

**Variability in Cochlear Implantation Outcomes
in a Large German Cohort with a Genetic Etiology of Hearing Loss**

Supplemental Digital Content 2.

Expression pattern of all genes (n=35) that contributed causative mutations to the genetic hearing loss cohort.

Gene expression is classified into five expression clusters designated as designated as (1) Neural, (2) Hair Cell (HC), (3) structural genes in the Tectorial Membrane (Structural – TM) and the Cochlear Duct (Structural – CD), (4) Stria Vascularis (SV) and (5) Mitochondria (Mito).

| Gene | Expression* | Function* | Cluster |
|----------------|--------------------------------------|---|-----------------|
| <i>ACTG1</i> | IHCs, OHCs, PCs | Cytoskeletal nonmuscle actin protein gamma. Localized in F-actin gap region of stereocilia. ESPN also observed in this region. | HC |
| <i>CDH23</i> | IHCs, OHCs, RM | Component of tip link and transient lateral links of stereocilia. Component of presynaptic region of IHCs and OHCs. | HC |
| <i>COCH</i> | ESCs, SLig, SL | Extracellular matrix protein. | Structural – CD |
| <i>COL11A1</i> | HCs, CCs, SLig, SV, TM, GER, LW | Structural components of the extracellular matrix of the tectorial membrane. | Structural – CD |
| <i>COL2A1</i> | TM, SL | Structural components of the extracellular matrix of chondrocytes and the tectorial membrane. | Structural – TM |
| <i>COL4A3</i> | SLig, RM, SL, BM | Structural components of the extracellular matrix of the basilar membrane and spiral ligament. | Structural – CD |
| <i>DIAPH1</i> | PCs, DCs, SGNs (Neuhaus et al. 2017) | Actin polymerization protein in hair cells of the inner ear. MITF regulates DIAPH1 expression (Nishio et al. 2015). Mutations in DIAPH1 are associated with auditory neuropathy (Wu et al. 2020). | Neural |
| <i>EDNRB</i> | SGNs (Ida-Eto et al. 2011) | EDNRB required for normal differentiation and development of melanocytes. SOX10 enhances the expression of EDNRB. EDNRB inhibits the Na-K transporter | Neural |

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| | | and activates G protein-coupled inwardly rectifying potassium channels (Kir-3). | |
| <i>EYA4</i> | SGNs, HCs, CCs, ESCs, LW, ISCs | Involved in innate immune response regulation by modulating the phosphorylation of signal transducers for intracellular pathogens. | Neural |
| <i>GJB2</i> | PCs, SuCs, HCs, CCs, ESCs, LW, SLig, SV, IDCs, SL, ISCs | Gap junction protein for potassium recirculation and the transport of other metabolites. | Structural - CD |
| <i>KCNE1</i> | SV | Voltage-gated potassium channel KQT-like subfamily protein. KCNE1 is expressed on the apical surface of the marginal cells in the stria vascularis and is involved in EP generation by the endolymph, particularly in its high potassium ion concentration. | SV |
| <i>LHFPL5</i> | IHCs, OHCs, PCs, SuCs, HCs, CCs, Tip of stereocilia | Tetraspan membrane protein of hair cell stereocilia and involved in the tip link complex. | HC |
| <i>LOXHD1</i> | IHCs, OHCs | Involved in the regulation of stereocilia elongation. Mutation of LOXHD1 causes “fused stereocilia” and “membrane ruffling” at the apical surface of hair cells. | HC |
| <i>MARVELD2</i> | IHCs, OHCs, SV | Tight junction protein that contributes to the structure and function of tricellular contacts between neighboring cells. | HC |
| <i>MITF</i> | | Important for the development of various types of neural crest-derived melanocytes. SOX10 and PAX3 directly regulate MITF gene expression. | Structural – CD |
| <i>MT-TL1</i> | IHCs, OHCs (Shen et al. 2015) | Mitochondrially encoded tRNA-Leu (UUA/G) 1 | Mito |
| <i>MYH14</i> | IHCs, OHCs, PCs, SuCs, HCs, CCs, ESCs, LW, SLig, SV | ATP-dependent molecular motors that interact with cytoskeletal actin. MYH14 is involved in the regulation of cytokinesis, cell motility, and cell polarity. | HC |
| <i>MYO15A</i> | IHCs, OHCs | MYO15A directly binds to WHRN to form the MYO15A-WHRN-EPS8 complex of stereocilia. This complex is essential for stereocilia elongation. | HC |

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| <i>MYO3A</i> | IHCs, OHCs | Myosin IIIA expression is restricted to the retina and IHCs and OHCs of the cochlea. MYO3A is localized in the tip density region of stereocilia and acts in the maintenance of stereocilia morphology. | HC |
| <i>MYO6</i> | IHCs, OHCs | MYO6 is expressed in the cuticular plate region of IHCs and OHCs. MYO6 is involved in stereocilia formation and may have an important role in anchoring stereocilia. | HC |
| <i>MYO7A</i> | IHCs, OHCs | MYO7A encodes a component of the USH complex (including CDH23, SANS, USH1C, and MYO7A) in the tip links of stereocilia. | HC |
| <i>OTOF</i> | IHCs, OHCs | Correlated with afferent synaptogenesis and involved in the late step of synaptic vesicle exocytosis. OTOF may act as the major Ca ²⁺ sensor for IHC ribbon synapses. | HC |
| <i>PAX3</i> | SGNs, SV | SOX10 and PAX3 strongly activate MITF gene expression, which is required for the differentiation and development of melanocytes. | Neural |
| <i>POU3F4</i> | SLig, RM | Transcription factor restrictedly expressed in the spiral ligament fibrocytes. POU3F4 may have a role in potassium ion homeostasis. | Structural – CD |
| <i>POU4F3</i> | IHCs, OHCs | POU family of transcription factors and is involved in the maintenance of inner ear hair cells. POU4F3 activates MYO7A gene expression. | HC |
| <i>PTPRQ</i> | IHCs, OHCs | Protein tyrosine phosphatase receptor protein and has an important role in shaft connector formation in hair bundles. PTPRQ is necessary for the long-term survival of high-frequency auditory hair cells. | HC |
| <i>RRM2B</i> | IHCs, OHCs, PCs, DCs (Liu et al. 2018) | RRM2B (Ribonucleotide Reductase Regulatory TP53 Inducible Subunit M2B) encodes for small subunit of p53 (191170)-inducible ribonucleotide reductase, that is essential for mitochondrial DNA synthesis. | Mito |

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| <i>SLC26A4</i> | ESCs, LW | Acts as a chloride, bicarbonate, and iodide ion transporter in the spiral prominence. Pendrin also contributes to pH homeostasis and mineralization in the organ of Corti and vestibular organs. | Structural – CD |
| <i>SMPX</i> | IHCs, OHCs, PCs, SuCs, LW | SMPX encodes small muscle proteins that may protect the hearing organs from mechanical stress. | HC |
| <i>SOX10</i> | SGNs, PCs, SuCs, HCs, CCs, ESCs, LW, SV, RM, IDCs, ISCs | PAX3 and SOX10 interact directly with the promoter of the MITF and RET genes, which encode the central melanocyte developmental transcription factors. | Neural |
| <i>TFAP2A</i> | Otic vesicle | TFAP2A is involved in the regulation of SGNs development via modulation of Fgf, Notch, and Bmp signaling (Kantarci et al. 2015). | Neural |
| <i>TMIE</i> | IHCs, OHCs, PCs, SuCs, HCs, CCs, SV, RM | TMIE is required for normal postnatal maturation of sensory hair cells in the cochlea, including the development of stereocilia bundles. | HC |
| <i>TMPRSS3</i> | IHCs, SGNs | Involved in the maturation of the epithelial amiloride-sensitive sodium channel (ENaC) and K ⁺ channel (KCNMA1). | Neural |
| <i>USH2A</i> | IHCs, OHCs | Scaffold protein and forms a complex with USH1C and VLGR1. Usherin is present in the ankle links in stereocilia. | HC |
| <i>WFS1</i> | IHCs, OHCs, SGNs, PCs, SuCs, HCs, CCs, ESCs, LW, SLig, SV, RM, IDCs, SL, ISCs | Encodes the endoplasmic reticulum protein and may act as an ER calcium channel or regulator of ER calcium channel activity. WFS1 may be involved in ER stress responses. | Neural |

Solving genes expressed in the inner ear. If possible, gene expression was localized into distinct cell types. Depending on the cell type, gene expression was classified into one of five expression clusters designated as designated as (1) Neural, (2) Hair Cell (HC), (3) structural genes in the Tectorial Membrane (Structural – TM) and the Cochlear Duct (Structural – CD), (4) Stria Vascularis (SV) and (5) Mitochondria (Mito). The classification into a particular cluster was based on the localization of gene expression, which is thought to be most influential on cochlear implant performance. *If not stated otherwise, the gene/protein expression and function is based on and taken or adapted from (Nishio et al. 2015).

BM, basilar membrane; CCs, Claudius' cells; CD, cochlear duct; DCs, Deiter's cells; EP, endocochlear potential; ER, endoplasmic reticulum; ESCs, external sulcus cells; GER, greater epithelial ridge; HCs, Hensen's cells; IDCs, interdental cells; IHCs, inner hair cells; ISCs, inner sulcus cells; LW, lateral wall; Mito, mitochondrial; OHCs, outer hair cells; PCs, pillar cells; RM, Reissner's membrane; SGNs, spiral ganglia neurons; SL, spiral limbus; SLig, spiral ligament; SuCs, supporting cells; SV, stria vascularis; TM, tectorial membrane.

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