The Notch Ligand JAG1 Is Required for Sensory Progenitor Development in the Mammalian Inner Ear

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In mammals, six separate sensory regions in the inner ear are essential for hearing and balance function. Each sensory region is made up of hair cells, which are the sensory cells, and their associated supporting cells, both arising from a common progenitor. Little is known about the molecular mechanisms that govern the development of these sensory organs. Notch signaling plays a pivotal role in the differentiation of hair cells and supporting cells by mediating lateral inhibition via the ligands Delta-like 1 and Jagged (JAG) 2. However, another Notch ligand, JAG1, is expressed early in the sensory patches prior to cell differentiation, indicating that there may be an earlier role for Notch signaling in sensory development in the ear. Here, using conditional gene targeting, we show that the Jag1 gene is required for the normal development of all six sensory organs within the inner ear. Cristae are completely lacking in Jag1-conditional knockout (cko) mutant inner ears, whereas the cochlea and utricle show partial sensory development. The saccular macula is present but malformed. Using SOX2 and p27kip1 as molecular markers of the prosensory domain, we show that Jag1 is initially expressed in all the prosensory regions of the ear, but becomes down-regulated in the nascent organ of Corti by embryonic day 14.5, when the cells exit the cell cycle and differentiate. We also show that both SOX2 and p27kip1 are down-regulated in Jag1-cko inner ears. Taken together, these data demonstrate that Jag1 is expressed early in the prosensory domains of both the cochlear and vestibular regions, and is required to maintain the normal expression levels of both SOX2 and p27kip1. These data demonstrate that JAG1-mediated Notch signaling is essential during early development for establishing the prosensory regions of the inner ear.

Introduction

The mammalian inner ear is a complex structure consisting of a coiled cochlea, three orthogonally positioned semicircular canals, a central vestibule, and a dorsally projecting endolymphatic duct and sac. With the exception of the endolymphatic duct and sac, the different parts of the ear all contain sensory organs populated by sensory hair cells and their associated supporting cells. There are three different categories of sensory organs: cristae, located at the base of each semicircular canal; maculae, housed within the central vestibule; and the organ of Corti, which lines the cochlear duct. Only one sensory organ, the organ of Corti, is required for hearing; the other five organs are important for balance. Unfortunately, in mammals, if these regions are damaged due to an environmental or genetic insult, they cannot regenerate, leaving a permanent hearing and/or balance impairment.

Although some progress has been made in understanding how the individual cell types within the sensory areas of the ear are formed [1,2], little is known about the molecular mechanisms that establish the prosensory lineage and how the different sensory organ types are formed. Interestingly, the molecular mechanisms that underlie sensory differentiation in the vertebrate inner ear demonstrate strong parallels with Drosophila sense organ development [3–5]. For example, during Drosophila external sense organ development, lateral inhibition mediated by Notch signaling is required to restrict the adoption of the sensory organ precursor cell fate, which then gives rise to the entire sensory organ [6–8]. Similarly, in the vertebrate ear, lateral inhibition mediated by Notch signaling appears to be important for restricting the number of cells that can adopt the hair cell fate [9–15]. Lineage analysis has also shown that, at least in the chicken, hair cells and supporting cells arise from a common progenitor [16], consistent with an equipotent epithelium that undergoes lateral signaling to specify cell fates. Unlike in Drosophila, which has a single Notch receptor and two ligands (Delta and Serrate/Jagged), in mammals Notch signaling pathway components include four receptors (Notch 1–4) and five ligands (Delta-like [DLL] 1, 3, and 4, and Jagged [JAG] 1 and 2; for reviews, see [8,17–19]). In the mouse, both DLL1 and JAG2 are expressed in nascent hair cells [10,20] and act synergistically during lateral inhibition [15]. Both DLL1 and JAG2 appear to signal through the NOTCH1 receptor [15].

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Malformation of the Inner Ear in Jag1-cko Mutants

To examine the morphology of the Jag1-cko inner ears, paintfilling of the inner ears of mutants and controls was
performed at E15.5 (Figure 4). Results of this analysis showed a severe disruption in the structure of the Jag1-cko inner ears compared to their littermate controls (Figure 4C and 4D). Specifically, the semicircular canals were largely absent, with the exception of a portion of the anterior and lateral semicircular canals. In addition, the utricle appeared small, the saccule was misshapen, and the cochlea was undercoiled. In contrast, the parts of the inner ear that are not associated with sensory formation, including the endolymphatic duct and sac and the common crus, appeared relatively unaffected.

Sensory Defects in Jag1-cko Mutant Inner Ears

Since the Jag1 gene is expressed in the sensory areas of the ear, and because the structural malformations observed in Jag1-cko mutant inner ears appeared to primarily affect regions of the ear that contained sensory organs, we examined the sensory regions of the ear for defects. We examined the organ of Corti, the sensory organ of the cochlea, at E18.5 by scanning electron microscopy (SEM) (Figure 5). By this stage, all hair cells within the organ of Corti have exited the cell cycle, and most are well-differentiated although not fully mature [36]. Severe hair cell patterning defects were apparent by SEM within the Jag1-cko mutant cochleae. This phenotype was most striking in the basal turns of the cochlea, where no hair cell formation was observed (Figure 5D). In the midbasal regions of the organ of Corti, hair cells formed in patches, within which there was no clear formation of rows or distinction between inner and outer hair cells (Figure 5F). More apically, hair cells appeared more continuous along the organ of Corti (Figure 5H). However, although hair cells were present in the apical region, their...
numbers were clearly reduced; rather than the normal, perfectly ordered four rows of hair cells, there were only two rows of loosely arranged hair cells of indistinct type.

Abnormal Hair and Supporting Cell Patterns in Jag1-cko Inner Ears

To determine which sensory cell types were differentiating in the Jag1-cko mutant cochleae, specific markers were used to identify hair cell and supporting cell subtypes throughout the ear (Figure 6). In the cochlea, we used an antibody against MYO7A to label all hair cells and an antibody against S100A1 to label inner hair cells, Deiter’s supporting cells, and inner phalangeal supporting cells [24]. When both markers were used in combination, inner hair cells, outer hair cells, and some supporting cell types could be distinguished (Figure 6A–6F). This analysis showed that in the apex of the cochlea, inner hair cells were present and usually formed as doublets (Figure 6B). Their associated supporting cells, the inner phalangeal cells, were also present. Outer hair cells and their associated supporting cells, the Deiter’s cells, were not present in this region. In the middle portions of the cochlea, both inner and occasionally outer hair cells were present, although their patterning was clearly abnormal (Figure 6D). In addition, the tunnel of Corti was not apparent, and there were often doublets of inner hair cells and increased numbers of outer hair cell rows without accompanying Deiter’s supporting cells. As shown by SEM, both hair cells and supporting cells were absent in the very basal regions of the cochlea (Figure 6F).

Using the same markers we also examined the vestibular sensory organs in jag1-cko mutant inner ears (Figure 6G–6J). Consistent with the lack of semicircular canal and ampulla
formation observed by paintfilling, there was no evidence of cristal formation. The Jag1-cko utricular macula was extremely small with very few differentiating hair cells (Figure 6H). Surprisingly, the saccule and its macula were only mildly affected in the Jag1-cko inner ears (Figure 6J). Hair cell differentiation appeared relatively unaffected, although the entire saccular structure was shaped differently than in the controls, a feature that was also observed in the paintfilled specimens (see Figure 4C and 4D). These data show that all sensory organs within the inner ear are affected to varying degrees in Jag1-cko inner ears. However, some sensory organs, such as the cristae, appear to be more sensitive to the loss of JAG1 function.

To examine whether aberrant hair cell patterning in Jag1-cko cochleae was due to defects in hair cell formation or in subsequent differentiation, we examined hair cell patterning at an earlier stage (E16.5). Using a lectin that binds to hair cell stereocilia, we examined whether the patterns of hair cell formation at E16.5 looked similar to the patterns at E18.5 (Figure 7). At E16.5 in wild-type cochleae, a gradient of hair cell differentiation was evident (Figure 7A, 7C, 7E, and 7G); in the basal regions both inner and outer hair cells could be recognized (unpublished data), while in the middle regions only inner hair cells were clearly detected by most markers (Figure 7A and 7C). In the more apical regions, little to no hair cell differentiation had taken place by this stage (Figure 7E and 7G). In Jag1-cko cochleae, the patterns looked similar to those at E16.5, with patches of hair cells in the midbasal regions (Figure 7B and 7D) and a complete absence of hair cells in the very basal regions (Figure 7B). These data suggest that the Jag1-cko mutants have defects in hair cell formation rather than differentiation. In addition, the apical regions in the Jag1-cko cochleae did not appear more differentiated than the controls (Figure 7E–7H), arguing against precocious differentiation as an explanation for the reduced numbers of hair cells observed in the mutant cochleae.

Disrupted Prosensory Development in Jag1-cko Inner Ears

To determine how the JAG1 ligand functions during sensory development, we used several markers of the
prosensory domain, including p27\(^{kip1}\) and SOX2, and examined their expression patterns in both wild-type and Jag1-cko mutant cochleae (Figure 8). At E14.5, the majority of hair cells and supporting cells in the organ of Corti have completed their final division, and hair cells are beginning to differentiate in the basal portions of the cochlea [36]. p27\(^{kip1}\), a cell-cycle inhibitor, is required for the cochlear sensory progenitors to exit the cell cycle on time, and is an established marker of the prosensory domain in the cochlea [22,37]. p27\(^{kip1}\) begins to be expressed in a discrete domain within the cochlea as the hair cells and supporting cells exit the cell cycle around E13.5 to E14.5 (Figure 8A, 8B, 8D, and 8E). Recently it has been shown that the SRY-related transcription factor SOX2 is required for establishment of the prosensory regions in the inner ear [25]. Using fluorescence immunocytochemistry double labeling, we examined the relationship between these markers and JAG1 protein expression in both wild-type and Jag1-cko cochleae. As previously reported [22], JAG1 was not expressed within the prosensory domain as assessed by p27\(^{kip1}\) expression at E14.5, but instead was expressed immediately adjacent (possibly with some slight overlap) in the inner (neural) portion of cochlea (Köllicher’s organ; Figure 8A and 8D). In contrast, SOX2 did show a largely overlapping domain with p27\(^{kip1}\) (Figure 8B and 8E), as originally described [25]. However, the SOX2 expression domain was slightly larger than the p27\(^{kip1}\) domain, extending into Köllicher’s organ and overlapping with the JAG1 domain. Despite the fact that JAG1 was not expressed within the prosensory domain at E14.5, both p27\(^{kip1}\) and SOX2 expression was absent in the basal regions of the cochlea (Figure 8C), indicating that prosensory formation is already disrupted in these ears. In the apex, weak expression of both markers was observed (Figure 8F), consistent with the fact that some sensory differentiation occurs in this region of the Jag1-cko cochlea.

In order to determine if JAG1 is ever expressed in the prosensory region of the cochlea, we examined an earlier age (E12.5) and compared the JAG1 domain to the SOX2 domain (since p27Kip1 is not expressed in the inner ear prior to E13.5 to E14.5). Adjacent sections from both wild-type and Jag1-cko cochleae were immunostained to detect either JAG1 or SOX2 protein (Figure 9). This analysis showed that in the basal regions of the wild-type cochlea, where sensory precursors were still dividing, JAG1 expression did overlap with the SOX2 domain (Figure 9A and 9B), indicating that JAG1 is initially expressed within the prosensory domain. However, in the apical regions, where the sensory precursors had ceased dividing, expression of JAG1 and SOX2 did not overlap (Figure 9D and 9E). In the Jag1-cko cochlea, SOX2 was absent from the basal regions and significantly down-regulated in the apical regions (Figure 9C and 9F). These data demonstrate that JAG1 is expressed within the prosensory domain of the cochlea at early stages, and that, in the absence of JAG1 function, sensory formation is disrupted prior to cell cycle exit and differentiation of sensory hair cells and nonsensory supporting cells.

We also compared JAG1 and SOX2 expression in the vestibular regions of the inner ear in both wild-type and Jag1-cko mutant embryos. JAG1 and SOX2 exhibited largely overlapping expression domains that corresponded to the locations of the five sensory organs in the vestibular portion of the ear (Figure 10). The two expression domains only
differed significantly in the anterior and posterior cristae, where JAG1 expression had a negative patch in the middle of its expression domain, whereas SOX2 expression did not show this same patch (Figure 10A, 10B, 10G, and 10H). The JAG1-negative region may correspond to the eminentia cruciatum, a nonsensory region present in the middle of both the anterior and posterior cristae, although it is not clear why SOX2 would be expressed there. In the Jag1-cko vestibular sensory patches, SOX2 expression was consistent with the patterns of sensory differentiation observed at E18.5. For example, the Jag1-cko saccule displayed fairly normal SOX2 expression (Figure 10C), consistent with the almost normal development of the saccular macula. In contrast, SOX2 expression in the utricle was very weak and the expression domain was much smaller than in controls (Figure 10F), consistent with the severe disruption of differentiation of the utricular macula in jag1-cko inner ears. There was no SOX2 expression in the Jag1-cko cristae, and in fact the entire ampullae appeared to be missing or severely disrupted even at this early stage (Figure 10C and 10I; dotted line regions), consistent with the lack of cristae and ampullae observed at later stages.

**Discussion**

We have demonstrated that Notch signaling, mediated by the JAG1 ligand, is required early in development for the formation of the sensory regions of the ear. By comparing expression of JAG1 to two markers of the prosensory domain, SOX2 and p27kip1, we have shown that JAG1 marks all prosensory regions of the ear from early time points (E12.5), but becomes down-regulated in the organ of Corti by E14.5, when the sensory progenitors exit the cell cycle and begin differentiating into hair cells and supporting cells. Both SOX2 and p27kip1 are down-regulated in the affected prosensory regions of the Jag1-cko inner ear, demonstrating that JAG1 is necessary for the development of early sensory progenitors in the inner ear.

Distinctive Patterns of Hair Cell Formation in Jag1-cko Inner Ears Suggest Progenitor Cell Numbers Are Reduced

One intriguing result from our studies was that the six sensory regions were not equally affected by the loss of Jag1 function. For example, in the Jag1-cko vestibular system, the cristae were lacking altogether, and only a small number of hair cells differentiated in the utricular maculae. In contrast, the saccular maculae exhibited little disturbance in hair cell formation, although the overall shape of the organ was abnormal. In the jag1-cko cochlea, hair cell differentiation patterns varied based on their apical or basal location. For example, in the apical regions of the cochlea only inner hair cells formed, and these were often arranged in multiple rows rather than the normal single row. In the middle and midbasal turns of the cochlea, patches of hair cells with nonsensory intervening regions were frequently observed. Within these patches, outer hair cells were sometimes present, although the patterning was abnormal and S100A1-labeled Dieter’s cells were not present. In the very basal regions of the cochlea, neither hair cells nor supporting cells were present.

The patches of hair cells found in the basal regions of the cochlea and the differential effect of the mutation on the basal and apical portions of the cochlea were particularly interesting, as similar defects have been found in at least two other mouse mutants of genes known to play a role in the generation of the sensory precursors of the ear. For example, both a hypomorphic allele and a conditionally deleted allele of the Fgfr1 gene exhibited patches of hair cells in portions of the cochlea [31]. Similar to the jag1-cko phenotype, these patches in the Fgfr1 conditional mutants contained mostly inner hair cells that were often arranged in multiple rows, with very few outer hair cells. Unlike the jag1-cko phenotype, Fgfr1 function was required only in the cochlea. Another mouse mutant, a hypomorphic allele of the Sox2 gene (yellow submarine; Sox2<sup>Sub</sup>), also displayed patches of hair cells in the basal portions of the cochlea and a milder phenotype in the apical regions of the cochlea [25]. More similar to the jag1-cko
A Prosensory Role for Notch in the Ear

An examination of early prosensory markers, including p27Kip1 and SOX2, demonstrated that prosenory establishment is disrupted in Jag1-cko inner ears, consistent with the suggestion that progenitors are reduced in these mutants. Our data show that JAG1 plays an early prosensory role in ear development, quite unlike the role played later during development by the other Notch ligands, DLL1 and JAG2, which are involved in lateral signaling and differentiation [10,15]. These data are consistent with an early role for Notch signaling in progenitor cell maintenance in the inner ear. In a number of other systems, including the nervous system and more recently in the intestinal epithelium, it has been demonstrated that Notch signaling is involved in maintaining cells in an undifferentiated state [42–46]. In the mammalian nervous system it has been shown that loss of Notch signaling leads to premature differentiation and a reduction in the progenitor pool [42]. Consistent with these findings, in vitro studies have demonstrated that the frequency of neurosphere production was reduced in Notch signaling mutants [47,48], indicating a loss of stem cell potential. Moreover, studies have also shown that Notch signaling promotes radial glial identity, a cell type that has been shown to act as a progenitor cell in the central nervous system [49–52]. Our results suggest that, similar to the nervous system, Notch signaling via JAG1 is important for sensory precursor formation or maintenance in the inner ear. However, unlike the nervous system, we see no evidence for precocious differentiation, suggesting instead that JAG1 may affect the specification, survival, or proliferative capacity of the sensory precursors.

Recent evidence from the chick indicates that JAG1 may be important for the initial sensory specification events. By expressing a constitutively active form of Notch (Notch1-ICD), Daudet et al. [14] demonstrated that ectopic sensory patches could be induced, indicating that early Notch signaling may be important for the induction of sensory areas, and not just for their maintenance. However, it should...
be noted that ectopic sensory areas formed only in certain areas of the ear, indicating that some sensory competence is required for this effect. A similar result was obtained by overexpressing an activated form of β-catenin, an essential component of the canonical Wnt signaling pathway, in the chicken inner ear [53]. As in the Notch1-ICD studies, ectopic sensory regions were obtained, but again, only in certain regions of the ear. However, unlike the Notch gain-of-function studies, overexpression of β-catenin also led to a change in sensory region character (i.e., cochlear to vestibular), indicating that Wnt signaling governs not only whether a sensory region will form but also the type of sensory region that will form. In Drosophila, interactions between Notch and Wingless, a member of the Wnt family of signaling molecules, are well established [54,55], and evidence of an interaction has begun accumulating in vertebrates as well [45,56,57]. Bone morphogenetic protein (BMP) signaling may also be important for sensory formation, particularly for the sensory cristae, as BMP4 has been shown to mark the mouse cristae from very early in development [58]. Experiments in the chicken have shown that blocking BMP signaling sometimes leads to disturbances in sensory development [59]. Taken together, these data indicate that, based on expression patterns, previous studies, and the evidence presented here, JAG1 is the ligand responsible for the prosensory function of the Notch pathway in the ear. Furthermore, the Notch pathway likely interacts with other signaling pathways such as the Wnt, FGF, and BMP pathways to create sensory organs of the proper size, organization and character.

**Sensory Formation Still Occurs in Jag1-cko Inner Ears**

One somewhat puzzling question is that, if JAG1 is important for sensory progenitor development, why does any sensory formation occur in Jag1-cko inner ears? One possibility is that another Notch ligand is compensating for the loss of JAG1 function. This explanation seems unlikely since none of the other Notch ligands shows a similar expression pattern to JAG1 in the ear. For example, both the Dll1 and Jag2 genes are expressed in nascent hair cells after they exit the cell cycle and begin differentiating. However, in addition to hair cell expression, there is also early expression of the Dll1 gene in the anteroventral portion of the otocyst at about E10.5 [4,20], that likely overlaps with at least part of the JAG1 domain (see Figure 2) [60]. This expression domain has previously been thought to be related to the formation of the neuroblasts that delaminate from the otic epithelium and later differentiate into the neurons that will innervate the hair cells [4]. It has been shown in zebrafish that correct neuroblast formation requires Notch-mediated lateral signaling [9]; however, in mammals it has not been shown definitively that this is the role that the Dll1 gene plays at early stages. This leaves open the possibility that this early domain of DLL1 expression may be at least partially involved in prosensory specification, similar to the JAG1 expression domain.

**Nonsensory Defects in Jag1-cko Inner Ears**

In addition to the defects in sensory formation in the Jag1-cko inner ears, the mutant inner ears also exhibited nonsensory defects. Specifically, the semicircular canals were largely absent, with the exception of portions of the anterior and lateral canals. In addition, all three ampullae were absent, the utricle was small, and the cochlea was undercoiled. Based on recent studies, it is likely that these defects are secondary to the sensory defects. For example, it has been shown that FGFs expressed in the sensory cristae promote semicircular canal formation through up-regulation of BMP2 [61]. Thus, loss of the cristae would be expected to have a severe affect on canal formation. Emerging evidence from mouse mutants has demonstrated that genes involved in sensory formation result in severely malformed inner ears. For example, mutations in the Sox2 gene lead to malformations very similar to those described here in Jag1-cko mutants. The inner ears of embryos homozygous for two different mutant alleles
of Sox2, Sox2<sup>loclcc</sup> and Sox2<sup>ysbysb</sup>, showed disrupted canal formation; smaller utricular and saccular compartments; and thinner, undercoiled cochleae [25]. In addition, FGF10 mouse knockouts also showed disrupted cristae development associated with loss of canal structures [62]. However, unlike the canal structures, cochlear formation does not appear to be strictly dependent on development of the organ of Corti, as a cochlea, albeit short and thin, will form in the absence of any sensory formation [25]. However, normal cochlear length appears to be dependent on sensory formation, at least partially through convergent extension. Recently, a number of genes have been found in the cochlea that lead to defects in planar cell polarity as well as a shortened cochlea, presumably because of defects in convergent extension [38,41]. Therefore it is likely that the shortened cochlea observed in Jag1-cko mutants is at least partially a result of failure of convergent extension caused by a reduction in the number of sensory precursors.

The data presented here demonstrate that the JAG1 gene is required for sensory precursor development in the inner ear. Further studies are required to establish the exact role that JAG1-mediated Notch signaling plays in early sensory progenitors, and also its relationship to the roles played by FGF signaling and SOX2 expression. Understanding how the sensory precursors form is an important prerequisite for regeneration studies that may provide molecular tools to treat hearing loss and vestibular disorders [63].

**Materials and Methods**

**Construction of the Jag1<sup>fl<sup> neo</sup></sup> allele.** To construct the Jag1<sup>fl<sup> neo</sup></sup> allele, bacterial artificial chromosome clones containing the Jag1 genomic locus were isolated from a RPCI-22 (129S6/SvEvTac) mouse bacterial artificial chromosome library (filters obtained from Research Genetics) by hybridization to a 1.8-kb mouse Jag1 cDNA probe. To make the shorter 5′ homology region of the targeting vector, a 2.2-kb KpnI fragment upstream of exon 4 was isolated, blunt-ended, and subcloned into the SmaI site of a modified pBS vector that contained a loxP-FRT-PGK<sup>neo</sup>-FRT cassette. A 1.5-kb KpnI fragment that contained exon 4 was also subcloned into the loxP-FRT-PGK<sup>neo</sup>-FRT cassette. To construct the longer 3′ homology region, a 3.5-kb KpnI–SmaI fragment containing exon 5 was blunt-ended and subcloned into the EcoRV site of another modified pBS vector that contained a single loxP site. A 3.5-kb SmaI–SalI fragment from this construct was then cloned into the SmaI–XhoI site of a pKO 905 vector containing a diphtheria toxin gene for negative selection. A 5.7-kb SalI–NotI fragment from the loxP-FRT-PGK<sup>neo</sup>-FRT construct was then cloned into the SalI–NotI sites of the pKO 905 vector containing the 3′ homology region to generate the final Jag1<sup>fl<sup> neo</sup></sup> targeting vector (see Figure 1).

**Generation of Jag1<sup>fl<sup> neo</sup></sup> mice.** The Jag1<sup>fl<sup> neo</sup></sup> targeting construct was linearized with NotI and electroporated into CJ7 embryonic stem (ES) cells, as described previously [64]. DNA from 288 ES cell clones was screened by PCR using an internal/external primer set, and positive clones were then confirmed by Southern blot by probing EcoRI-digested DNA with an external 1.7-kb StuI–EcoRI fragment located 3′ to the targeting construct (see Figure 1). This probe also detected partial recombination events in which the distal loxP site was lost; in these cases a slightly larger fragment (11 kb rather than 9.3 kb) was obtained (see Figure 1A). The presence of the distal loxP site was further confirmed by PCR using primers that flanked the loxP site (DSLF and J1LoxR1; see below for sequences). Correctly targeted clones were injected into C57BL/6j (B6) blastocysts, and chimeric

**Figure 10. JAG1 and SOX2 Mark the Prosensory Regions of the Vestibule, and SOX2 Expression Correlates with Impaired Sensory Formation in the Jag1-cko Vestibule**

(A and B, D and E, G and H) Alternate sections demonstrating either JAG1 or SOX2 expression in the vestibular regions of control inner ears. (C, F, I) Similar sections through the Jag1-cko inner ear demonstrating SOX2 expression. Dotted lines indicate regions where the cristae and ampullae are missing in the Jag1-cko inner ear. ac, anterior cristae; lc, lateral cristae; pc, posterior cristae; sac, saccular macula; ut, utricular macula.

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were used: Cre1, Cre2, 5′-TCAGGCATGATAAACCCTAGC-3′ (forward) and 5′-TGGATGTGGAATGTGTGC-3′ (reverse). A different reverse primer, J1GKO2, 5′-TGATGAGGTTCGCAAGAACC-3′ was used to detect the wild-type allele.

Mouse husbandry and genotyping. Foss1-Cre mice [30; gift of Rob Burgess] were maintained on an outbred Swiss Webster background. ZP3-Cre mice [34; gift of Mimi de Vries and Barbara Knowles] were maintained on a B6 background. Typically, males that were heterozygous for both a Foss1-Cre allele and our previously constructed Jag1 null allele (Jag1floxx) [35] (maintained on a B6 background), were crossed to Jag1floxx J1floxx females that were maintained on a B6/129 background. Mice of the genotypes Foss1-Cre/Jag1floxx/Jag1floxx and Foss1-Cre+/Jag1floxx/Jag1floxx were used interchangeably, and are designated as Jag1-cko mice in this report.

To genotype the Jag1floxx mice, the primers used were: DSFL, 5′-TCAGGCATGATAAACCCTAGC-3′ (forward) and J1loxR, 5′-CTA CATACGAGTCTCACAGGTC-3′ (reverse); these primers flank the 5′ LoxP site. To genotype for CRE-mediated recombination, a primer upstream of the 5′ LoxP site was used: J1FPl, 5′-CAGGT TAGGCTGACAGT-3′, along with the J1loxR reverse primer. To genotype for the Foss1-Cre and ZP3-Cre alleles, Cre-specific primers were used: Cre1, 5′-TGATGAGGTTCGCAAGAACC-3′ (forward) and Cre2, 5′-CCATGAGTGGAGAACCTGG-3′ (reverse). Jag1floxx primers were as follows for the mutant: JGKO1, 5′-TCTACTCTAGCCGATGA TAAACC-3′ (forward) and SOL1, 5′-TGATGAGGTGGATGTGG-3′ (reverse). A different reverse primer, JGKO2, 5′-TAAACGG GACTCCGG ACAGGG-3′ was used to detect the wild-type allele. Littermates (wild-type, Jag1floxx/Jag1floxx) were used as controls for all experiments.

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