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Short communication

LDLR expression in the cochlea suggests a role in endolymph homeostasis and cochlear amplification

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ABSTRACT

There is now growing evidence that hypercholesterolemia and high serum levels of low-density lipoproteins (LDL) predispose to sensorineural hearing loss. Circulating LDL-cholesterol is delivered to peripheral tissues via LDL receptor (LDLR) -mediated endocytosis. Recently, it has been shown that *LDLR* gene polymorphisms are associated with higher susceptibility to sudden deafness. These findings suggested that we should investigate the expression of LDLR from the postnatal maturation of the mouse cochlea until adulthood. In the cochlea of newborn mice, we observed that LDLR is mostly expressed in the lateral wall of the cochlea, especially in a band of cells directly facing the cochlear duct. Moreover, LDLR is expressed in the inner and outer hair cells, as well as in the adjacent greater epithelial ridge. In early postnatal stages, LDLR is expressed in the marginal cells of the immature stria vascularis, in the root cells of the spiral ligament, and in the adjacent outer sulcus cells. At the same time, LDLR begins to be expressed in the pillar cells of the immature organ of Corti. From the onset of hearing, LDLR is expressed in the marginal cells of the stria vascularis, in the outer sulcus cells, and in the capillaries of the adjacent spiral ligament. In the organ of Corti, LDLR is expressed in outer pillar cells and Deiters' cells, i.e. in the non-sensory supporting cells that directly surround the outer hair cells. These cells are believed to provide a mechanical coupling with the outer hair cells to modulate electromotility and cochlear amplification. In the stria vascularis of three-month-old mice, LDLR is further expressed in both marginal and intermediate cells. Overall, our results suggest that LDLR is mostly present in cochlear cells that are involved in endolymph homeostasis and cochlear amplification. Further functional studies will be needed to unravel how LDLR regulates extracellular and intracellular levels of cholesterol and lipoproteins in the cochlea, and how it could influence cochlear homeostasis.

1. Introduction

Hearing loss is the fourth highest cause of disability globally. The most recent estimations of the World Health Organization (WHO) suggest that approximately 466 million people (or 6.1% of the world's population) were living with disabling hearing loss in 2018 (WHO, 2018). With the rise and aging of the global population, the number of people with hearing loss is growing at a rapid pace. WHO projections suggest that unless action is taken, there will be 630 million people living with disabling hearing loss by the year 2030, with that number expected to grow to over 900 million by 2050 (WHO, 2018). Sensorineural hearing loss (SNHL) can be caused by damage to any portion of the peripheral and central auditory systems. The main causes of SNHL are degenerative processes associated with aging, genetic mutations, noise exposure, exposure to therapeutic drugs that have ototoxic side effects, and chronic diseases (Cunningham and Tucci, 2017). Among these chronic conditions, there is now growing evidence that hypercholesterolemia predisposes to SNHL (Chang et al., 2014; Cunningham and Goetzinger, 1974; Lowry and Isaacson, 1978; Quaranta et al., 2015; Shargorodsky et al., 2010; Spencer, 1973). Thus, targeting cholesterol homeostasis could be a key issue to prevent SNHL (Malgrange et al., 2015).

Cholesterol is delivered to peripheral tissues via low-density lipoproteins (LDL). It is worth noting here that cholesterol biosynthesis can occur *de novo*. About 80% of daily cholesterol production occurs in the liver and in the intestines, other sites of higher synthesis rates include the brain, the adrenal glands and the reproductive organs (Luo et al., 2020). High serum levels of LDL have been associated with an increased risk of sudden SNHL with poor recovery (Oreskovic et al., 2011; Lin et al., 2015; Shao et al., 2021; Weng et al., 2013; Zhang et al., 2019). LDL receptor (LDLR)

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promotes the endocytosis of cholesterol-rich LDL and thus maintains the plasma level of LDL. This occurs in all nucleated cells, but mainly in the liver, which removes more or less 70% of LDL from the circulation. LDLR is a cell-surface receptor that recognizes the apoprotein B100, which is embedded in the outer phospholipid layer of LDL particles (Young, 1990). The receptor also recognizes the apoE protein found in chylomicron remnants, very-low-density lipoprotein (VLDL), indermediate-density lipoprotein (IDL) and a subclass of high-density lipoprotein (HDL) (Mahley et al., 2009). Upon binding to LDL, LDLRs are clustered in clathrin-coated pits, and coated pits pinch off from the surface to form coated endocytic vesicles that carry LDL into the cell (Goldstein and Brown, 2009). After internalization, the receptors dissociate from their ligands when they are exposed to lower pH in endosomes. After dissociation, the receptor folds back on itself to obtain a closed conformation and recycles to the cell surface (Rudenko et al., 2002). The rapid recycling of LDLRs provides an efficient mechanism for delivery of cholesterol to cells (Basu et al., 1981; Brown et al., 1983).

Hypercholesterolemia can be caused by a variety of environmental and genetic factors. On the one hand, environmental factors include obesity, poor diet, smoking, physical inactivity and stress (Pirillo et al., 2021; Zhao et al., 2019). On the other hand, loss-of-function mutations in the gene encoding LDLR are likely to cause familial hypercholesterolemia (Brautbar et al., 2015). Mice lacking *Ldlr* are particularly vulnerable to oxidized LDL-induced hepatic inflammation and further liver damages (Bieghs et al., 2012). Within the vasculature, LDL can undergo different types of oxidative modifications, such as esterification and lipid peroxidation. The resulting oxidized LDL has been found to have antigenic potential and contribute heavily to atherosclerosis-associated inflammation, activating both innate and adaptive immunity (Rhoads and Major, 2018).

Little is known about the mechanisms causing SNHL due to hypercholesterolemia, except that it reduces otoacoustic emissions, which reflect the electromotile reponse of sensory outer hair cells (Preyer et al., 2001). One reason could be that cholesterol is known to influence the fluidity/stiffness of the lateral wall of these cells. Hearing is powered by the outer hair cell electromotility, a membrane-based motor mechanism that resides in the lateral wall of the outer hair cells. Cholesterol levels modulate the distribution of the motor protein prestin and functionally tune the outer hair cells (Rajagopalan et al., 2007). Experimental observations of hypercholesterolemic animal models revealed the presence of edemas in the sensory outer hair cells, as well as in the stria vascularis of the cochlea (Gratton and Wright, 1992; Satar et al., 2001). Recently, it has been shown that *LDLR* gene polymorphisms are associated with higher susceptibility to sudden deafness (Cui et al., 2020). Although hypercholesterolemia is a chronic condition that mainly affects adult people, LDLR also regulates some aspects of cell proliferation and differentiation (Feng et al., 2012; Parada et al., 2008). These findings suggested that we should investigate the expression of LDLR from the postnatal maturation of the cochlea until adulthood. This could help us to understand how cholesterol and lipoproteins could influence cochlear homeostasis.

2. Material and methods

2.1. Animals

Mice of the BALB/c strain were grouped-housed in the animal facility of the University of Liège under standard conditions with food and water *ad libitum* and were maintained on a 12h light/dark cycle. All animals were taken care in accordance with the Declaration of Helsinki and following the guidelines of the Belgian ministry of agriculture in agreement with the EC laboratory animal care and use regulation (2010/63/UE, 22 September 2010).

2.2. Tissue processing and immunostaining

Cochleae were dissected from newborn (postnatal day 0, P0), P6, P12, P25 and P90 mice. After rapid decapitation, inner ears were removed and fixed for 2h in 4% formaldehyde. If necessary, tissues were decalcified prior to cryosectioning. Sections were cut parallel to the modiolus (mid-modiolar cut). Tissue sections of the cochlea were incubated overnight at 4°C with primary antibodies directed against LDLR (rabbit monoclonal antibody [EP1553Y]; 1:50; Abcam; ab52818), myosin VIIa (mouse monoclonal antibody; 1:50; Santa Cruz Biotechnology; sc-74516), Kir4.1 (mouse monoclonal antibody; 1:50; Santa Cruz Biotechnology; sc-293252) and VE-cadherin (goat polyclonal antibody; 1:50; R&D Systems; AF938). The anti-LDLR antibody used in this study was found to give a positive signal in western-blot performed with liver extracts of wild-type mice, but not with liver extracts of *Ldlr*$^{-/-}$ mice (Li et al., 2021). Tissues were then incubated for 1h with Rhodamine Red X-, Cy5- and/or FITC-conjugated anti-mouse, anti-goat and anti-rabbit IgGs secondary antibodies (Jackson Immunoresearch Laboratories). F-actin staining was obtained using Phalloidin-iFluor 647 reagent (1:50; Abcam; ab176759). Antibody specificity was tested by omitting each of the primary antibodies. Pictures were representative of three age-matched cochleae, from three different animals.

2.3. Confocal microscopy and imaging

Confocal fluorescence images were acquired using the Olympus Fluoview FV1000 confocal system (Olympus Europa GmbH) with objective LUCPLFLN 40X numerical aperture 0.60, working distance 2.7–4 mm, oil immersion.

3. Results and discussion

3.1. LDLR expression in the postnatal development of the cochlea

In the newborn (P0) mouse cochlea, we observed that LDLR was mostly expressed in the cells of the lateral wall bordering the cochlear duct (or scala media), but also in the organ of Corti (Fig. 1**A-C**). In the lateral wall, LDLR was particularly expressed in the cells that will differentiate over time to form the future stria vascularis. At this stage of development, the subcellular distribution of LDLR appeared to be mostly apical, directly facing the cochlear duct (yellow arrowheads in Fig. 1**D-G**). It is worth noting that capillaries of the lateral wall, which were labelled by an anti-VE-cadherin antibody, were not positive for LDLR (white arrowheads in Fig. 1**D-G**). In the cochlear sensory epithelium, LDLR was expressed in the inner and outer hair cells, as well as in the apical region of the greater epithelial ridge, which refers to the thickened ridge of epithelial cells that extends laterally from the inner border of the organ of Corti to the modiolar edge of the cochlear duct (Fig. 1**H-J**). These cells are connected via Cx26/Cx30 gap junction channels, which are excluded from cholesterol-rich lipid raft domains (Defourny et al., 2019b; Defourny and Thiry, 2021). This should be due to low affinity of Cx26 for cholesterol (Hung and Yarovsky, 2011; Locke and Harris, 2009). Gap junction activity is critical for cochlear homeostasis and hearing. Thus, LDLR-mediated cholesterol endocytosis into these cells could have an effect on gap junction biogenesis and stability.

In the six-day-old (P6) mouse cochlea, LDLR continued to be expressed in the developing stria vascularis, in the adjacent spiral ligament, as well as in the organ of Corti (Fig. 2**A-C**). To discriminate between different cell types present in the stria vascularis, we used Kir4.1 immunolabelling as a relevant marker of the intermediate cells (Ando and Takeuchi, 1999). In the immature stria

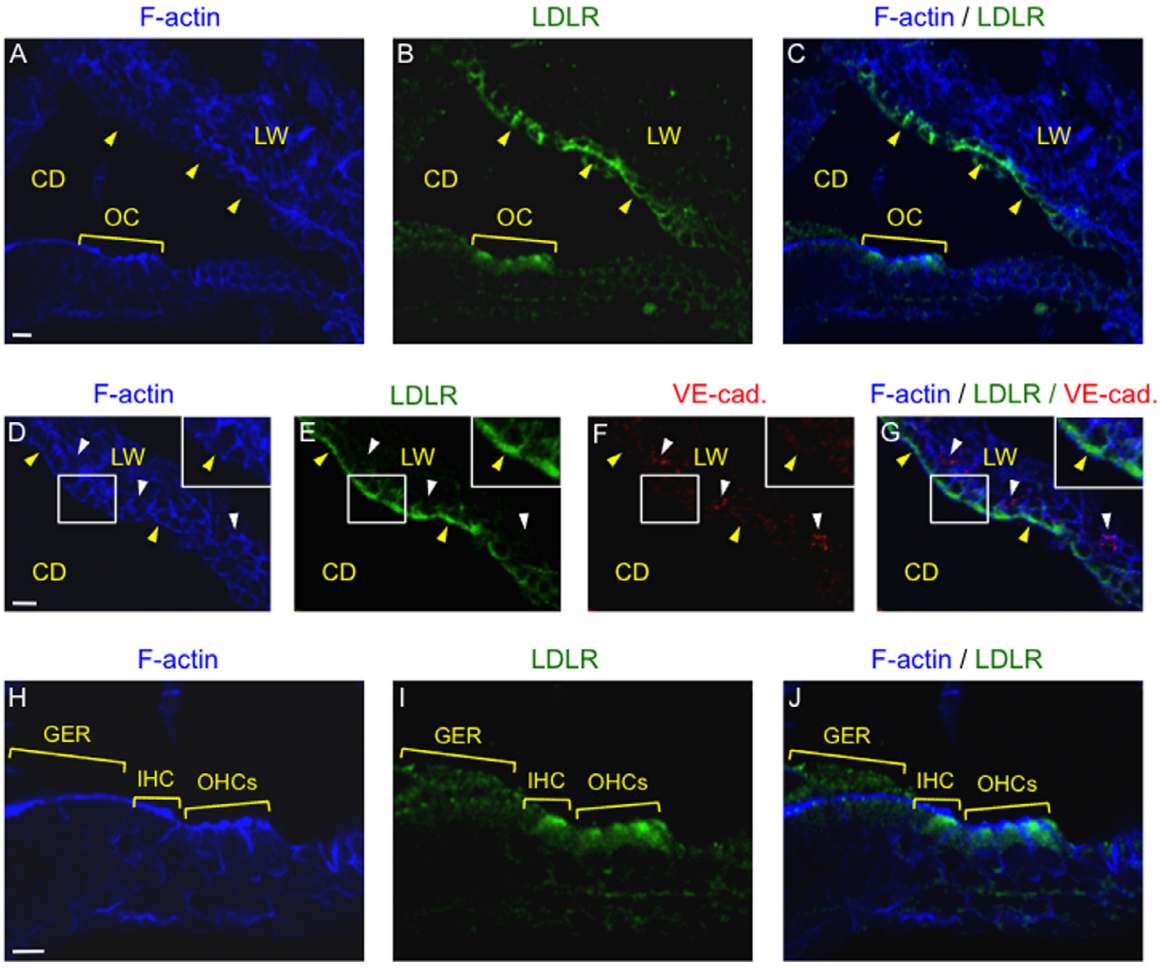

Fig. 1. LDLR expression pattern in the newborn (P0) mouse cochlea. (**A-C**) F-actin (blue) staining combined with LDLR (green) immunolabelling of a transverse section of a newborn mouse cochlea. LDLR is mostly expressed in the lateral wall of the newborn mouse cochlea, as well as in the organ of Corti. (**D-G**) F-actin (blue) staining combined with LDLR (green) / VE-cadherin (red) co-immunolabelling focused on the lateral wall of a transverse section of a newborn mouse cochlea. LDLR is expressed in a thin band of cells directly facing the cochlear duct (yellow arrowheads), but is not expressed in the capillaries present in the lateral wall of the cochlea (white arrowheads). (**H-J**) F-actin (blue) staining combined with LDLR (green) immunolabelling focused on the organ of Corti of a transverse section of a newborn mouse cochlea. LDLR is expressed in the inner and outer sensory hair cells, as well as in the apical region of the greater epithelial ridge. Scale bars in (A), (D) and (H) = 10 μm in (A-C), (D-G) and (H-J), respectively. CD = cochlear duct, GER = greater epithelial ridge, IHC = inner hair cell, LW = lateral wall, OC = organ of Corti, OHCs = outer hair cells.

vascularis, we observed that LDLR was mostly expressed in Kir4.1-negative cells directly facing the cochlear duct, which undergo differentiation to produce the marginal cell layer (yellow arrowheads in Fig. 2**D-G**). At this stage, only a few cells co-expressed LDLR and Kir4.1 (orange arrowhead in Fig. 2**D-G**). Of note is that the capillaries present in the immature stria vascularis did not express LDLR (white arrowheads in Fig. 2**D-G**). Besides the stria vascularis, LDLR was also expressed in the outer sulcus cells (orange arrowhead in Fig. 2**H-K**), which are epithelial cells covering the luminal side of the outer spiral sulcus of the cochlea. Moreover, LDLR was also expressed in the adjacent root cells, which extend into the spiral ligament (yellow arrowheads in Fig. 2**H-K**). At the lateral extremity of the outer hair cell layer lie the root cells, which reside in part within the outer sulcus region, and also within the connective tissue forming the spiral ligament. The root cells take their name from characteristic finger-like basolateral projections that extend from their cell bodies and infiltrate between the fibrocytes of the spiral ligament. These cells are highly polarized, and display structural and functional specializations of both the apical membrane, which is exposed to the endolymph within the cochlear duct, and the basolateral extensions provided by the root processes. Electrophysiological recordings of root cells have shown that membrane properties are consistent with a role in cochlear K^+ cycle (Jagger et al., 2010). Light micrographs revealed that the root processes lie in close proximity with a rich capillary network in the spiral ligament (Jagger and Forge, 2013) (white arrowheads in Fig. 2**H-K**). Thus, the proximity with these blood vessels could be required to provide sufficient LDL-derived cholesterol amounts for specific metabolic needs of the root cells.

In the organ of Corti, LDLR expression was still present in inner hair cells and in the first row of outer hair cells. At this stage however, LDLR immunolabelling was also observed in the pillar cells (Fig. 2**L-O**). In the cochlear sensory epithelium, inner and outer hair cell layers are separated by two parallel rows of non-sensory inner and outer pillar cells. At immature stages, all cells of the organ of Corti are closely connected and the inner pillar cells abut on the outer pillar cells. As development progresses, the apical ends of the pillar cells remain connected, whereas the lateral membranes become no longer apposed, being separated with fluid spaces. When mature, these pillar cells form the boundaries of a functionally important triangular fluid-filled space referred to as the tunnel of Corti. The somatic motility of sensory outer hair cells produces oscillatory fluid flow in the tunnel of Corti, which is critical for cochlear amplification (Karavitaki and Moun-

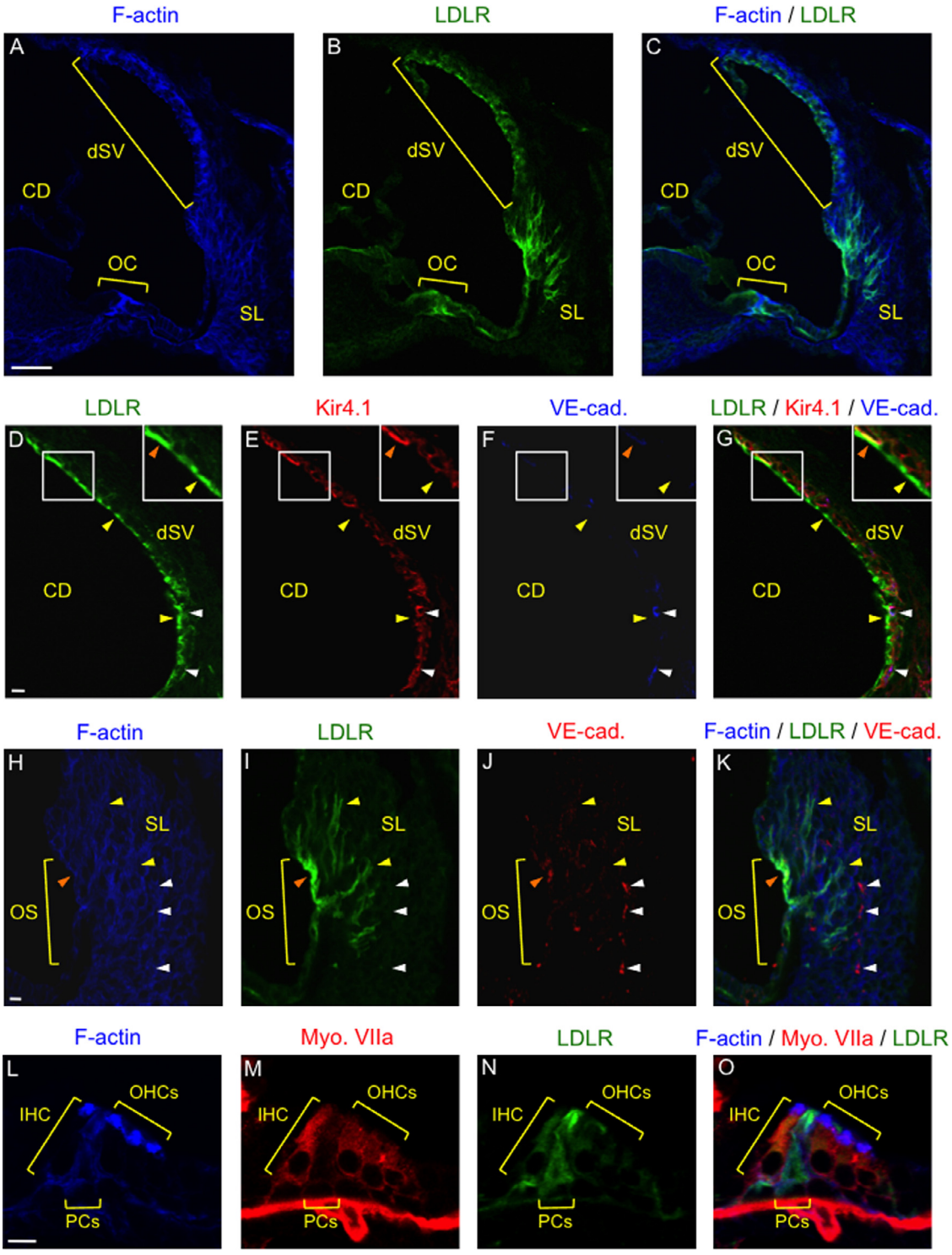

Fig. 2. LDLR expression pattern in the the pre-hearing onset (P6) mouse cochlea. (**A-C**) F-actin (blue) staining combined with LDLR (green) immunolabelling of a transverse section of a P6 mouse cochlea. LDLR is expressed in the developing stria vascularis, in the adjacent spiral ligament, as well as in the organ of Corti. (**D-G**) LDLR (green) / Kir4.1 (red) / VE-cadherin (blue) co-immunolabelling focused on the developing stria vascularis of a transverse section of a P6 mouse cochlea. LDLR is mostly expressed in Kir4.1-negative marginal cells of the immature stria vascularis (yellow arrowheads). Only a few cells co-express Kir4.1 and LDLR (orange arrowhead). Intrastrial capillaries do not express LDLR (white arrowheads). (**H-K**) F-actin (blue) staining combined with LDLR (green) / VE-cadherin (red) co-immunolabelling focused on the outer sulcus and adjacent spiral ligament of a transverse section of a P6 mouse cochlea. LDLR is expressed in the outer sulcus cells (orange arrowhead), and in the adjacent root cells (yellow arrowheads), but not in the capillaries present at the proximity of the root processes (white arrowheads). (**L-O**) F-actin (blue) staining combined with myosin VIIa (red) / LDLR (green) co-immunolabelling focused on the organ of Corti of a transverse section of a P6 mouse cochlea. LDLR is mostly expressed in the inner hair cells and in the pillar cells. Scale bar in (A) = 50 µm in (A-C), scale bars in (D), (H) and (L) = 10 µm in (D-G), (H-K) and (L-O), respectively. CD = cochlear duct, dSV = developing stria vascularis, IHC = inner hair cell, OC = organ of Corti, OHCs = outer hair cells, OS = outer sulcus, PCs = pillar cells, SL = spiral ligament.

tain, 2007). Recent advances have been made in the characterization of the molecular mechanisms that regulate its development (Defourny et al., 2019a). Cholesterol depletion experiments using the chelator 2-hydroxylpropyl-beta-cyclodextrin recently revealed a severe loss of pillar cells (Ding et al., 2021). Thus, these findings suggest that cholesterol is critical to ensure pillar cell integrity and survival.

3.2. LDLR expression in the cochlea at the onset of hearing (P12)

At the onset of hearing in mice (twelve-day-old, P12), we observed that LDLR was expressed in the stria vascularis, in the adjacent spiral ligament, as well as in the organ of Corti (Fig. 3**A-C**). In the stria vascularis, we observed that LDLR was mostly expressed in Kir4.1-negative marginal cells, which directly border the cochlear duct (yellow arrowheads in Fig. 3**D-G**), whereas only a few cells co-expressed LDLR and Kir4.1. The capillaries present in the mature stria vascularis still did not express LDLR (white arrowheads in Fig. 3**D-G**). In the spiral ligament, LDLR was no more expressed in the root cells themselves, but rather in the capillaries which lie in proximity to the root processes (white arrowheads in Fig. 3**H-K**). In addition, LDLR was also expressed in the outer sulcus cells (orange arrowhead in Fig. 3**H-K)**. A growing body of evidence now suggests that outer sulcus cells and root cells regulate the solute content of the endolymph, and may be essential in safe-guarding the global homeostasis of the cochlea (Jagger and Forge, 2013). An attractive hypothesis could be that LDLR regulates the amount of lipoprotein components present in the endolymph of the cochlear duct. It should be remembered here that the endolymph has a charge of +80-90 mV, while the perilymph is not charged. This endocochlear potential is critical for sensory hair cell mechanotransduction. In contrast to the perilymph, for which apolipoproteins constitute the second major group of macromolecules (Swan et al., 2009), the endolymph contains very low amounts of apolipoproteins (Thalmann and Thalmann, 1999). One reason could be that apolipoproteins are negatively charged (Sparks et al., 2008). Thus, the amount of apolipoproteins should be kept minimal in the cochlear duct to ensure a high positive potential in the endolymph. Finally, LDLR was mostly expressed in the outer pillar cells of the organ of Corti (Fig. 3**L-O**).

3.3. LDLR expression in the cochlea after the onset of hearing (P25)

In the post-hearing-onset cochlea (twenty-five-day-old, P25), we observed that LDLR was expressed in the stria vascularis, in the adjacent spiral ligament, as well as in the organ of Corti (Fig. 4**A-C**). At this stage, LDLR was expressed in the marginal cells of the stria vascularis (yellow arrowheads in Fig. 4**D-G**), but neither in the intermediate cells nor in intrastrial capillaries (white arrowheads in Fig. 4**D-G**). In contrast, LDLR was expressed in the capillaries of the spiral ligament (white arrowheads in Fig. 4**H-K**), as well as in the adjacent outer sulcus cells (orange arrowheads in Fig. 4**H-K**). In the organ of Corti, LDLR was expressed in the outer pillar cells, as well as in the Deiters' cells, i.e. the non-sensory supporting cells which directly surround the outer hair cells (Fig. 4**L-O**). *In situ*, each outer hair cell is constrained by phalangeal processes of Deiters' cells and outer pillar cells. Deiters' cells have been shown not only to provide a physical scaffold to support the outer hair cells, but also to directly modulate outer hair cell electromotility through a mechanical coupling (Yu and Zhao, 2009). Cholesterol depletion has been associated with dramatic decrease in cochlear electromotility of guinea pigs (Brownell et al., 2011). Thus, LDLR-mediated uptake of cholesterol into these supporting cells could be needed to regulate their membrane fludity and stiffness. These specific membrane properties would therefore be necessary to provide an optimal mechanical coupling with the adjacent outer hair cells. As mentioned above, otoacoustic emissions are reduced in patients with hypercholesterolemia and high levels of LDL-cholesterol (Preyer et al., 2001). In the case where hypercholesterolemia is due to a dysfunction of LDLR, which increases LDL plasma levels, it is tempting to speculate that hearing loss could come from a deficient LDLR-mediated uptake of cholesterol into outer pillar cells and Deiters' cells. This would lead to lack of membrane fluidity and stiffness, which in turn affects the mechanical coupling with the outer hair cells. Finally, a compromised cochlear amplification is expected to increase the susceptibility to SNHL. Indeed, without amplification, the auditory system is effectively deaf (Ashmore, 2008). Alternatively, the presence of oxidized LDL at the proximity of LDLR-expressing cells could initiate a local inflammatory response. This could explain why hypercholesterolemic animals develop edemas in the sensory outer hair cells, as well as in the marginal cell layer of the stria vascularis (Gratton and Wright, 1992 ; Satar et al., 2001). These alterations suggest that chronic hypercholesterolemia mainly causes metabolic stress to each of these two regions of the cochlea. The presence of edema in the sensory outer hair cells is certainly likely to reduce electromotility and otoacoustic emissions.

3.4. LDLR expression in the cochlea of adult mice (P90)

In adult mice (three-month-old, P90), we observed that LDLR was still expressed in the stria vascularis, in the adjacent spiral ligament, as well as in the organ of Corti (Fig. 5**A-C**). In the stria vascularis, it is worth noting that, at this stage, LDLR was expressed not only in the marginal cells, but also in Kir4.1-positive intermediate cells (yellow and orange arrowheads in Fig. 5**D-G**, respectively). As mentioned above, intermediate cells are present in close vicinity with intrastrial capillaries, which themselves barely express LDLR (white arrowheads in Fig. 5**D-G**). Thus, the intermediate cells could play a role in removing excessive LDL levels from these blood vessels. Experimental data have shown that the endocochlear potential is critically dependent on the voltage jump across the plasma membrane of the intermediate cells (Takeuchi et al., 2000). The presence of Kir4.1 K^+ channels in the intermediate cells is crucial for the generation of this endocochlear potential (Marcus et al., 2002). Interestingly, *in vitro* experiments revealed that Kir4.1 channel activity is inhibited by cholesterol depletion (Hibino and Kurachi, 2007). Moreover, recent observations based on a lipid bilayer membrane model suggested that inclusion of high levels of cholesterol increases membrane conductance (Alobeedallah et al., 2020). Thus, it is tempting to speculate that LDLR-mediated uptake of cholesterol into intermediate cells could be needed to increase K^+ channel activity, membrane conductance, and hence the generation of endocochlear potential. In the spiral ligament, LDLR was expressed in the capillaries (white arrowheads in Fig. 5**H-K**), as well as in the adjacent outer sulcus cells (orange arrowheads in Fig. 5**H-K**). Finally, we observed that LDLR was still expressed in the outer pillar cells and Deiters' cells of three-month-old mice (Fig. 5**L-O**). This finding supports a role for LDLR in cochlear amplification throughout adulthood in mice.

4. Conclusion

Overall, our results suggest that LDLR is largely expressed in cochlear cells that are involved in endolymph homeostasis. After the onset of hearing, LDLR is also expressed in non-sensory supporting cells that could provide a mechanical coupling with the adjacent outer hair cells to modulate electromotility and cochlear amplification. Further functional studies will be needed to unravel how LDLR regulates extracellular and intracellular levels of cholesterol and lipoproteins in the cochlea.

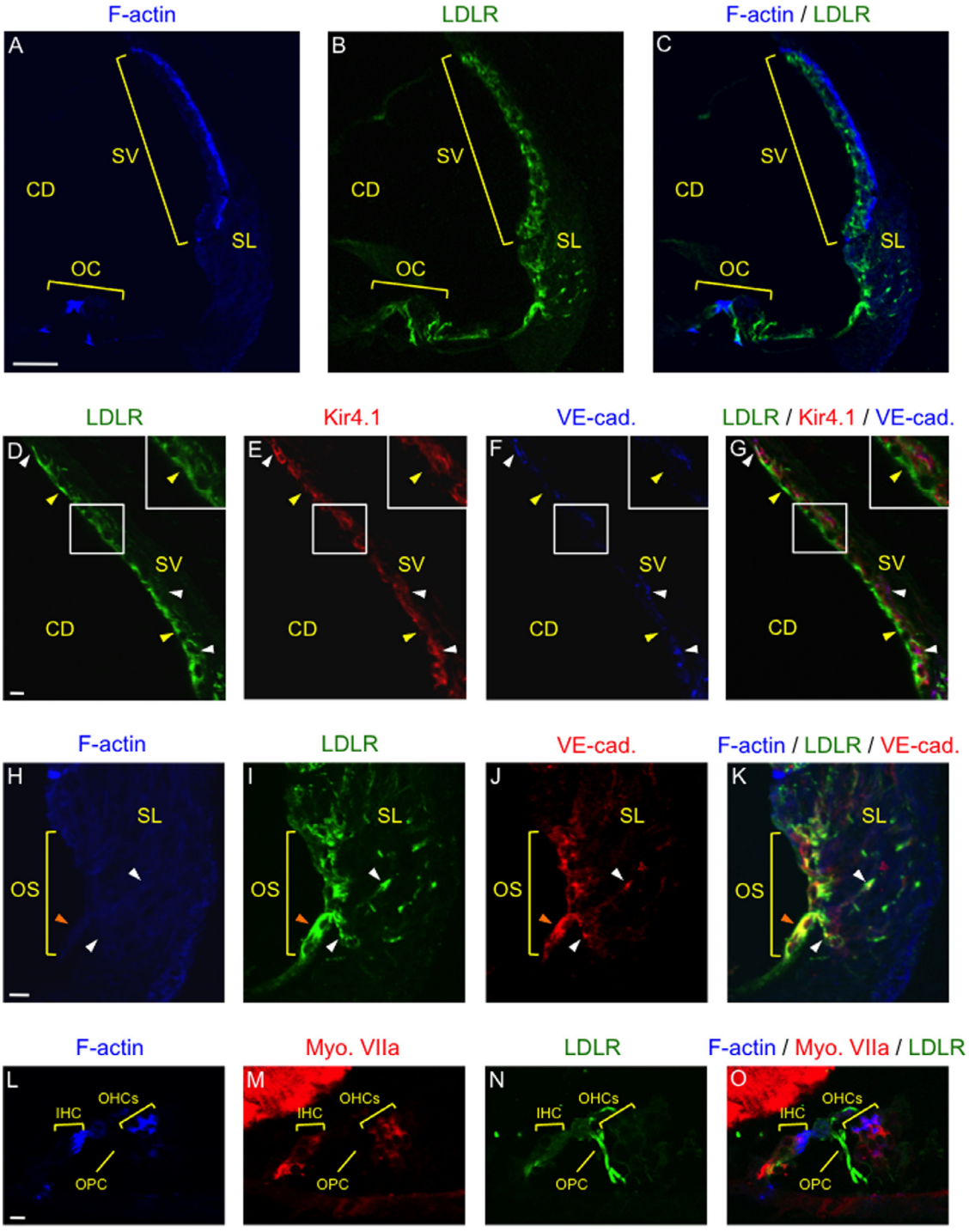

Fig. 3. LDLR expression pattern at the onset of hearing (P12) in the mouse. (**A-C**) F-actin (blue) staining combined with LDLR (green) immunolabelling of a transverse section of a P12 mouse cochlea. LDLR is expressed in the stria vascularis, in the adjacent spiral ligament, as well as in the organ of Corti. (**D-G**) LDLR (green) / Kir4.1 (red) / VE-cadherin (blue) co-immunolabelling focused on the stria vascularis of a transverse section of a P12 mouse cochlea. LDLR is mostly expressed in Kir4.1-negative marginal cells of the stria vascularis (yellow arrowheads). Most of the intrastrial capillaries do not express LDLR (white arrowheads). (**H-K**) F-actin (blue) staining combined with LDLR (green) / VE-cadherin (red) co-immunolabelling focused on the outer sulcus and adjacent spiral ligament of a transverse section of a P12 mouse cochlea. LDLR is expressed in the outer sulcus cells (orange arrowhead), as well as in the capillaries of the spiral ligament (white arrowheads). (**L-O**) F-actin (blue) staining combined with myosin VIIa (red) / LDLR (green) co-immunolabelling focused on the organ of Corti of a transverse section of a P12 mouse cochlea. LDLR is mostly expressed in the outer pillar cells. Scale bar in (A) = 50 µm in (A-C), scale bars in (D), (H) and (L) = 10 µm in (D-G), (H-K) and (L-O), respectively. CD = cochlear duct, IHC = inner hair cell, OC = organ of Corti, OHCs = outer hair cells, OPC = outer pillar cell, OS = outer sulcus, SL = spiral ligament, SV = stria vascularis.

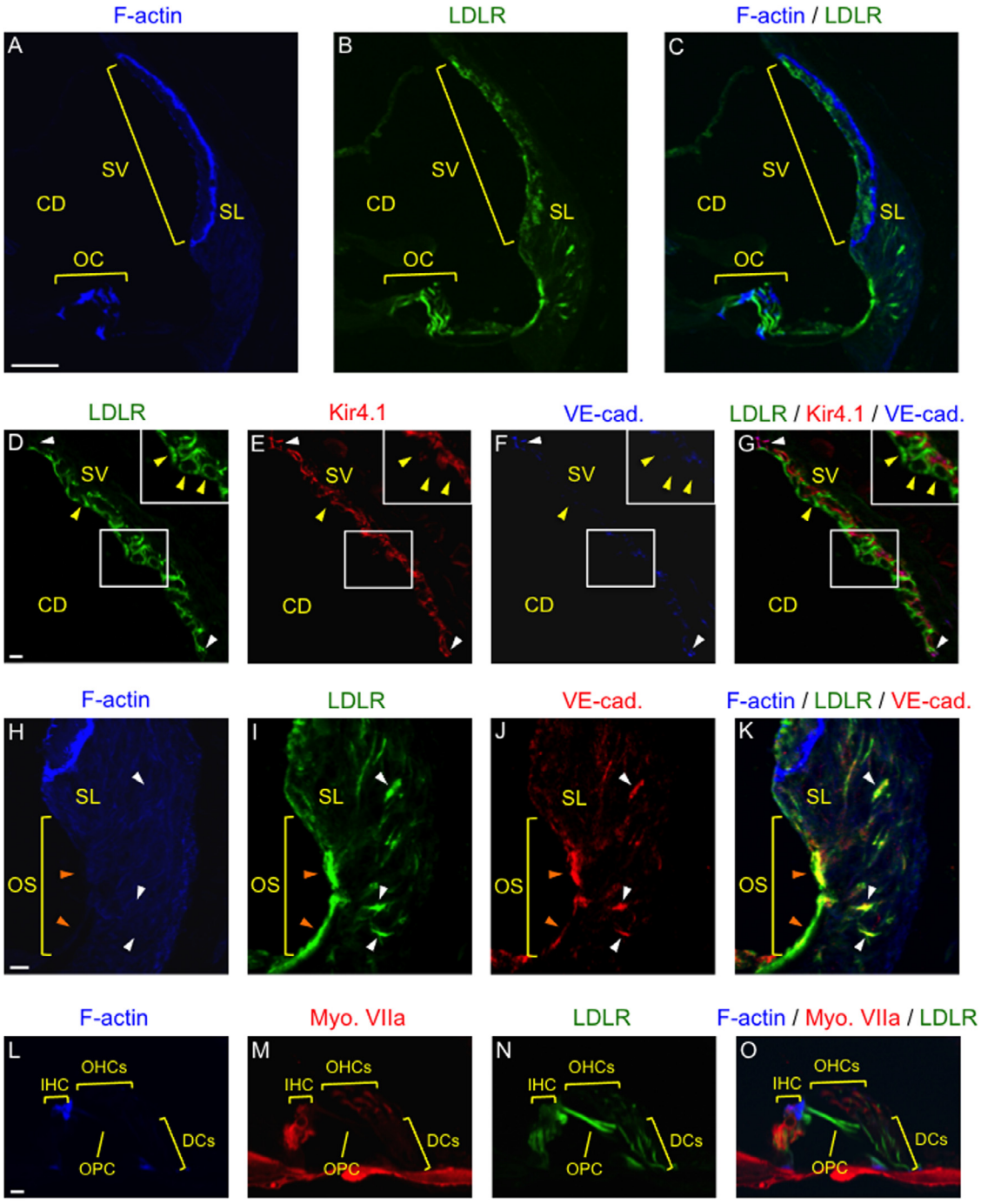

Fig. 4. LDLR expression pattern after the onset of hearing (P25) in the mouse. (**A-C**) F-actin (blue) staining combined with LDLR (green) immunolabelling of a transverse section of a P25 mouse cochlea. LDLR is expressed in the stria vascularis, in the adjacent spiral ligament, as well as in the organ of Corti. (**D-G**) LDLR (green) / Kir4.1 (red) / VE-cadherin (blue) co-immunolabelling focused on the stria vascularis of a transverse section of a P25 mouse cochlea. LDLR is expressed in Kir4.1-negative marginal cells (yellow arrowheads), but not in the intermediate cells of the stria vascularis. Most of the intrastrial capillaries do not express LDLR (white arrowheads). (**H-K**) F-actin (blue) staining combined with LDLR (green) / VE-cadherin (red) co-immunolabelling focused on the outer sulcus and adjacent spiral ligament of a transverse section of a P25 mouse cochlea. LDLR is expressed in the outer sulcus cells (orange arrowheads), as well as in the capillaries of the spiral ligament (white arrowheads). (**L-O**) F-actin (blue) staining combined with myosin VIIa (red) / LDLR (green) co-immunolabelling focused on the organ of Corti of a transverse section of a P25 mouse cochlea. LDLR is mostly expressed in the outer pillar cells and in the Deiters' cells. Scale bar in (A) = 50 μm in (A-C), scale bars in (D), (H) and (L) = 10 μm in (D-G), (H-K) and (L-O), respectively. CD = cochlear duct, DCs = Deiters' cells, IHC = inner hair cell, OC = organ of Corti, OHCs = outer hair cells, OPC = outer pillar cell, OS = outer sulcus, SL = spiral ligament, SV = stria vascularis.

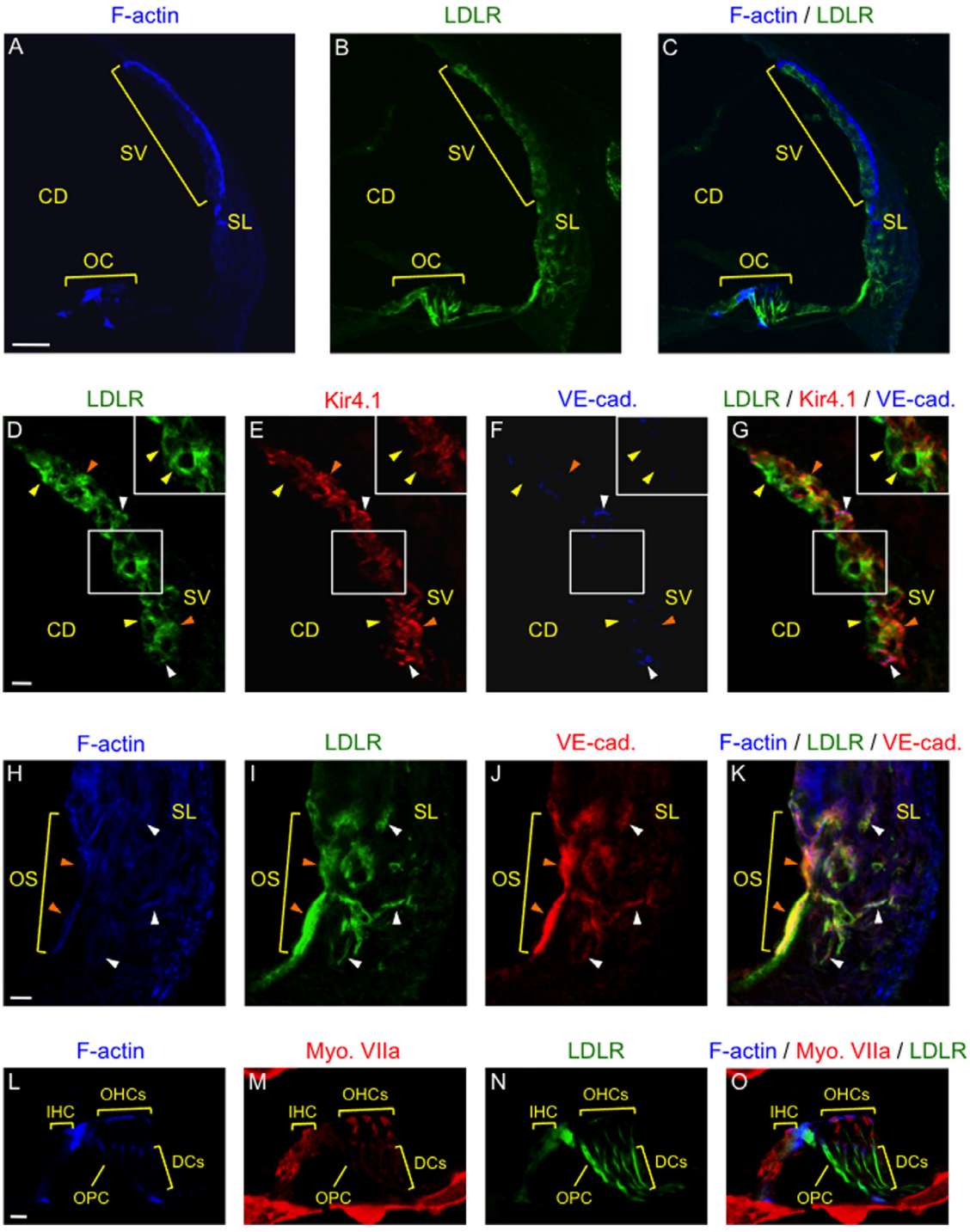

Fig. 5. LDLR expression pattern in the cochlea of a three-month-old (P90) mouse. (**A-C**) F-actin (blue) staining combined with LDLR (green) immunolabelling of a transverse section of a P25 mouse cochlea. LDLR is expressed in the stria vascularis, in the spiral ligament, as well as in the organ of Corti. (**D-G**) LDLR (green) / Kir4.1 (red) / VE-cadherin (blue) co-immunolabelling focused on the stria vascularis of a transverse section of a P90 mouse cochlea. LDLR is expressed in both Kir4.1-negative marginal cells (yellow arrowheads) and in Kir4.1-positive intermediate cells (orange arrowheads) of the stria vascularis. In contrast, intrastrial capillaries barely express LDLR (white arrowheads). (**H-K**) F-actin (blue) staining combined with LDLR (green) / VE-cadherin (red) co-immunolabelling focused on the outer sulcus and adjacent spiral ligament of a transverse section of a P90 mouse cochlea. LDLR is expressed in the outer sulcus cells (orange arrowheads), as well as in the capillaries of the spiral ligament (white arrowheads). (**L-O**) F-actin (blue) staining combined with myosin VIIa (red) / LDLR (green) co-immunolabelling focused on the organ of Corti of a transverse section of a P90 mouse cochlea. LDLR is mostly expressed in the outer pillar cells and in the Deiters' cells. Scale bar in (A) = 50 µm in (A-C), scale bars in (D), (H) and (L) = 10 µm in (D-G), (H-K) and (L-O), respectively. CD = cochlear duct, DCs = Deiters' cells, IHC = inner hair cell, OC = organ of Corti, OHCs = outer hair cells, OPC = outer pillar cell, OS = outer sulcus, SL = spiral ligament, SV = stria vascularis.

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CRediT authorship contribution statement

Aurore Saume: Formal analysis, Investigation. **Marc Thiry:** Writing – review & editing, Funding acquisition. **Jean Defourny:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition.

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