gene, gene-protein, and protein-protein interaction are present in the regulatory pathway of the phenotype (**Figure 1B**). The nonadditive effect is called "epistasis" in technical terms of genetics, stating that there is a direct interaction between two or more genes [19, 20]. The additive effect states that phenotypic acceleration and modification occur by accumulation of mutations in genes of similar function in different molecular pathways (**Figure 1B**) [20].

### 2.2. Gene-environment interaction

In a complex disease, the expression of phenotype is also influenced by environmental factor(s), along with the interaction between the environment and genes in genetic background. A model is shown in **Figure 2A**. Let us assume that we performed an experiment of noise exposure to mice. We performed the experiment in strains R and S and then measured ABR. The ABR responses between the two strains were different. The peaks of the ABR

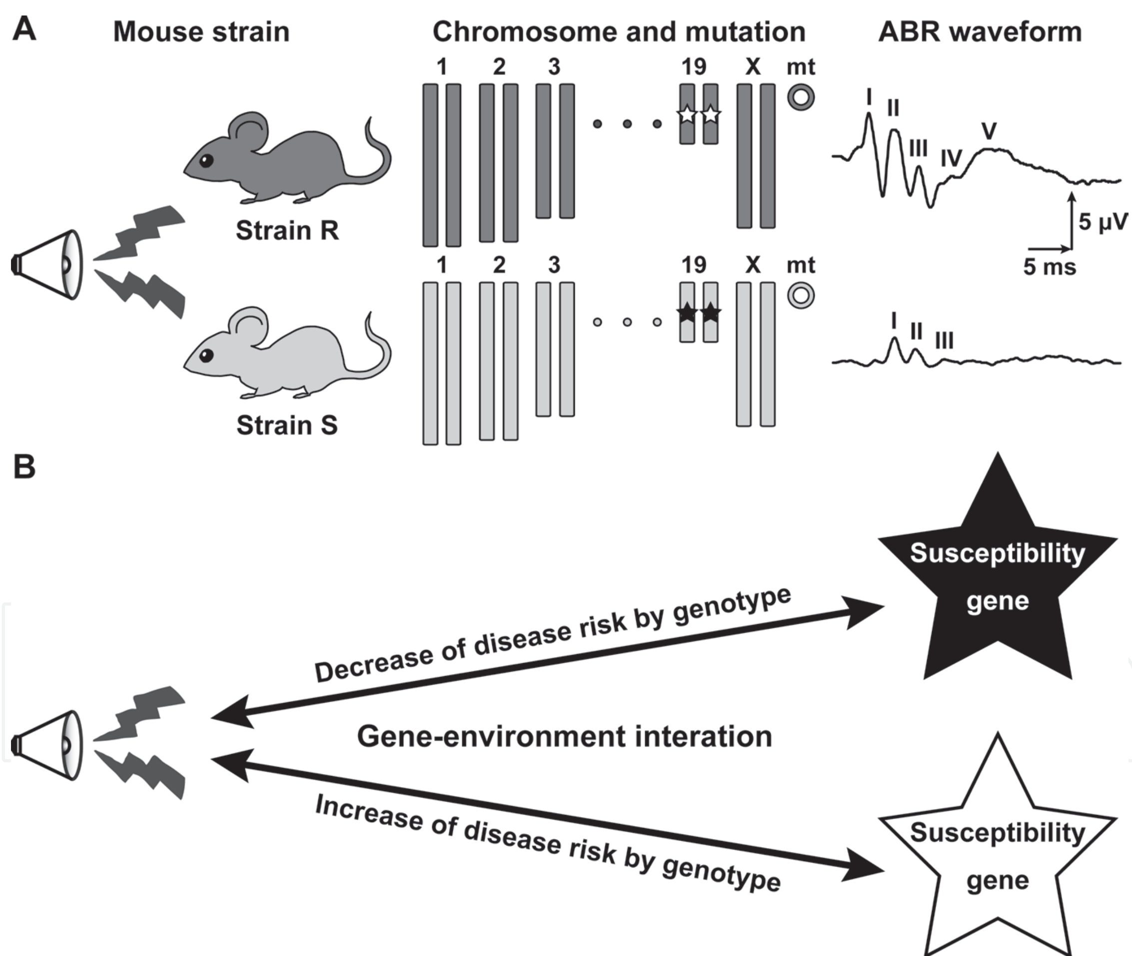


**Figure 2.** Simple models for genetic background effect in the susceptibility of environmental factors. (A) Schematic representation of the genetic background effect in noise-induced hearing loss of mice. Illustrations show the differences between strain R and S of ABR waveforms by the effects of the noise exposure and susceptible allele (black stars). The locations of ABR peaks I–V are indicated with ranges (μV) of the negative wave apex and latency (ms). (B) a model for genetic background effect in the susceptibility of environmental factors.

waveform in strain S were significantly lower than those in strain R. This result suggests that strain R is a noise-resistant strain, whereas strain S is a noise-susceptible strain. In addition, the genetic mechanism for noise sensitivity in both strains can be explained by the presence of resistance and susceptibility alleles in addition to the risk of noise exposure, namely, a gene-environment interaction contributes to the development of NIHL (**Figure 2B**) based on a genetic background effect. Thus, the genetic background effect is important in the study of the influence of environmental factors on complex diseases.

There is one thing to note. Although we explained the gene-environment interaction using a model that would be easy to understand, this model is admittedly simple because it only considers the effect of one gene and one environmental factor. In the case of human diseases, the gene-environment interactions are more complicated because the interactions involve multiple genes, multiple environmental factors, genetic heterogeneity, and heterogeneity of environmental exposure [20].

## 3. Phenotypic variations of hearing caused by genetic backgrounds in mouse inbred strains

Laboratory mice are one of the best experimental models to investigate the genetic background effect for ARHL as mentioned in Section 1. Moreover, the classical inbred strains have been established in large numbers and exhibit variable hearing ability and onset time of ARHL caused by genetic background [21, 22]. **Figure 3** shows the means of ABR thresholds to tone-pip stimuli at 4, 8, 16, and 32 kHz in mice from MSM/Ms, C3H/HeN, C57BL/6J, A/J, and DBA/2J at 4 months of age, as cited in our previous studies [23–25]. The hearing phenotypes of these strains can be classified into two groups: normal hearing and early onset ARHL. The MSM/Ms and C3H/HeN comprise the normal hearing group. The ABR thresholds are stable at all frequencies. The C57BL/6J mice also show normal ABR thresholds at 4, 8, and 16 kHz. However, the ABR threshold at 32 kHz of C57BL/6J mice is significantly higher than that of MSM/Ms and C3H/HeN mice, indicating that C57BL/6J developed high-frequency-specific ARHL. The A/J mice exhibit an ARHL that is more severe for high-frequency stimuli. In addition, the ABR thresholds of the A/J mice at other frequencies are clearly increased when compared with those of C57BL/6J mice. Moreover, the DBA/2J mice developed more severe hearing loss. The ABR thresholds of the DBA/2J mice exhibited levels of severe (71–90 dB SPL) and profound (<91 dB SPL) hearing loss in sound stimuli at 4/8 and 16/32 kHz, respectively, which were significantly higher than those of A/J mice at 4, 8, and 16 kHz.

The difference between the normal hearing and early onset ARHL groups can be explained by a mutation of the Cadherin 23 gene (*Cdh23*). The responsible $Cdh23^{c.753G>A}$ mutation was identified at one base before the splice-donor site, leading to partial skipping of a single exon [21, 24, 26] and age-related stereocilia degeneration in cochlear hair cells [23, 24, 27]. The A/J mice have another strain-specific mutation (p.His55Asn) in the citrate synthase gene (*Cs*) [28]. By identifying $Cs^{p.His55Asn}$ mutation, A/J mice were shown to have developed severe ARHL

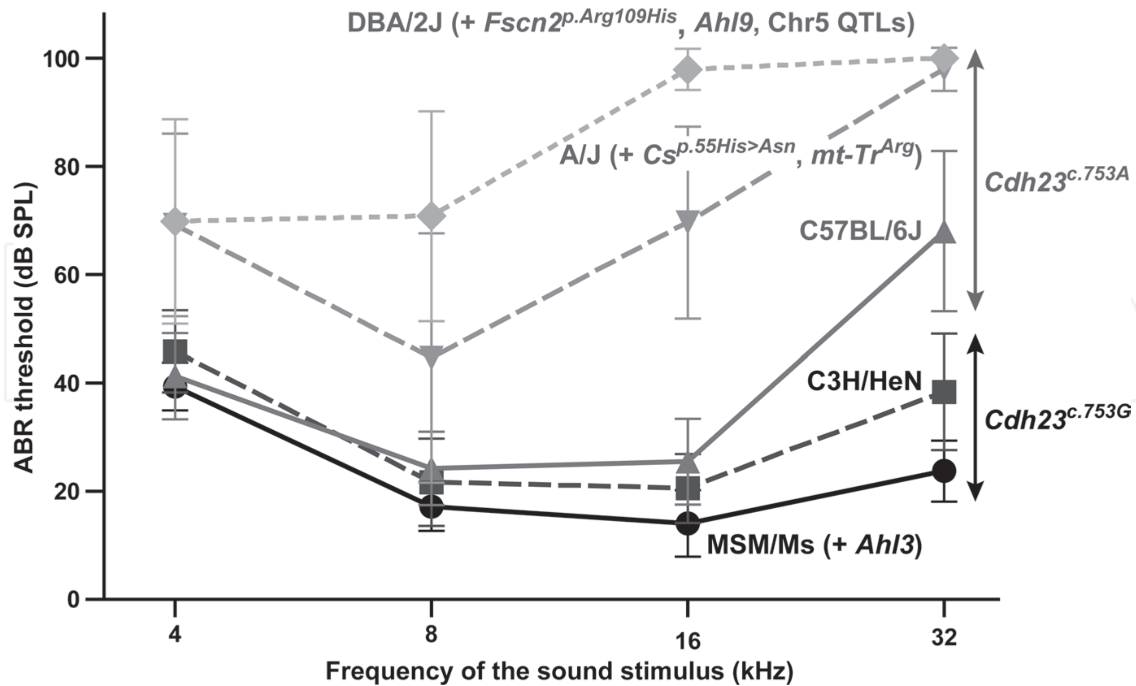


**Figure 3.** Comparison of hearing levels among the mouse inbred strains. The means (circles, squares, diamonds, and upper and lower triangles) and standard deviations (error bars) of ABR thresholds for 4, 8, 16, and 32 kHz sound stimuli are shown for MSM/Ms, C3H/HeN, DBA/2J, C57BL/6J, and A/J mice at 4 months of age. The graph was created by using data from our previous studies [23–25].

by interaction between *Cdh23*$^{c.753G>A}$ and *Cs*$^{p.His55Asn}$ mutations. The CDH23 is a member of the calcium-dependent cell-cell adhesion and tip link component [21, 29, 30]. In contrast, CS is the first enzyme of the tricarboxylic acid cycle, generating citrate and free coenzyme A [31]. Therefore, both proteins seem to contribute different functions in the inner ear for hearing, suggesting that A/J mice develop early onset hearing loss from additive effects of different functional mutations, as described in the previous section (**Figure 1B**). In addition, A/J mice carried a single adenine insertion in the mitochondrial tRNA-Arg gene (*mt-Tr*$^{Arg}$) [32]. CS is transported into the mitochondrial matrix and plays an important role in condensing mitochondrial acetyl-coenzyme A and oxaloacetate for transporting of acetyl-coenzyme A from the mitochondrial matrix to the cytosol [31], suggesting that mitochondrial dysfunction by epistasis (**Figure 1B**) between *Cs*$^{p.His55Asn}$ and *mt-Tr*$^{Arg}$ mutations is accelerated in ARHL of A/J mice. The DBA/2J mice also have a strain-specific mutation (p.Arg109His) in the fascin 2 gene (*Fscn2*) [33]. DBA/2J mice exhibit progressive shortening of the stereocilia, and this phenotype only develops with homozygosity of both the *Cdh23*$^{c.753G>A}$ and *Fscn2*$^{p.Arg109His}$ mutations [34]. FSCN2 is an actin crosslinking protein and localizes along the length of stereocilia at especially high concentration around the stereocilia tips [33, 34]. Although the pathological mechanisms in the genetic interaction between the *Cdh23*$^{c.753G>A}$ and *Fscn2*$^{p.Arg109His}$ mutations are widely unknown, the degeneration of stereocilia in DBA/2J mice may be explained by epistasis. Moreover, the other QTLs related to ARHL were detected in DBA/2J mice. The QTLs, *Ahl9* [35] and *Chr5* QTL [25, 36], are likely to contribute to frequency-specific ARHL. Although the causative genes and mutations are still unknown, these QTLs lead to severe hearing loss by

additive or epistatic interaction with the *Cdh23*$^{c.753G>A}$ and *Fscn2*$^{p.Arg109His}$ mutations. Thus, the differences of hearing levels among the inbred strains are regulated by background effects through the epistatic and additive effects.

## 4. Identification of the genetic modifiers in mice

We have described in the previous section that several mouse inbred strains developed ARHL by genetic background effects. In this section, we introduce approaches to identify genetic modifiers and susceptibility loci of hearing loss underlying the genetic backgrounds.

### 4.1. Classical forward genetics approach

The forward genetics approach is phenotype-driven, with a foundation that associates the detection of chromosomal location with phenotype by linkage analysis. The start of the experiments included production of chromosomal recombinants, $F_2$ and $N_2$ mice, by crossing between the susceptible and resistant strains in phenotype (**Figure 4A**). The linkage analysis is based on meiotic recombination events that occur in sperm and egg precursor cells of $F_1$ hybrid, which have heterozygous chromosomes derived from both the susceptible and resistant strains. Accordingly, the $F_2$ and $N_2$ progenies were produced by intercrossing between $F_1$ mice and backcrossing of $F_1$ mice to one parental strain, respectively, and inherited chromosomes that underwent recombination events. The recombinant region on the chromosomes was detected by using a genetic marker, such as microsatellites and SNPs, which recognized genetic polymorphisms compared to parental strains. Finally, the phenotypes of $F_2$ and $N_2$ mice were investigated to determine whether there is linkage with the recombinant regions of $F_2$ and $N_2$ mice. This approach has been a powerful and productive method to identify QTL-associated ARHL. Johnson et al. [37] detected the first QTL *ahl* locus for ARHL that displayed a *Cdh23*$^{c.753G>A}$ mutation by using $N_2$ backcross mice between ARHL-susceptible C57BL/6J and -resistant CAST/Ei.

As mentioned earlier, there are many modifiers in the genome of inbred strains. To evaluate the effect of a single QTL identified in the mapping by avoiding the effects from other modifiers, congenic mice have become a powerful tool. Congenic mice are defined as having part of the mutation or a chromosomal segment from one inbred genetic background (donor) to another (host) [38] (**Figure 4B**). The creation of congenic mice is based on backcrossing the system for at least seven times. Although this process is long, the resolution of the phenotype greatly improves when compared with $F_2$ and $N_2$ mice. The most successful example of this strategy is the study of *moth1* locus [39, 40]. The *tubby* mice, which are a mutant of tubby bipartite transcription factor gene (*Tub*), exhibit severe hearing loss caused by cochlear degeneration in C57BL/6J background [39]. However, some $F_2$ mice produced by intercrossing with AKR/J and CAST/Ei showed normal hearing. Ikeda et al. [39] mapped the modifier, *moth1*, via linkage analysis using both $F_2$ mice and confirmed the locus by creating congenic mice. This study led to the successful identification of the association of the modification of hearing loss with a strain-specific mutation in microtubule-associated protein 1 gene (*Map1a*), which was the first elucidated causative gene caused by the background effect in hearing [40].

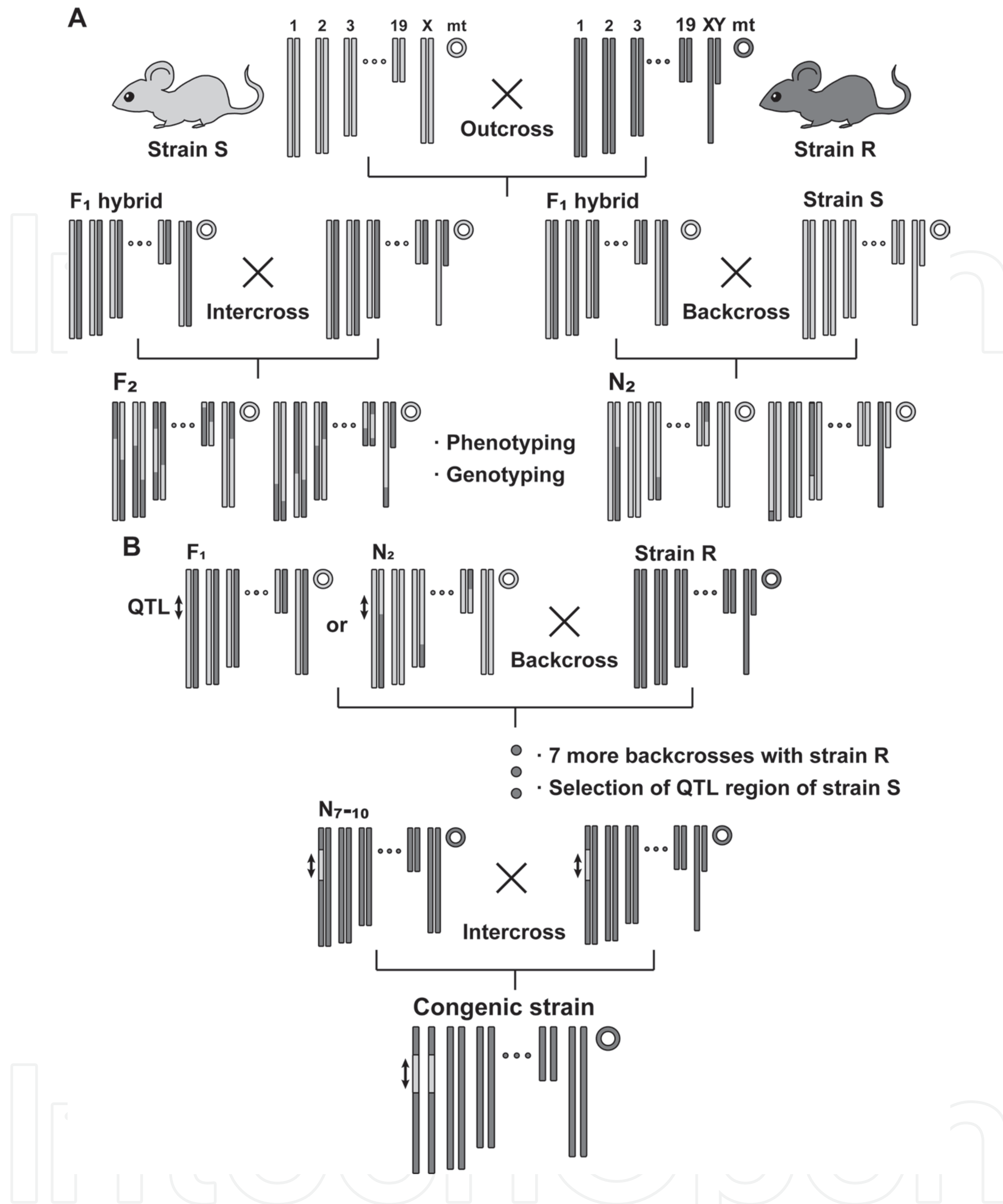


**Figure 4.** Schematic representation of the virtual genomic structures of experimental cross ($F_2$ and $N_2$) (A) for genetic mapping and congenic mouse (B). The rectangles and circles represent chromosome and mitochondrial DNA, respectively. The different strain-derived chromosomal regions are distinguished by light and dark gray colors. Bidirectional arrows indicate quantitative trait locus (QTL) regions.

## 4.2. Forward genetics approaches using genetic reference populations of mice

Currently, we believe that a classical forward genetics approach using $F_2$, $N_2$, and congenic mice is useful to identify the modifiers from genetic background in mice. However, productions of the $F_2$, $N_2$, and congenic mice consume great amount of time and costs for breeding. Large numbers of mice are required to increase mapping resolution. Moreover, the $F_2$ and $N_2$ mice must be used for genome-wide genotyping because their genome architectures are uniquely mixed between parental chromosomes. Therefore, the genotyping cost is enormous. Here, we

introduce the public genetic reference populations of mice. These populations have several advantages owing to established QTL mapping.

*4.2.1. Recombinant inbred strain (RIS)*

Recombinant inbred strain (RIS) panel is a genetic reference population of mice and can serve as a powerful tool for QTL mapping. It is produced by mating sibling $F_2$ mice until the resulting progeny, at least 20 generations later, is fully inbred and displays a mosaic of parental genomes (**Figure 5A**) [41, 42]. RIS panel has several advantages for QTL mapping; if the genotyping is performed once, it does not require genotyping in each individual and is available in public databases; individual, environmental, and measurement variability can be reduced; it has greater mapping resolution because the breakpoints in the genome are denser than those that occur in any one meiosis, such as $F_2$ and $N_2$ mice [41].

RIS panels have been successfully applied to several QTL mappings for ARHL. The strategy includes only evaluating the hearing abilities of each individual RIS panel performed by

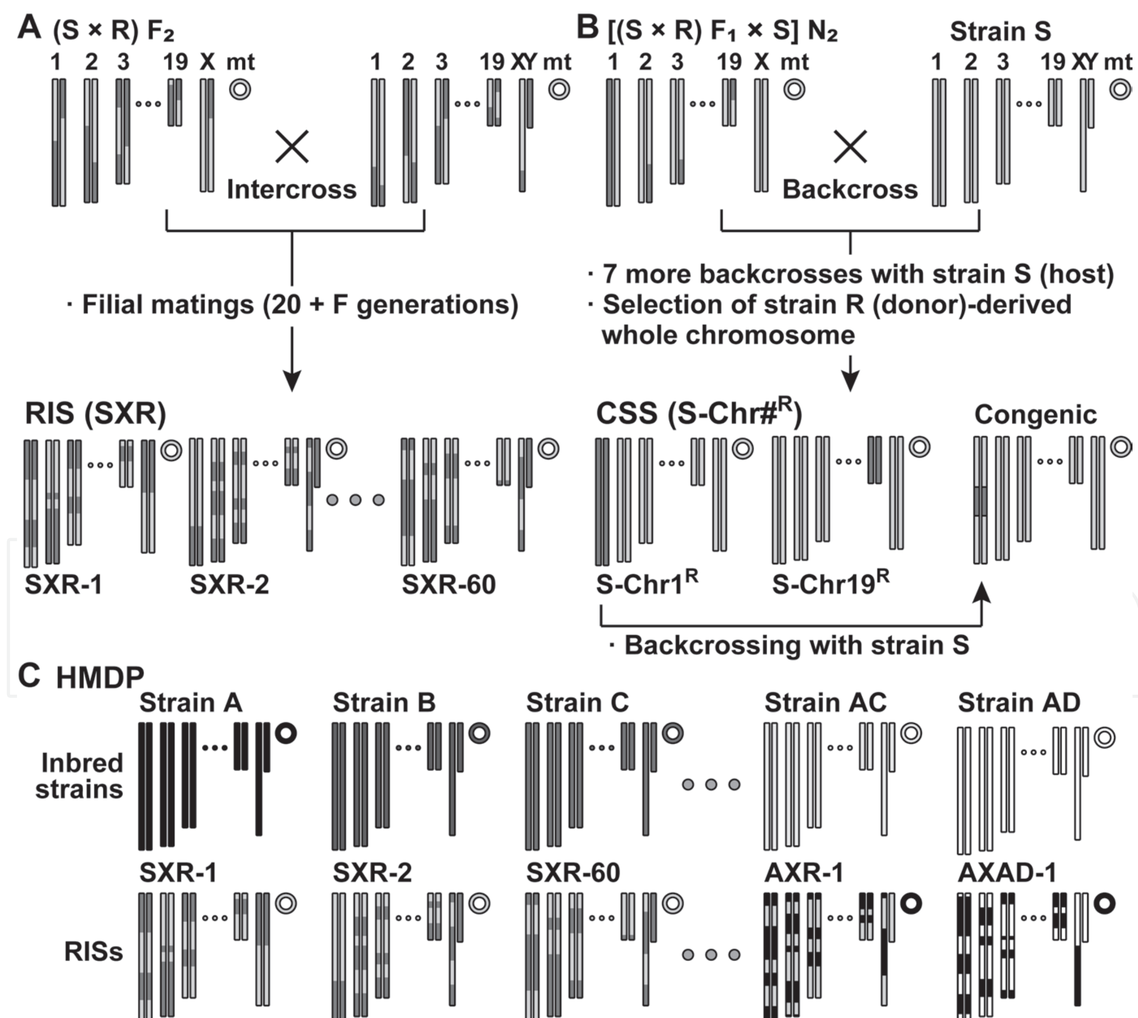


**Figure 5.** Schematic representation of the virtual genomic structures of the recombinant inbred strains (RIS) (A), consomic strain (CSS) (B), and hybrid mouse diversity panel (HMDP) (C) for QTL analysis and genome-wide association study (GWAS). The rectangles and circles represent chromosomes and mitochondrial DNA, respectively. Each strain-derived chromosomal region is distinguished by a different color.

measurements of ABR thresholds and then performing QTL linkage analysis using WebQTL [43], which collects genotypes of microsatellite and SNPs in each RIS panel. By this strategy, the QTLs *ahl4* (*Cs*$^{p.His55Asn}$) [44], *ahl8* (*Fscn2*$^{p.Arg109His}$) [45], *Ahl9* [35], and *Snhl* [18] were mapped using the AXB/BXA [46], BXD [47], BXD and LXS [48], and RIS panels, respectively (**Table 1**). The other RIS panels, such as CXB [49], BXH [50], and SMXA [51], have been established by sibling mating between several inbred strains and will become useful resources to identify new loci associated with ARHL and NIHL.

#### *4.2.2. Consomic strains (CSS)*

Consomic strains (CSS), also called chromosome substitution strains, are combined genomes of two founder inbred strains that have a substitution of one whole chromosome pair from the donor strain into the genetic background of the host strain (**Figure 5B**) [52, 53]. The productive strategy is the same with congenic mice. Usually, a full set of CSSs will consist of 22 strains, which includes 19 pairs of autosomal chromosomes, X and Y sex chromosomes, and a mitochondrial genome, although the introgression into the host background of a whole chromosome from the donor is difficult in some cases [53]. The main advantage of CSS is mapping of the phenotype to single chromosomes. The *Ahl3* [54] and *ahl4* [44] loci have

| Population | Panel | Origin | QTL or QTN | Gene |
|---|---|---|---|---|
| Recombinant inbred strain (RIS) | AXB [46] | (A/J × C57BL/6J) $F_1$ | *ahl4* [44] | *Cs* [28] |
| | BXA [46] | (C57BL/6J × A/J) $F_1$ | | |
| | BXD [47] | (C57BL/6J × DBA/2J) $F_1$ | *ahl8* [45] | *Fscn2* [34] |
| | | | *Ahl9* [35] | Unknown |
| | LXS [48] | * (ILS × ISS) $F_1$ | *Snhl* [18] | Unknown |
| Consomic strain (CSS) | C57BL/6J-Chr#$^{A/J}$ [52] | Host: C57BL/6J, donor: A/J | *ahl4* [44] | *Cs* [28] |
| | C57BL/6J-Chr#$^{MSM/Ms}$ [53] | Host: C57BL/6J, donor: MSM/Ms | *Ahl3* [54] | Unknown |
| | | | *mjs*** [24] | *Cdh23* [24] |
| Inbred strain population | Hybrid mouse diversity panel (HMDP) [56] | 30 classic inbred strains and 70 recombinant inbred strains | rs33652818 [17] | *Nox3* [17] |
| | | | rs37517079 [60] | Unknown |
| Outbred stock (OS) | Black Swiss [62] | NIH Swiss, C57BL/6J | *Ahl5* [63] | *Gipc3* [14] |
| | | | *Ahl6* [63] | Unknown |
| | NIH Swiss [64] | NIH GP colony | *Hfhl1* [15] | Unknown |
| | | | *Hfhl2* [15] | Unknown |
| | | | *Hfhl3* [16] | Unknown |

*Inbred long sleep (ILS) and inbred short sleep (ISS) mouse strains, which were derived from a multi-generation cross of eight inbred strains.

**Modifier of *js* (tentative symbol).

**Table 1.** Genetic reference population contributed for identification of the loci and SNPs associated with hearing loss underlying in genetic background of mice by QTL analysis and GWAS.

been mapped to a single chromosome by ABR measurements in CSSs of partial and full sets (**Table 1**). Moreover, congenic mice for QTL mapping can be easily and quickly produced by intercrossing (CSS × host strain) $F_1$ and backcrossing (CSS × host strain) $F_1$ × host strain (**Figure 5B**). The genotyping is only required in one chromosome for progeny. By this strategy, we have previously mapped the *Ahl3* [54] and a modifier [24] responsible for the acceleration of ARHL of heterozygotes of Jackson shaker mice (*Ush1g*$^{js}$) (**Table 1**).

In addition, CSS may be used to study epistasis in detail. An example is *Ahl3* [54], which was mapped to chromosome 17 using C57BL/6J-Chr17$^{MSM/Ms}$ CSS. The donor strain, MSM/Ms, is an inbred derived from the Japanese wild mouse, *Mus musculus molossinus*. By introgression of the genomic segment including *Ahl3*, the ARHL of C57BL/6J was dramatically suppressed despite having the homozygous *Cdh23*$^{c.753A}$ allele in the genome. Although we predicted that *Ahl3* is the resistance allele for ARHL in MSM/Ms mice, the resistant effects of *Ahl3* were not detected in the other mapping system of the C57BL/6J and MSM/Ms strain [24]. The genetic divergence between C57BL/6J and MSM/Ms is extremely high [55]; therefore, the resistant effect of *Ahl3* was caused by incompatibility by chromosomal substitution. We detected a similar situation in CSSs of another chromosome [54] (Yasuda et al. unpublished data). There is some possibility that the incompatibility is caused by a *cis*/*trans* change via chromosomal substitution; therefore, the analysis of CSS may allow for the study of cross talk between genes on different chromosomes, namely, *cis*- and *trans*-regulation of the variation of gene expression in hearing research.

#### *4.2.3. Hybrid mouse diversity panel (HMDP)*

Although the classical genetic crosses, RIS and CSS, are powerful tools to identify modifiers in genetic backgrounds, the phenotypic and genetic variations are low because of the inclusion of only two parental strains. Moreover, the low genetic variation is not suitable for GWAS. The hybrid mouse diversity panel (HMDP) was developed to increase the statistical power and resolution of the classical QTL mapping [56]. The HMDP consists of 30 classical inbred strains and four set of RISs (AXB/BXA, BXD, BXH, and CXB) (**Figure 5C** and **Table 1**), which are genotyped with 140,000 SNPs [57]. By using the HMDP, a high statistical power and high resolution of QTL mapping were provided from the RISs and classical inbred strains, respectively.

HMDP contributed to the identification of the gene and locus associated with NIHL. Lavinsky et al. [17] investigated the noise susceptibility in five-week female mice in 64 strains selected from HMDP. The noise susceptibilities of strains varied widely, and GWAS analysis picked up five quantitative trait nucleotides (QTNs) with statistically significant p values ($p < 4.1E{-}06$) [17]. Consequently, candidate genes were screened by expression QTL (eQTL) analysis after which NADPH oxidase-3 gene (*Nox3*) was selected as a candidate (**Table 1**). Moreover, the study identified that the *Nox3* KO mice showed noise sensitivity [17]. This is the first study to have reported the NIHL-related gene. Till date, noise sensitivity of 100 stock strains from HMDP has been reported [58], and phenotype data have also been collected continuously [59]. Additionally, a QTN at chromosome 6 associated with noise susceptibility was detected (**Table 1**) [60].

#### *4.2.4. Outbred stock (OS)*

QTL analysis using inbred strains is limited to the phenotypes and alleles associated with ARHL and NIHL. Although the genotyping is required, outbred stock (OS) is a colony with

maintained phenotypic and genetic diversity kept in laboratory settings and thus exhibits a high degree of both genetic and phenotypic diversity, allowing high-resolution genetic mapping for a wide variety of traits by crossing with another population [42, 61].

In a study of ARHL, Black Swiss [62], which is derived from NIH Swiss outbred stock and C57BL/6J, was first used in OS for QTL linkage mapping. Drayton and Noben-Trauth [62] performed QTL linkage mapping using [(Black Swiss × CAST/Ei) $F_1$ × Black Swiss] $N_2$ backcross mice and mapped two QTLs: *Ahl5* and *Ahl6*. Subsequently, the responsible mutation for *Ahl5* was detected in the GIPC PDZ domain containing family member 3 gene (*Gipc3*), which is associated with audiogenic seizures and sensorineural hearing loss in mice and humans [14]. The *Gipc3*$^{p.Gly115Arg}$ mutation was absent in inbred strain, indicating that OS is a powerful tool to identify new ARHL-related genes. Moreover, NIH Swiss mice [63] also contributed to the detection of three loci (*Hfhl1–3*) associated with high-frequency hearing loss (HFHL) (**Table 1**) [15, 16].

Heterogeneous stock (HS) and diversity outcross stock (DO) could be considered variants of OS and display a similar advantage with OS for QTL mapping [42, 61]. These populations are available and probably will contribute to the identification of QTL and genes responsible for ARHL and NIHL.

#### 4.3. Elucidation of genetic background effects by using reverse genetics approaches

When the candidate gene and mutation are detected by forward genetics approach, the next step is elucidation of the real causative gene and mutation. The reverse genetics technique is immensely helpful with this elucidation. An approach is production and phenotypic analysis of KO mice of candidate gene *Nox3* as mentioned earlier [17]. However, rescue of phenotype is required for full proof.

Transgenic expression of bacterial artificial chromosome (BAC) clones in mice is commonly used for *in vivo* complementation tests. The test BAC contains wild-type allele and is simple since BAC contains the wild-type allele to be injected into the susceptible strain with the candidate mutation. The *Fscn2*$^{p.Arg109His}$ mutation of DBA/2J is confirmed by this complementation test [33]. The disadvantage of BAC transgenesis is that genes of large sizes exceeding the BAC clone (average size between 120 and 250 kb) cannot be rescued. Moreover, the rescue of the phenotype caused by dominant-negative and gain-of-function mutations is difficult. In addition, the strains of available BAC libraries are limited [64, 65].

An advanced approach is rescue by the knock-in (KI) method mediated by the CRISPR/Cas9 genome editing system. This system can efficiently and quickly repair the candidate mutation of the KI donor oligonucleotide containing the wild-type allele via a homology-directed repair (HDR) [66]. We previously reported utility of this method [24]. C57BL/6J-*Ush1g*$^{js/+}$ heterozygous mice exhibit severe early onset ARHL caused by progressive degeneration in the stereocilia of outer hair cells. We mapped a locus associated with early onset ARHL of *Ush1g*$^{js/+}$ mice in an interval of chromosome 10 that harbors the *Cdh23*$^{c.753G>A}$ mutation, which is also responsible for ARHL of C57BL/6J mice as mentioned above. We injected Cas9 mRNA, single guide RNA (sgRNA) and a donor oligonucleotide that contained *Cdh23*$^{c.753G}$ (**Figure 6A**) to produce KI mice. In KI mice, early onset ARHL and stereocilia degeneration were completely rescued (**Figure 6B**). This is the first report that confirms the phenotypic effect of modifiers at the mutation level in hearing research.

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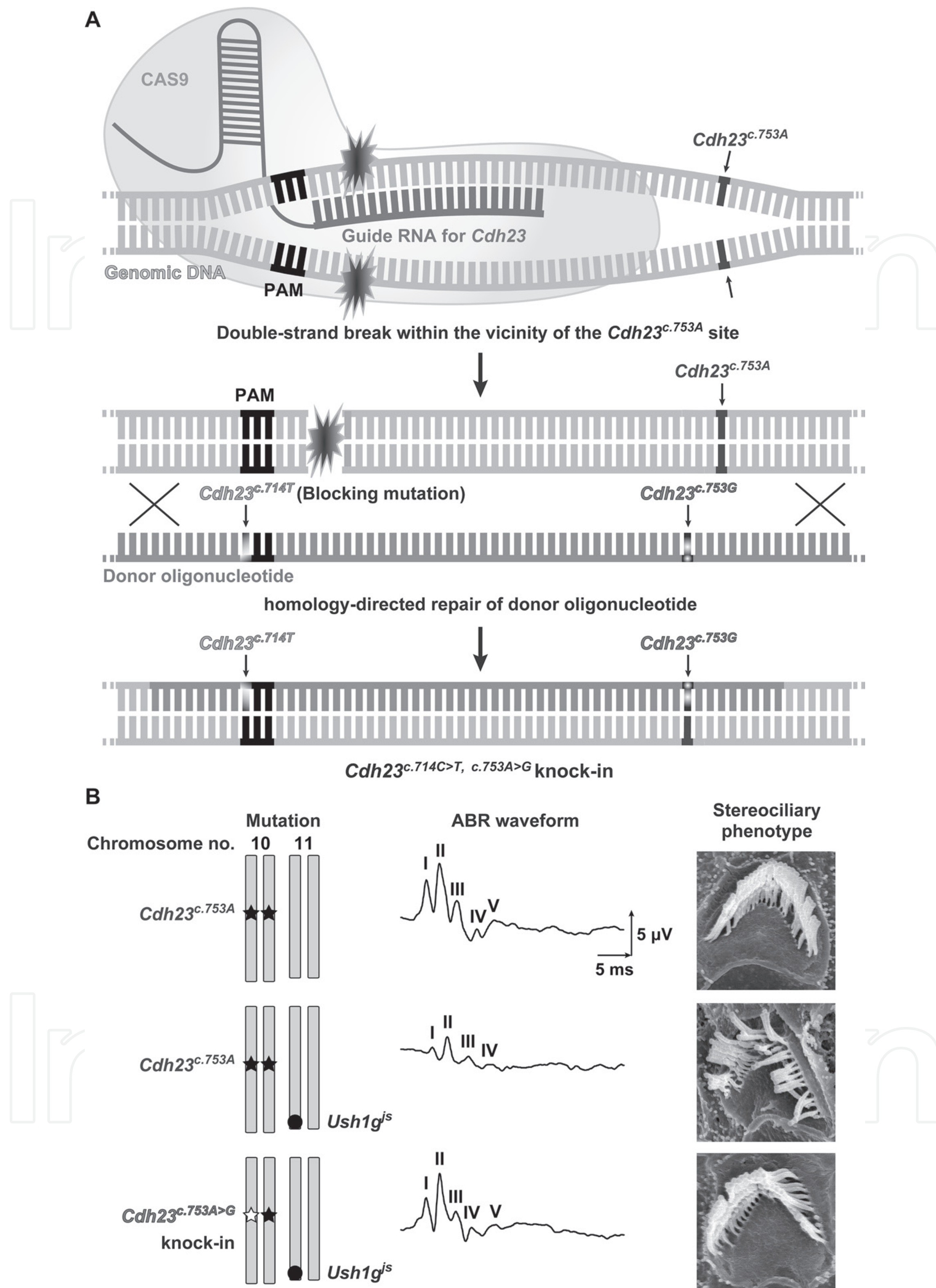


**Figure 6.** Phenotypic rescue of hearing loss in mice caused by genetic background effect using the CRISPR/Cas9-mediated KI method. (A) Schematic representation of the *Cdh23*$^{c.753A>G}$ KI using double-strand break within the vicinity of the *Cdh23*$^{c.753A}$ site of C57BL/6J mice by CRISPR/Cas9 and homology-directed repair of single-stranded donor oligonucleotide. The donor oligonucleotide was designed to include c.753A>G with a synonymous blocking of the c.714C>T mutation to avoid cleavage by Cas9 [24]. (B) Comparison of the hearing phenotypes among the C57BL/6J (top), C57BL/6J-*Ush1g*$^{js/+}$ (middle) and -*Ush1g*$^{js/+}$ *Cdh23*$^{c.753A/G}$ (bottom) mice. Illustrations of left panels represent combination of the *Cdh23*$^{c.753A>G}$ (★>☆) on chromosome 10 and *Ush1g*$^{js}$ (●) on chromosome 11. Middle and right panels show ABR waveforms and stereocilia phenotypes, respectively, in each mouse.

## 5. Conclusions

In this chapter, we attempt to highlight several issues regarding the identification of background effects in mice. To identify the modifiers underlying genetic background effects, several strains were established for QTL and GWAS. Thus, the technologies for forward genetics in mice have enabled important breakthroughs and will contribute to the identification of new loci and genes associated with hearing loss. The reverse genetics approach has also been developed based on technological innovations, that is, genome editing and will be increasingly applied. An increasing number of studies have reported that the hearing loss phenotype resulting from a single gene mutation in humans is modulated by the genetic background in which the mutation is maintained (e.g., see [67].). Using genome editing, mutations discovered in the genetic background of patients with hearing loss can be conveniently manipulated in mice, and the effects of the candidate mutation can be confirmed *in vivo* by phenotypic analyses of mice.

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## Index of technical terms

μV: microvolts

ABR: auditory brainstem response

*ahl*, *Ahl3*, *ahl4*, *Ahl5*, *Ahl6*, *ahl8* and *Ahl9*: loci for age-related hearing loss in mice

A/J, C3H/HeN, C57BL/6J, CAST/Ei, DBA/2J and MSM/Ms: mice inbred strains

ARHL: age-related hearing loss

AXB, BXA, BXD, BXH, CXB, LXS and SMXA: mice recombinant inbred strains

BAC: bacterial artificial chromosome

Black Swiss and NIH Swiss: mice outbred strains

C57BL/6J-Chr17$^{MSM/Ms}$: a consomic (chromosome substitution) strain, which contain MSM/Ms-derived chromosome 17 in the genetic background of the C57BL/6J strain

Cas9: CRISPR associated protein 9

*Cdh23*: cadherin 23 gene

*Cdh23*$^{c.753G>A}$: a guanine-to-adenine substitution at nucleotide position 753 of cadherin 23 gene

*Chr5* QTL: QTL on chromosome 5

CRISPR: clustered regularly interspaced short palindromic repeat

*Cs*: citrate synthase gene

*Cs*$^{p.His55Asn}$: a histidine-to-asparagine substitution at amino acid position 55 of citrate synthase (CS) protein

CSS: consomic (chromosome substitution) strains

dB SPL: decibel sound pressure level

DPOAE: distortion product otoacoustic emission

DO: diversity outcross stock

eQTL: expression QTL

*Fscn2*: fascin 2 gene

*Fscn2*$^{p.Arg109His}$: an arginine-to-histidine substitution at amino acid position 109 amino acid of fascin 2 (FSCN2) protein

*Gipc3*: GIPC PDZ domain containing family member 3

GWAS: genome-wide association study

HDR: homology-directed repair

HFHL: high-frequency hearing loss

*Hfhl1, 2* and *3*: loci for high-frequency hearing loss in mice

HMDP: hybrid mouse diversity panel

HS: heterogeneous stock

kHz: kilohertz

KI: knock-in

KO: knock-out

*Map1a*: microtubule-associated protein 1

*moth1*: modifier of tubby hearing

ms: milliseconds

*mt-Tr*$^{Arg}$: mitochondrial tRNA-Arg

NIHL: noise-induced hearing loss

*Nox3*: NADPH oxidase-3

OS: outbred stock

QTL: quantitative trait loci

QTN: quantitative trait nucleotides

RIS: recombinant inbred strain

sgRNA: single guide RNA

*Snhl*: a locus for sensorineural hearing loss in mice

SNP: single nucleotide polymorphism

*Tub*: tubby bipartite transcription factor gene

*Ush1g*: USH1 protein network component sans gene

*Ush1g*$^{js}$: Jackson shaker mouse

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