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The role of insulin-like growth factor-I in the physiopathology of hearing

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Insulin-like growth factor-I (IGF-I) belongs to the family of polypeptides of insulin, which play a central role in embryonic development and adult nervous system homeostasis by endocrine, autocrine, and paracrine mechanisms. IGF-I is fundamental for the regulation of cochlear development, growth, and differentiation, and its mutations are associated with hearing loss in mice and men. Low levels of IGF-I have been shown to correlate with different human syndromes showing hearing loss and with presbyacusis. Animal models are fundamental to understand the genetic, epigenetic, and environmental factors that contribute to human hearing loss. In the mouse, IGF-I serum levels decrease with aging and there is a concomitant hearing loss and retinal degeneration. In the *Igf1*$^{-/-}$ null mouse, hearing loss is due to neuronal loss, poor innervation of the sensory hair cells, and age-related stria vascularis alterations. In the inner ear, IGF-I actions are mediated by intracellular signaling networks, RAF, AKT, and p38 MAPK protein kinases modulate the expression and activity of transcription factors, as AP1, MEF2, FoxM1, and FoxP3, leading to the regulation of cell cycle and metabolism. Therapy with rhIGF-I has been approved in humans for the treatment of poor linear growth and certain neurodegenerative diseases. This review will discuss these findings and their implications in new IGF-I-based treatments for the protection or repair of hearing loss.

Keywords: animal models, deafness, human genetics, insulin-like factors, IGF1R signaling, organ of Corti

IGF SYSTEM AND SIGNALING PATHWAYS

Early studies have provided evidences that the insulin-like growth factor (IGF) system exerts influence on almost every organ system in the body, playing an important function in growth, development, and metabolism. The mammalian IGF system is a complex family of proteins that includes three factors (insulin, IGF-I, and IGF-II) and their cell surface receptors, which are the insulin receptor (IR), the IGF receptors 1 and 2 (IGF1R, IGF2R), and hybrid IGF1R/IR receptors. Six high-affinity binding proteins (IGFBP1–6) and their IGFBP proteases modulate the interactions between factors and receptors (**Figure 1**).

Insulin-like growth factor-I is a single-chain 70 amino acid peptide, mainly secreted by the liver (Foulstone et al., 2005). At the surface of target cells, the biological actions of the IGFs are mediated by receptors responsible for the transmission to a highly regulated intracellular signaling network. IGF1R, a transmembrane tyrosine kinase receptor, shares functional and structural homology with the IR, and can bind IGF-I and insulin, the latter only with a 100-fold lower affinity than IGF-I (Annunziata et al., 2010). The IGF1R is a tetramer with an intracellular protein tyrosine kinase domain (Adams et al., 2000; De Meyts and Whittaker, 2002). The close homology between IGF1R and IR allows the formation of functional hybrid receptors (Pandini et al., 2002; Torres-Aleman, 2010). The biological role of hybrid receptors has not been extensively tested and needs further investigation (Belfiore et al., 2009; Beauchamp et al., 2010). Finally, IGF2R, the cation-independent mannose-6-phosphate receptor, is a transmembrane glycoprotein without tyrosine kinase activity (Morgan et al., 1987). The IGF2R binds IGF-II but is unable to bind IGF-I (Brown et al., 2009). IGF system actions are regulated by a family of six high-affinity secreted binding proteins (IGFBPs1–6) that act modulating the half-life and distribution of the IGFs and competing with receptors for free ligands (Federici et al., 1997; Firth and Baxter, 2002). The IGFBP family also includes a group of low-affinity IGFBP-related proteins (Jiang et al., 2008).

Insulin-like growth factor signaling occurs via a complex intracellular network of molecules, where the cascade(s) triggered depend on the cellular spatiotemporal context and is determined by the ligands and receptors involved (**Figure 2**). It was already known that signaling mediated by these tyrosine kinase receptors

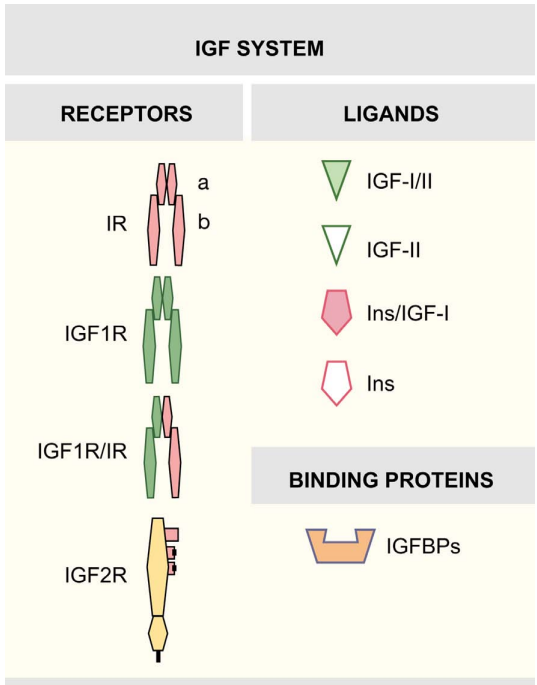

FIGURE 1 | Insulin-like growth factor system.

regulates pancreatic β cell function, but it has been recently concluded that IGF1R and IR signaling modulate insulin secretion and cell proliferation differently through their actions on IRS1 or IRS2, respectively (Xuan et al., 2010). IGF-I binding to IGF1R results in its autophosphorylation and activation of docking sites for receptor substrates (Shc/IRS; Laviola et al., 2008). Activation of IGF1R triggers two main downstream pathways: the PI3K–Akt and the RAF/ERK cascade. Both have been shown to play an important role in the transmission of signals from cell membrane receptors to the nucleus through interactions with other proteins (Mccubrey et al., 2011). On the first pathway, one of the critical targets is the serine/threonine kinase Akt that regulates protein synthesis and antiapoptotic effects of IGF1R (Laviola et al., 2008). In turn, activation of the RAF/ERK cascade participates in cell growth, proliferation, and differentiation (Downward, 2003; Mebratu and Tesfaigzi, 2009). Other well-documented kinases modulated in response to IGF-I are SAPK (stress-activated protein kinase) and the p38 MAP kinase activated by different stress stimuli (Cuenda and Nebreda, 2009; Vardatsikos et al., 2009).

MUTATIONS OF THE GENES OF THE IGF SYSTEM AND HUMAN DEAFNESS

Main molecular defects in the IGF system include mutations of the *IGF1* gene and mutations of the IGF-I high-affinity receptor gene (*IGF1R*).

MUTATIONS OF THE IGF1 GENE

Homozygote mutations of the *IGF1* gene are extremely rare and only four cases have been reported (Woods et al., 1996; Bonapace et al., 2003; Walenkamp et al., 2005; Netchine et al., 2009). These patients have in common the features of severe intrauterine growth retardation, and some degree of microcephaly. However, sensorineural deafness is not constant and the intensity of delayed psychomotor development is variable. Severe or profound hearing loss has been described in three of the four cases. **Table 1** shows the main features of homozygous mutations of the *IGF1* gene. In the fourth case reported, an extensive hearing test performed at 9 years of age was normal. This child also showed the mildest phenotype since the mutation allows IGF-I synthesis although with reduced affinity for its receptor (Netchine et al., 2009).

Accordingly, heterozygosis for *IGF1* mutations is associated with a modest decrease of height, but hearing loss has not been reported in these patients. A case of partial IGF-I deficiency has recently been described and was associated with pre and postnatal growth retardation and microcephaly, but the developmental delay was mild and hearing tests were normal (Van Duyvenvoorde et al., 2010). Sensorineural hearing loss is associated with poor growth rates in infancy and adolescence (Welch and Dawes, 2007), adult short stature (Barrenas et al., 2005), and Turner's syndrome (Barrenas et al., 2000).

MUTATIONS OF THE IGF-I RECEPTOR GENE (IGF1R)

IGF1R mutations are characterized by IGF-I resistance causing impaired fetal and postnatal growth. Ester et al. (2009) reported hearing problems in two cases of *IGF1R* mutations. No data about audiograms or electrophysiological tests were provided. The fact that craniofacial anomalies were present, and tympanostomy tubes were inserted in both cases suggests that hearing loss could be conductive, and the link to IGF-I resistance remains unproved. Other authors reporting human *IGF1R* mutations did not find marked hearing loss (Klammt et al., 2011). The fact that no profound sensorineural deafness has been reported in patients with heterozygous *IGF1* or *IGF1R* defects suggest that partial IGF-I signaling is sufficient for normal development of the inner ear.

NERVOUS SYSTEM ALTERATIONS ASSOCIATED WITH Igf1 MUTATIONS IN MICE

Studies of mutant mice with altered IGF-I expression show that this factor exerts a variety of actions that take place in the nervous system during neural cell development as well as in adulthood, and highlights the fact that IGF-I contributes to neurogenesis by promoting proliferation, differentiation, and survival of neural cells (D'ercole et al., 2002; Liu et al., 2009).

The *Igf1*$^{-/-}$ null mice present reduced brain size and altered brain regions, where the hippocampus, cerebellum, and cerebral cortex are the most affected, along with alteration of myelination processes (Ye et al., 2002a,b). Postnatal *in vivo* imaging by RMN has shown that the olfactory bulb is reduced and disorganized, in addition to reduced brain size and other brain and cranium malformations (Riquelme et al., 2010). The number of myelinated fibbers is normal, but whole fibber size is reduced (Gao et al., 1999). In mice with absent or reduced IGF-I levels, motor, and sensory conduction velocities are significantly decreased (Sullivan et al., 2008). These mice also present a decrease in dendrite length and complexity in the cortex, together with a smaller pyramidal neuron soma size (Cheng et al., 2003). Previous reports showed that the

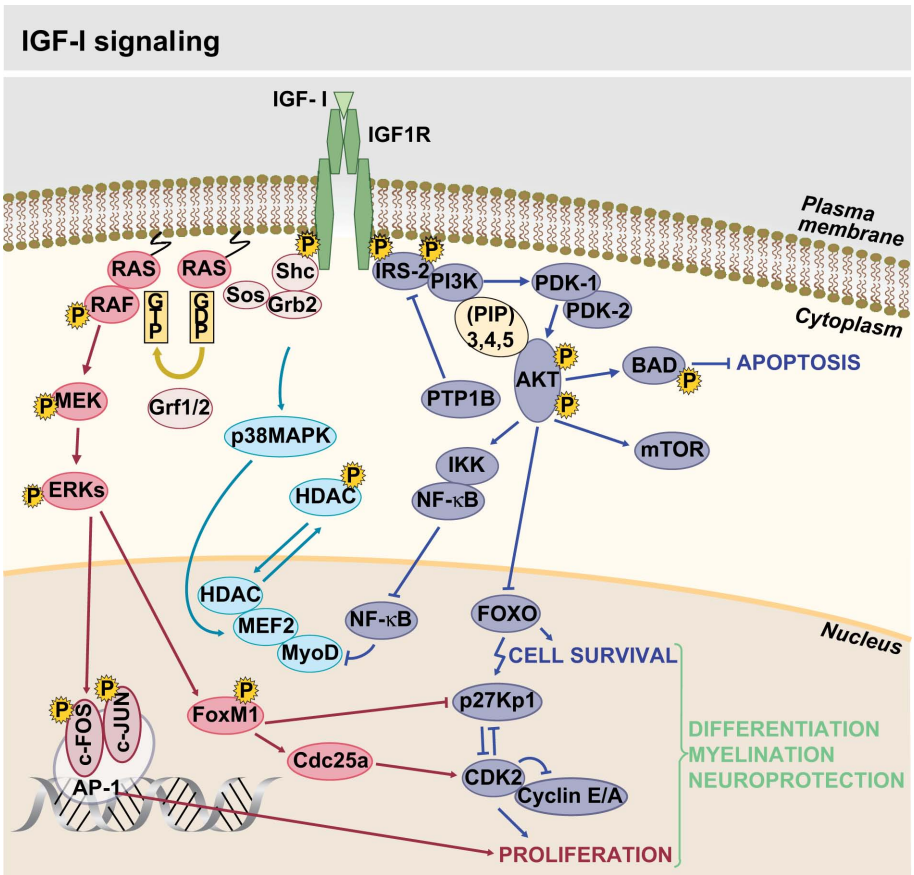

FIGURE 2 | Insulin-like growth factor-I signaling.

Table 1 | Reported cases with homozygous mutations of the *IGF1* gene.

	Woods et al. (1996)	Bonapace et al. (2003)	Walenkamp et al. (2005), Walenkamp and Wit (2007)	Netchine et al. (2009)
Sex	Male	Male	Male	Male
Consanguinity	Yes	Yes	Yes	Yes
Birth weight SDS/g	−3.9/1400	−4.0/1480	−2.5/1420	−2.5/2350
Birth length SDS/cm	−5.4/37.8	−6.5/41	−3/39	−3.7/44
Growth SDS	−6.9 at 16 years	−6.2 at 1.6 years	−9 at 5 years	−4.5 at 3 years
Sensorineural hearing loss	Profound	Severe	Profound	No
IGF-I levels	Undetectable	1.0 ng/mL	+7.3 SDS	Variable
IGF-I affinity-for IGF1R	Zero	Not studied	Extremely low	Partially reduced
Molecular defect	Deletion of exons 4–5	Polymorphism	Missense mutation V44M	Missense mutation R36Q

IGF-I knockout has a lower number of neurons and oligodendrocytes in the olfactory bulb, dentate gyrus, and striatum (Liu et al., 1993; Beck et al., 1995; Cheng et al., 1998) along with a decrease in the cochlear ganglion neurons (Camarero et al., 2001). This appears to be the result of various processes such as decreased cell proliferation, delayed development, and increased cell death (Beck et al., 1995; Camarero et al., 2001; Ye et al., 2002b; Ye and D'ercole, 2006; Sanchez-Calderon et al., 2010). Studies with *Igf1*$^{-/-}$ mice during embryonic and adult neurogenesis suggest possible new functions of IGF-I as the regulation of neuronal migration and disposition in the olfactory bulb, the neuroblast output from the subventricular zone and incorporation into the rostral migratory stream, as well as the entry of new neurons from subventricular zone to the olfactory bulb (Hurtado-Chong et al., 2009). A conditional mouse mutant (Δ*Igf1r*), with targeted manipulation of *Igf1r* gene by CaMKIIα-Cre recombination in the central nervous

system, in which the oligodendrocyte progenitors do not accumulate, proliferate, or survive, has been the preferred model to study the role of IGF-I signaling in remyelinating lesions (Mason et al., 2003).

Otherwise the transgenic mouse with IGF-I overexpression in the brain presents increased brain size and cell numbers in all neural lineages during embryonic and early postnatal development, whereas hippocampus and cerebellum sizes are increased postnatally. Previous studies have shown a proportional increase in the number of neurons, astrocytes, and oligodendrocytes in the dentate gyra of mice expressing an IGF-I transgene specifically in astrocytes, indicating that the development of these cell lineages was similarly affected by the IGF-I transgene (Ye and D'ercole, 2006). The potential role of IGF-I during embryonic and early postnatal brain development was investigated in a transgenic mice (IGF-I nestin Tg mice) with IGF-I overexpression driven from early in embryogenesis by regulatory sequences from the nestin gene. In this case, the neuron progenitor proliferation in the cerebral cortical ventricular and subventricular zones is performed by shortening the length of the cell cycle G1 phase and an accumulation of progenitors in the mitotic cell cycle. IGF-I promotes growth of the central nervous system in these stages of development by mechanisms that involve both neural progenitor proliferation and reduced apoptosis during the postnatal phase (Hodge et al., 2004; Popken et al., 2004).

On the other hand, single and double mutant mice for IGF-I and leukemia inhibitory factor (LIF) have been used to analyze the neural embryonic phenotype, and the results suggest that both factors decrease motoneuron number in specific brain stem nuclei (Vicario-Abejon et al., 2004). Finally, the comparison of the brain phenotype of *Igf1* mutants carrying a deletion either in the liver gene or in the forebrain neurons have lead to the conclusion that circulating IGF-I plays an important role in brain function (reviewed in Torres-Aleman, 2010).

In conclusion, transgenic mouse models are valuable tools for better understanding of IGF-I mechanisms in the nervous system, albeit further investigation is needed to understand the organ-specific actions and to explore possible therapies.

Sensory systems are also affected by IGF-I deficit, particular attention has been devoted to the study of hearing because of the human phenotype (**Figure 3**).

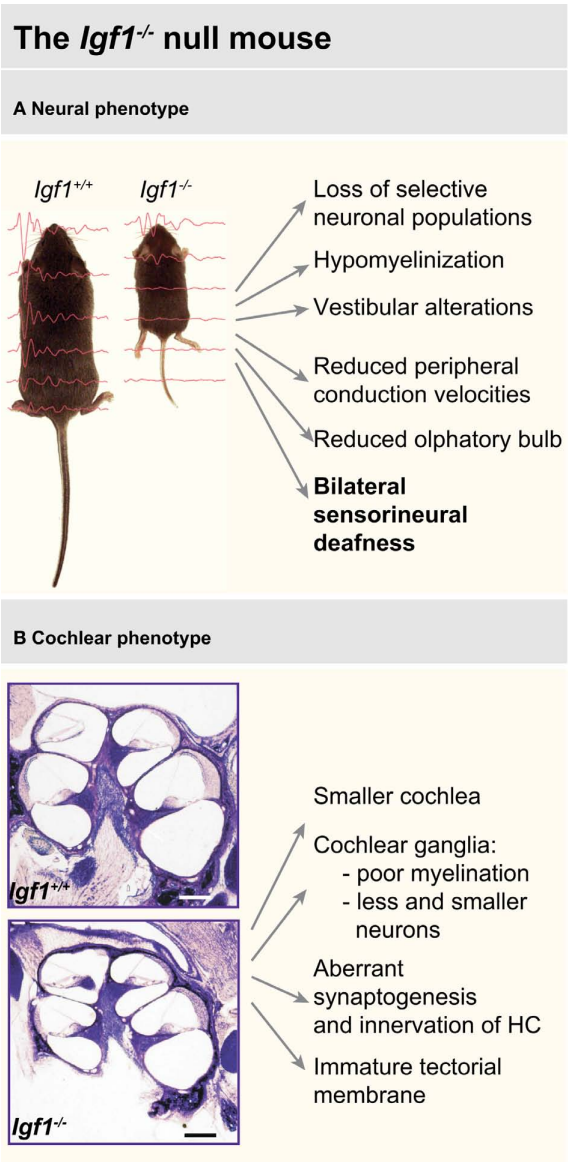

FIGURE 3 | Main features of the neural (A) and cochlear (B) phenotype of the *Igf1*$^{-/-}$ null mouse. Data have been compiled from Liu et al. (1993), Beck et al. (1995), Cheng et al. (1998), Gao et al. (1999), Camarero et al. (2001, 2002), Ye et al. (2002a,b), Cediel et al. (2006), Sullivan et al. (2008), Riquelme et al. (2010).

ANIMAL MODELS FOR THE STUDY OF IGF-I ACTIONS ON THE INNER EAR

The mammalian inner ear develops from an ectodermal patch, the otic placode, which invaginates to form the otic cup that subsequently closes and forms the otic vesicle. This autonomous structure contains the information required to generate the adult inner ear (Bissonnette and Fekete, 1996; Sanchez-Calderon et al., 2007). Organogenesis involves a dynamic balance of cell proliferation, differentiation, survival, and death, biological processes that are tightly regulated by a network of extrinsic and intrinsic factors (Sanchez-Calderon et al., 2007; Magarinos et al., 2010). Among them, the IGF-I plays a key role in promoting proliferation and survival of otic progenitor cells and in supporting neurogenesis and late differentiation (Frago et al., 2003; Varela-Nieto et al., 2007). During cochlear development, this factor and its specific receptor IGF1R are expressed in abundance in several species (Groigno et al., 1999; Varela-Nieto et al., 2003; Sanchez-Calderon et al., 2007, 2010; Gross et al., 2008); however the transcription of both ligand and receptor declines significantly after birth (Baker et al., 1993; Stratikopoulos et al., 2008; Riquelme et al., 2010; Sanchez-Calderon et al., 2010). In a variety of neural cell types, IGF-I is able to induce growth and DNA synthesis, i.e., in the chicken otic precursors (Leon et al., 1995; Rodriguez-De La Rosa et al., submitted), to short the length of the cell cycle, to increase the number of

progenitors and to prevent apoptosis (Varela-Nieto et al., 2003; Ye and D'ercole, 2006). Thus IGF-I is essential for the proliferation, survival, and early differentiation of inner ear neuronal precursors (Varela-Nieto et al., 2004, 2007).

The lack of IGF-I causes significant defects in the inner ear in different species, including zebra fish (Schlueter et al., 2007), chicken (Camarero et al., 2003), rat (Zheng et al., 1997), mice (Camarero et al., 2001, 2002; Cediel et al., 2006), and men (Walenkamp and Wit, 2007). Currently, there are several animal models available with mutations in different IGF-I signaling pathway components, which could be used to study the effects of the deficit in inner ear development. Regarding to mice, there are genetically modified strains with mutations in the factors (IGF-I and II), the receptor (IGF1R, IGF2R, and insulin receptor), and the IGF binding proteins (IGFBP1–5 and IGFBP7). Detailed information about the category, allelic composition and phenotype of the mutant lines can be obtained at the Mouse Genome Database at the Mouse Genome Informatics website (http://www.informatics.jax.org). Most of the IGF system mutant mice present a phenotype that includes some of these features: increased mortality, premature aging, growth deficits small size, infertility, endocrine changes, muscle–skeletal abnormalities, and neural alterations. Hearing impairment has been described only in the mutant mouse with a targeted (knockout) mutation in the *Igf1* gene ($Igf1^{tm1Arge}/Igf1^{tm1Arge}$; Liu et al., 1993). It was generated by Argiris Efstratiadis' group by replacement of sequences encoding part of exon 4 with a neomycin resistance gene. The homozygous mutant mouse maintained in a hybrid background (129S/SvEv*MF1) presents delayed inner ear development, abnormal cochlear, and otic capsule morphology, degeneration of the cochlear ganglion, aberrant innervation patterns, increased neural apoptosis, and deficits in myelination (Camarero et al., 2001, 2002; Sanchez-Calderon et al., 2010). As a consequence, $Igf1^{-/-}$ null mice develop a profound syndromic sensorineural hearing loss with increased hearing threshold assessed by auditory brainstem response recording. Furthermore, as discussed above for the nervous system, the cochlea and the auditory central pathway present poor myelinization and this is reflected in the delayed latencies of the auditory brainstem responses (Cediel et al., 2006; Riquelme et al., 2010). IGF-I deficiency induces caspase-dependent neuronal apoptosis in the brain and also in the cochlea (Sanchez-Calderon et al., 2007). However, the impact of IGF-I deficit on the neuronal populations of the central nuclei of the auditory pathway have not been yet studied.

In addition to deafness, $Igf1^{-/-}$ null mice also show other sensorial deficits including olfactory and visual function (Vicario-Abejon et al., 2003; Otaegi et al., 2006; Scolnick et al., 2008; Hurtado-Chong et al., 2009; Riquelme et al., 2010; Rodriguez-De La Rosa et al., submitted). Accordingly, the transcriptome of the $Igf1^{-/-}$ cochlea when compared to that of the wild type mouse showed expression changes in multiple genes associated with hearing and seeing (**Table A1** in Appendix). It is remarkable that genes as *harmonin*, *Rp1h*, and *tubby*, whose mutations have been associated to retinitis pigmentosa and to the human Usher syndrome are IGF-I targets in the mouse cochlea (Sanchez-Calderon et al., 2010). Unfortunately, there are no data available on the hearing phenotype of other IGF system mouse mutants.

PERSPECTIVES OF IGF-I THERAPY IN THE TREATMENT OF DEAFNESS

In recent years, advances in the fields of human genetics and the sequencing of the human and other species genomes had prompted a step forward in the knowledge of hearing physiopathology (Dror and Avraham, 2009). In parallel, the use of animal models of deafness (Lewis and Steel, 2010) and the developments in the fields of nanotechnology (Danti et al., 2009) and stem cells (Rivolta, 2010) have opened new possibilities of therapeutic intervention. Finally, advances in microsurgery and cochlear implants technology have facilitated research on local drug delivery to the cochlea for hearing loss treatment (Rivera et al., submitted). Local recombinant human IGF-I application has been reported to be useful for the treatment of noise induced hearing loss in animals (Iwai et al., 2006; Lee et al., 2007). Recently, Nakagawa et al. (2010) have demonstrated that topical IGF-I application using gelatine hydrogels is well tolerated and may be efficacious for hearing recovery in patients with sudden sensorineural hearing loss that is resistant to systemic glucocorticoids. These recent data open new perspectives for IGF-I in the treatment of deafness. Still, further studies are required to confirm the therapeutic potential of IGF-I in hearing loss and the prognostic value of plasmatic IGF-I levels.

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APPENDIX

Table A1 | Insulin-like growth factor-I deficiency alters sensory gene expression in the developing cochlea.

Gene name	FC arrays[1]	Function	Expression in the inner ear	Expression in the retina/synapsis	MIM number Orphanet number MIM disorder
CALCIUM BINDING PROTEINS					
S100 g (S100 calcium binding protein G) [CABP1; Calb3; Cabp9k]	−4, 1	Calcium binding protein. S100 family participates in microtubule assembly, neurite extension, cell cycle progression, and cell proliferation.	S100 proteins are expressed in different structures of the inner ear of many species including human, monkey, rat, and mouse (Buckiova and Syka, 2009).	nf	nf
CELL–CELL ADHESION					
Cldn18 (Claudin 18)	−6	Integral membrane protein. Forms tight junction strands in epithelial cells.	In adult mice, the protein is located in the organ of Corti, marginal cells of the stria vascularis, Reissner's membrane, spiral limbus, and vestibular sensory epithelium (Kitajiri et al., 2004).	nf	nf
CELL MIGRATION					
Mash1 (Mammalian achaete-scute homolog 1) [Ascl1, Hash1]	2, 9	Basic helix-loop-helix (bHLH) transcription factor, involved in the development of specific neural lineages in most regions of the CNS, and of several lineages in the PNS. This family is highly conserved through the evolution and is involved in hair cell differentiation, Atoh1, and in sensory neurons differentiation, Neurog1, and Neurod1 (Fritzsch et al., 2006).	nf	bHLH genes, such as *Mash1* and *Math3*, and the homeobox gene *Chx10* are essential for generation of bipolar cells in the inner nuclear layer of the retina. In the presence of *Chx10 Mash1* and *Math3* only promote the bipolar cell fate, which suggests they can play a role in neuronal subtype specification.	MIM: 100790 ORPHA159769 Congenital central hypoventilation syndrome (CCHS) also known as "Ondine's curse." CCHS has been associated with several disorders classified as neurocristopathies (aberrant phenotypes arising from a defect of migration or differentiation of neural crest cells).
ION TRASPORT/ION CHANNELS					
Cacna1f (calcium channel, voltage-dependent, alpha 1F subunit) [CSNB2, CORDX3, CSNB2A, AIED, OA2]	−2,1	Voltage-sensitive calcium channel. Mediates the entry of Ca^{2+} into excitable cells and it is also involved in a variety of calcium-dependent processes, including muscle contraction, hormone, or neurotransmitter release, gene expression, cell motility, cell division, and cell death.	nf	Mutations of human *CACNA1F* gene cause the incomplete form of X-linked congenital stationary night blindness (CSNB2) characterized by a defective transmission of light signals from photoreceptors to second order neurons. In the mouse, the loss of function result in electroretinogram b-wave reduction and changes at the photoreceptor synapse (Specht et al., 2009).	MIM: 300110 ORPHA119148 X-linked congenital stationary night blindness; X-linked cone-rod dystrophy-3; Aland Island eye disease.

Kcnd2 (potassium voltage-gated channel, Shal-related family, member 2) [Kv4.2]	−1, 3	Pore-forming (alpha) subunit of voltage-gated rapidly inactivating A-type potassium channels. It is transported by Kif17 (Chu et al., 2006).	Kv4.2 gene and protein expression has been found in the auditory hair cells of P15 chicken (Sokolowski et al., 2004). In mouse, apex neurons had a preponderance of Kv4.2 subunits, whereas base neurons possessed greater levels of KCa, Kv1.1, and Kv3.1 subunits (Adamson et al., 2002).	nf	MIM: 605410
Kcnmb1 (potassium large conductance calcium-activated channel, subfamily M, beta member 1) [BKb1]	−1, 1	Voltage-activated K(+) channels are important for shaping the receptor potentials of cochlear hair cells.	BK-type beta subunits are expressed strong in the IHC and weaker in the OHC. BK channels underlie the large K(+) conductance in IHC of mammals. BKb1 −/− mice present normal hearing function and cochlear structure (Langer et al., 2003; Ruttiger et al., 2004; Teijido et al., 2007).	nf	MIM: 603951 Resistance to diastolic hypertension.
Mlc1 (megalencephalic leukoencephalopathy with subcortical cysts 1 homolog (human))	1, 3	Membrane protein of unknown function with low homology to potassium channels. *MLC1* is the first gene responsible for Megalencephalic leukoencephalopathy with subcortical cysts. This disease is an autosomal-recessive neurological disorder in children characterized clinically by macrocephaly, deterioration in motor functions, cerebellar ataxia, and mental decline.	In the mouse, MLC1 showed postsynaptic localization on the IHC and in afferent fibers of the IHC in the organ of Corti as evidenced by co-localization with calretinin. MLC1 was strongly expressed in the SG in non-myelinated and myelinated parts of the auditory nerve. In the SG, only very few neuronal cell bodies were MLC1-positive (Teijido et al., 2007).	MLC1 was also detected in several sensory epithelia, as retina or saccula maculae. MLC1 immunostaining was detected in the retina, mainly in the ganglionar cells that send their axons along the optic nerve (Teijido et al., 2007).	MIM: 605908 ORPHA123257 Vacuolating megalencephalic leukoencephalopathy with subcortical cysts.
LIPID METABOLISM					
Alox12 (arachidonate 12-lipoxygenase)	−1,8	Oxygenase and 14,15-leukotriene A4 synthase activity. Belongs to the lipoxygenase family.	Its expression is increased in cells of the mouse Organ of Corti treated with cisplatinum (Previati et al., 2004).	nf	MIM: 152391

(Continued)

Table A1 | Continued

Gene name	FC arrays[1]	Function	Expression in the inner ear	Expression in the retina/synapsis	MIM number Orphanet number MIM disorder
NEUROTRANSMITTER TRANSPORT AND METABOLISM					
Slc5a7 (solute carrier family 5 [choline transporter], member 7) [Cht1]	−2, 2	High-affinity choline transporter involved in the synthesis of acetylcholine.	*Slc5a7* is expressed in efferents terminals of the IHC and OHC in adult rat (Bergeron et al., 2005).	Choline transporters (Specht et al., 2009) like SLC5A7 play an important role in presynaptic ACh synthesis. A better understanding of the CHT's subcellular localization together with the cloning of CHTs, have revealed a large reserve of CHTs on synaptic vesicles (Ferguson and Blakely, 2004).	MIM: 608761
Slc18a3 (solute carrier family 18 (vesicular monoamine), member 3) [VAT; VAChT]	−1, 7	Participates in acetylcholine transport in synaptic vesicles.	In the adult rat, it is expressed in efferent terminals of the inner and outer hair cells (Bergeron et al., 2005).	nf	MIM: 600336
ORGAN DEVELOPMENT					
Fgf15 (Fibroblast growth factor 15) [Ortholog of chicken and human Fgf19]	3, 6	Growth factor. Regulates cell division and patterning within specific regions of the embryonic brain, spinal cord and sensory organs. It is an early otic placode inducer (Ladher et al., 2000).	In the developing chicken, it is expressed in the sensory epithelium and neurons of the cochlear–vestibular ganglion (Sanchez-Calderon et al., 2007).	Fgf15 is first expressed in the distal optic vesicle at 9.5 dpc and in dividing retinal progenitor cells in the optic cup during retinal histogenesis. At 10.5 dpc Fgf15 appears in central presumptive retina (Behesti et al., 2009).	MIM: 603891
PERCEPTION OF SOUND AND LIGHT					
Six6 (sine oculis-related homeobox 6 homolog [Drosophila])	2, 9	Transcription factor. It is involved in eye development. *Six6*, in association with *Dach* corepressors, regulates proliferation by directly repressing cyclin-dependent kinase inhibitors, including the p27Kip1 promoter.	*The Six-1*$^{-/-}$ null mouse presents alterations in inner ear early development (Zheng et al., 2003).	Microarray analysis of human retinal progenitor cells have shown the expression of early retinal developmental genes like Six6 (Schmitt et al., 2009). The LIM homeobox transcription factor Lhx2 and Pax6 synergistically activate Six6 expression in retinal stem cells and Lhx2 is also required for Six6 expression in the optic vesicle (Tetreault et al., 2009).	MIM: 606326 ORPHA118728 Microphthalmia, cataract, and nystagmus.

Tub (tubby candidate gene)	−2, 3	Membrane-bound transcription regulator that translocates to the nucleus in response to G-protein activation-dependent phosphoinositide hydrolysis.	The homozygous Tubby (tub/tub) mutant mouse presents an early progressive hearing loss and photoreceptor degeneration (Carroll et al., 2004). These alterations are similar to those reported for Usher type I patients.	nf	MIM: 601197
Rp1h (retinitis pigmentosa 1 homolog [human])	2, 1	Photoreceptor specific protein. Mutations in human RP1 are a common cause of dominant retinitis pigmentosa. The human Usher syndrome is an inherited condition characterized by hearing impairment and progressive vision loss.	nf	The severity of the retinal degeneration caused by the Rp1h allele depends on genetic background (Liu et al., 2009).	MIM: 603937 ORPHA791 Retinitis pigmentosa 1; Susceptibility to hypertriglyceridemia.
Ush1c (Usher syndrome 1C homolog [human]) [Harmonin]	1, 4	Stereocilia protein. Harmonin interacts with cadherin 23 and myosine VIIA in growing stereocilia of the inner ear, to shape the functional stereocilia bundle. Mutations in the Harmonin gene cause Usher syndrome type I subtype C, characterized by hearing impairment and retinitis pigmentosa (Boeda et al., 2002).	Human and mouse stereocilia of sensory hair cells express Harmonin (Boeda et al., 2002).	The deaf circler (dfcr) mice, which possess mutant Ush1cA, present a significant concentration of Harmonin in the synapses of the rod photoreceptor cells, presynaptic region, and in the postsynaptic processes of the horizontal and bipolar cells. Retinas of the mutant mice remain unaffected while patients with USH1C show regions of normal central retina surrounded by degeneration (Williams et al., 2009).	MIM: 605242 ORPHA120433 Usher syndrome type 1C, Acadian variety; Autosomal-recessive non-syndromic sensorineural hearing loss, DFNB18.
Rorb (RAR-related orphan receptor beta)	1, 66	Transcription factor. ROR-beta is such an orphan nuclear receptor, forming a subfamily with the closely related nuclear receptors ROR-alpha. $Rorb^{-/-}$ mice are blind, yet their circadian activity rhythm is still entrained by light-dark cycles (Andre et al., 1998).	nf	Rorb, a clock gene, shows a evident circadian oscillation of their expression level in the rat retina (Kamphuis et al., 2005).	MIM: 601972

(Continued)

Table A1 | Continued

Gene name	FC arrays[1]	Function	Expression in the inner ear	Expression in the retina/synapsis	MIM number Orphanet number MIM disorder
VESICLE-MEDIATED TRANSPORT					
Kif17 (Kinesin family member 17)	−2,9	Motor protein. Transports vesicles containing NMDA receptor 2B along microtubules. It has a role in the transport of Kv4.2.	nf	Kif17 is expressed in all retinal layers, including the photoreceptor layer and retinal pigment epithelium in zebrafish. The outer segment formation and targeting of the visual pigment protein is severely disrupted in the Kif17 knockdown, showing that Kif17 is essential for photoreceptor outer segment development (Insinna et al., 2008).	MIM: 605037
Vamp1 (vesicle-associated membrane protein 1)	−2	Membrane protein. Involved in the targeting and/or fusion of transport vesicles to their target membrane. It is involved in vesicle fusion at both poles of the cell (Safieddine and Wenthold, 1999).	Vamp1, is present in hair cells and efferents fibers in the organ of Corti of guinea pig.	Vamp1 is selectively expressed in the outer segments of the photoreceptors, in the outer and inner plexiform layers, and in a subset of ganglion cells of the mouse retina (Nystuen et al., 2007).	MIM: 185880
VITAMIN TRANSPORT					
Slc19a2 (solute carrier family 19 [thiamine transporter], member 2)	−1, 4	High-affinity thiamine transporter. *Slc19a2* mutations underlie the clinical syndrome known as thiamine-responsive megaloblastic anemia characterized by anemia, diabetes, and sensorineural hearing loss.	Selective expression in IHC, which are lost in the *Slc19a2*$^{-/-}$ null mouse (Liberman et al., 2006).	Some results demonstrate the existence of a specialized and regulated uptake process for thiamine in a cellular model of human retinal pigment epithelia that involves hTHTR-1 (the product of *Slc19a2* gene) and hTHTR-2 (Subramanian et al., 2007).	MIM: 603941 ORPHA118762 Thiamine-responsive megaloblastic anemia syndrome.
XENOBIOTIC METABOLISM					
Akr1c13 (Aldo-keto reductase family 1, member C13)	3, 1	Reductase. Catalyzes the reduction of gluconic acid derivatives.	In a cochlear microarray study of the *sh2/sh2 null* mouse this gene was related to age progressive hearing loss (Gong et al., 2006).	nf	nf

REGULATION OF TRANSCRIPTION					
Esrrb (estrogen related receptor, betav)	−1, 9	Orphan nuclear receptors. Closely related to the estrogen receptors (ERs). ERRs bound to estrogen response elements and interfered in the ER signal pathway.	Is expressed and controls the development of the endolymph-producing cells of the inner ear: the strial marginal cells in the cochlea and the vestibular dark cells in the ampulla, and utricle. Mutations of ESRRB cause autosomal-recessive non-syndromic hearing impairment DFNB35 (Chen and Nathans, 2007;Collin et al., 2008).	nf	MIM: 602167 Deafness, autosomal-recessive 35, DFNB35.
SULFATE METABOLISM					
Papss2 (3′-phosphoadenosine 5′-phosphosulfate synthase 2)	−1	Bifunctional enzyme with both ATP sulfurylase and APS kinase activity. In mammals, PAPS is the sole source of sulfate; APS appears to be only an intermediate in the sulfate-activation pathway. May have a important role in skeletogenesis during postnatal growth.	It is expressed in the otic vesicle at E12.5 in the mouse (Stelzer et al., 2007).	nf	MIM: 603005 Spondyloepimetaphyseal dysplasia (SEMD), Pakistani type.
RESPONSE TO STRESS					
Hspb1 (heat shock protein 1) [Hsp27]	−1, 1	Stress-inducible molecular chaperone and regulator of actin polymerization.	In the rat, Hsp27 staining is localized to the cuticular plate and lateral wall of OHC. Hsp27-like immunostaining is also found in tension fibroblasts, in the root cells of the spiral limbus and in Reissner's membrane. The presence of Hsp27 in the actin-rich tension fibroblasts and OHC suggests a potential role in the regulation and maintenance of the actin cytoskeleton in these cells. The presence of high levels of constitutive Hsp27 may also provide a mechanism for pre-protecting these cells against environmental stressors (Leonova et al., 2002).	An increased expression of HIF-1 and HSPs like Hsp27 indicates that the inner retina is subjected to ischemic stress. The differential expression of HSPs in morphologically damaged and intact retinas of different stroke models suggests that HSPs have distinct roles in the protection against ischemia (Kalesnykas et al., 2008).	MIM: 602195 ORPHA122526 Distal hereditary motor neuronopathy type 2B; Charcot–Marie–Tooth disease type 2F.

(Continued)

Table A1 | Continued

Gene name	FC arrays[1]	Function	Expression in the inner ear	Expression in the retina/synapsis	MIM number Orphanet number MIM disorder
INSULIN RECEPTOR SIGNALING PATHWAY					
Insr(insulin receptor)	−1, 1	Insulin receptor is a tetramer of two alpha and two beta subunits. The alpha and beta subunits are coded by a single gene and are joined by disulfide bonds, a mechanism parallel to that of its ligand, insulin.	*In situ* hybridization analysis in *Xenopus* shows that InsR expression is restricted to regions of ectodermal and mesodermal origin, notably the encephalon, otic vesicles, optic vesicles, gills, somites, and the pronephros (Groigno et al., 1999). In the *Igf1*$^{-/-}$ null mouse *Igf1* and *Igf1r* are expressed in the developing cochlea with complementary cellular patterns (Sanchez-Calderon et al., 2010).	The retinal insulin receptor (IR) exhibits high constitutive activity that is reduced in diabetes. IR activation has been shown to rescue retinal neurons from apoptosis through a phosphoinositide 3-kinase and protein kinase B (Akt) survival cascade. The study of the retinal IR signaling in sorbitol-treated retinas *ex vivo* shows that sorbitol activates both the IR and IGF1R tyrosine kinases, which leads to the activation of PI3K and Akt survival pathway in the retina (Rajala et al., 2009).	MIM: 147670 ORPHA122664 Donohue syndrome; Rabson–Mendenhall syndrome; Insulin-resistant diabetes mellitus with acanthosis nigricans; Familial hyperinsulinemic hypoglycemia-5.
SIGNAL TRANSDUCTION					
Camk4 (calcium/calmodulin-dependent protein kinase IV)	−1, 3	CaMKIV–CREB pathway is crucial for osteoclast differentiation and function.	In sections of the adult gerbil inner ear, moderate to strong immunoreactivity for Camk4 was present along the lateral borders of OHCs (Koyama et al., 1999) and in the SG (Bok et al., 2007).	nf	MIM: 114080
INMUNE RESPONSE/INFLAMMATORY RESPONSE					
Ccl11 (small chemokine (C–C motif) ligand 11) [Scya11; eotaxin]	−1, 5	Chemokine. In response to the presence of allergens, this protein directly promotes the accumulation of eosinophils (a prominent feature of allergic inflammatory reactions), but not lymphocytes, macrophages, or neutrophils.	In the eosinophilic otitis media (EOM), chemokines such as ecalectin and eotaxin are also produced in the middle ear (Iino et al., 2005).	nf	MIM: 601156 Susceptibility to human immunodeficiency virus type 1. Susceptibility to asthma.
RESPONSE TO OXIDATIVE STRESS					
Mpo (myeloperoxidase)	1, 2	Lysosomal hemoprotein located in the azurophilic granules of polymorphonuclear leukocytes and monocytes. In response to stimulation, MPO is activated into a transient intermediate with potent	MPO could be detected after 3 days of the application of cisplatin, in the lateral wall, the organ of Corti, supporting cells of the sensory epithelium and dark cells. These results suggest that MPO and	nf	MIM: 606989 Myeloperoxidase deficiency; Susceptibility to Alzheimer disease.

		antimicrobial oxidizing abilities (Goedken et al., 2007).	reactive oxygen species are involved in the inner ear dysfunction after the application of cisplatin (Watanabe and Yagi, 2000).		
CELL CYCLE PROGRESSION					
FoxM1 (Forkhead box M1)	1.5	Transcription factor. FoxM1 is essential for mitotic progression and for the transcriptional response during DNA damage/checkpoint signaling (Wang et al., 2005; Zhang et al., 2006; Tan et al., 2007).	In mice, FoxM1 protein is located in the organ of Corti, the stria vascularis, and the auditory ganglia at postnatal day 15 (Sanchez-Calderon et al., 2010).	FoxM1 expression in *Xenopus laevis* is found in the spinal cord, the rhombencephalon, the retina, and in the branchial arches (Pohl et al., 2005).	MIM:602341
FoxG1 (Forkhead box G1)	-8.9×10^{-5}	This transcription Factor has essential roles in the development of ears, eyes, olfactory system, and telencephalon. It is a strong candidate gene for determining forebrain size in vertebrates due to its role in the development of the telencephalon, where it promotes progenitor proliferation and suppresses premature neurogenesis.	FoxG1 mouse mutants show vestibular and cochlear defects, between then a shortened cochlea with multiple rows of hair cells and supporting cells and the lack of horizontal crista. Zebrafish foxg1 has been reported to have similar roles as the mouse orthologue Foxg1 (Pauley et al., 2006).	Zebrafish foxg1 paralogue (Foxg1b) is expressed in a regionally restricted pattern within the developing eye, mainly in the dorsal–nasal retina, which is similar to the retinal expression of mouse Foxg1 (Zhao et al., 2009).	MIM: 164874 ORPHA167854 Congenital variant of Rett syndrome.
Incenp (Inner centromere protein)	−2.2	INCENP is a member of the chromosomal passenger complex (CPC). INCENP appears be a scaffold that interacts with the three other members of the complex, Aurora B, Survivin and Borealin (Ruchaud et al., 2007).	nf	nf	MIM: 604411

Differentially expressed genes in the E18.5 cochlea of the Igf1$^{-/-}$ mutant mouse ranked by gene ontology and biological process annotations. Genes were selected according to reported inner ear expression, their relation with deafness, biological function determined with the PANTHER and FATIGO programmes, as well as fold-change and low-variance calculated with multi-mgMOS software. nf, not described previously in the literature.

[1]Average fold-change from microarray experiments. Adapted from Sanchez-Calderon et al., 2010, Supplementary table S4 (http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0008699#s5). References related to gene expression/function in the inner ear can be found in the previous link.

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