IQGAP1 Deficiency Induces Progressive Hearing Loss With Early Onset

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Introduction:

IQ motif-containing GTPase-activating protein 1 (IQGAP1) is a scaffolding protein that is ubiquitously expressed. It has roles in many different aspects of cell physiology and interacts with numerous proteins. Recent studies have shown a direct interaction between IQGAP1 and deafness-associated proteins (Pendrin, Pejvakin). To investigate the role of IQGAP1 in hearing function, we used an *Iqgap1* knock-out (*Iqgap1*$^{-/-}$) mouse model.

Methods:

Auditory function was assessed at 1, 3, 6, 9, and 12 months in littermate *Iqgap1*$^{-/-}$, *Iqgap1*$^{+/-}$ and wild-type (WT) mice by measuring auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). IQGAP1 expression was evaluated by Western blot and immunolabeling. Cochlear hair cell loss was quantified by immunolabeling methods at 12 months of age.

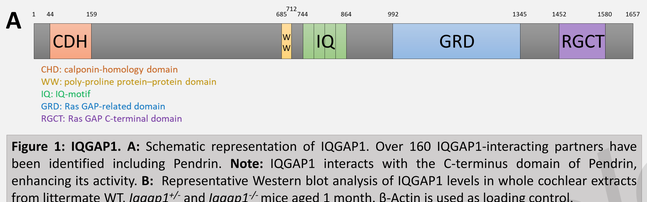

Figure 1: IQGAP1. A: Schematic representation of IQGAP1. Over 160 IQGAP1-interacting partners have been identified including Pendrin. **Note:** IQGAP1 interacts with the C-terminus domain of Pendrin, enhancing its activity. **B:** Representative Western blot analysis of IQGAP1 levels in whole cochlear extracts from littermate WT, *Iqgap1*$^{+/-}$ and *Iqgap1*$^{-/-}$ mice aged 1 month. β-Actin is used as loading control.
Note: Two bands are observed for IQGAP1 due to post-translational modifications.

Increased IQGAP1 expression in *Slc26a4*$^{-/-}$ mice

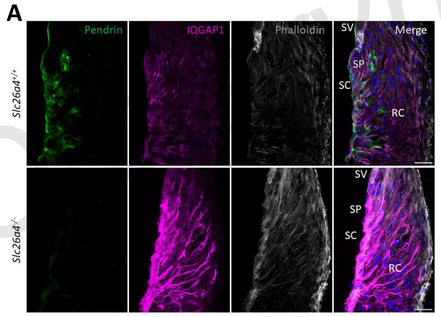

Figure 2: Increased IQGAP1 expression in *Slc26a4*$^{-/-}$ mice. **A:** Representative images of the spiral ligament area of *Slc26a4*$^{+/+}$ and *Slc26a4*$^{-/-}$ mice aged 1 month, immunolabeled for IQGAP1 (magenta), pendrin (green), phalloidin (grey) and nuclei (cyan). Scale bar: 20µm. (OS: outer sulcus, RC: root cells, SC: spindle cells, SP: spiral prominence, SV: stria vascularis). **B:** Box plot showing relative fluorescence intensity of IQGAP1 in Pendrin-expressing cells. t-test: *** p<0.0005. **Note:** In *Slc26a4* mice, IQGAP1 levels are increased in the SC and RC compared to WT mice; this increase may reflect a compensatory mechanism.

IQGAP1 expression in the inner ear

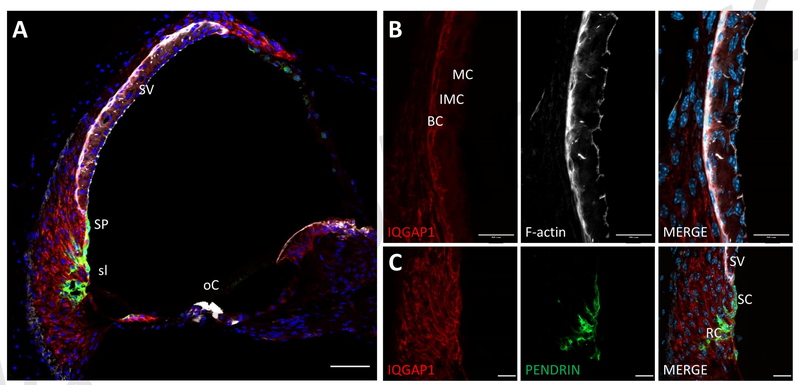

Figure 3: IQGAP1 expression in the cochlea. A: Representative confocal image of a transverse cochlear section from WT mice aged 1 month immunolabeled for IQGAP1 (Red), Pendrin (green), phalloidin (grey) and nuclei (cyan). **B,C:** High magnification of the stria vascularis (**B**) and Spiral Ligament (**C**) regions. **Note:** IQGAP1 is highly expressed in the spiral ligament cells expressing Pendrin and in the basal cells of the stria vascularis. Scale bar: 50µm in **A**, 20µm in **B,C**. (oC: Organ of Corti, sl: Spiral ligament, SP: Spiral prominence, SV: stria vascularis, RC: root cells, SC: spindle cells, BC: Basal cells, IMC: intermediate cells, MC: Marginal cells).

IQGAP1 is expressed in the Organ of Corti

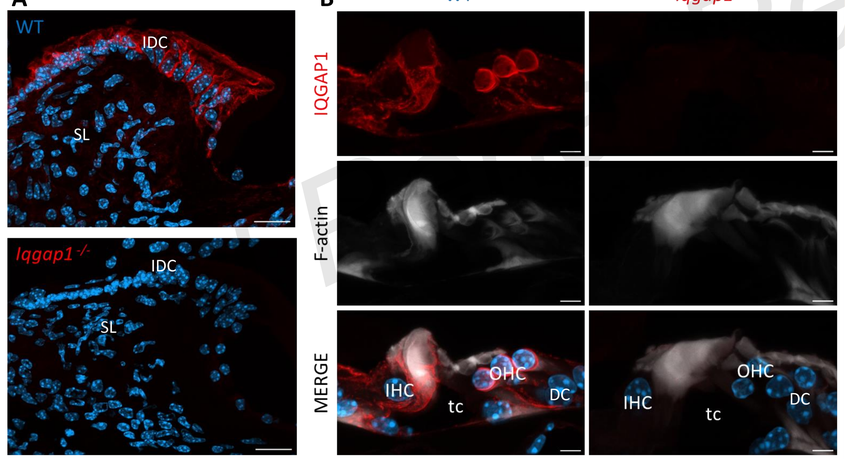

Figure 4: IQGAP1 expression in the Organ of Corti. A: Representative confocal images from a transverse section of the spiral limbus from WT and *Iqgap1*$^{-/-}$ mice aged 1 month, immunolabeled for IQGAP1 (red) and nuclei (cyan). **Note:** IQGAP1 is highly expressed in the interdental cells of the spiral limbus. **B:** High magnification of the Organ of Corti from WT (Right) and *Iqgap1*$^{-/-}$ (Left) mice aged 1 month immunolabeled for IQGAP1 (Red), F-actin (grey), and nuclei (cyan). **Note:** In the OHC, IQGAP1 is localized in the basal portion of the cell. Scale bar: 20µm in **A**, 5µm in **B**. (SL: Spiral Limbus, IDC: Interdental cells, IHC: Inner hair cell, OHC: outer hair cells, tc: tunnel of Corti, DC: Deiters cells).

IQGAP1 deficiency induces progressive hearing loss

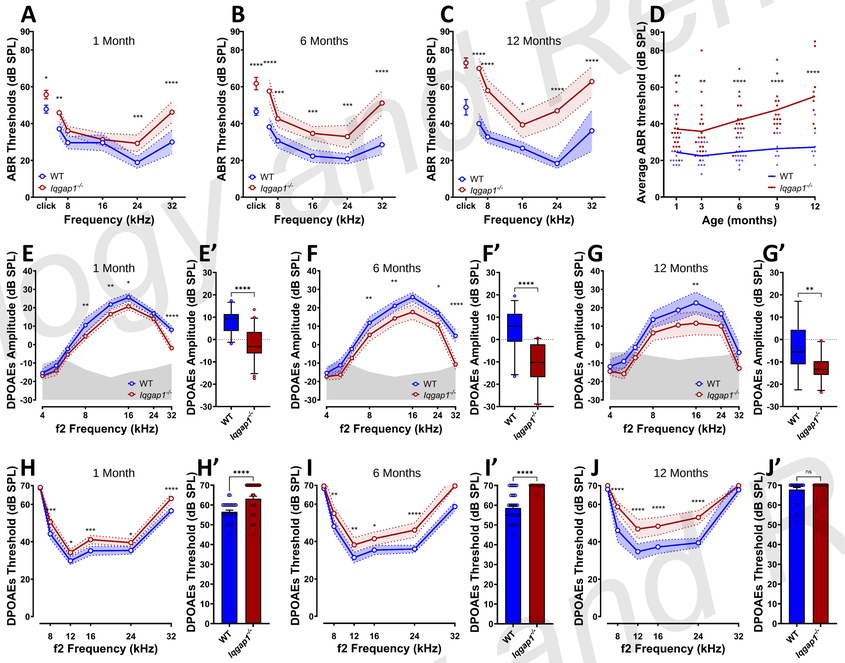

Figure 5 : IQGAP1 deficiency induces early onset progressive hearing loss. **A-C:** Auditory brainstem response (ABR) thresholds recorded in WT (blue) and *Iqgap1*$^{-/-}$ (red) mice aged 1 month (**A**), 6 months (**B**) and 12 months (**C**). **D:** Mean ABR thresholds in the higher frequencies from 1 to 12 months of age. **E-J:** DPOAE amplitude (**E-G**) and DPOAE threshold (**H-J**) recorded in WT (blue) and *Iqgap1*$^{-/-}$ (red) mice aged 1 month (**E, H**), 6 months (**F, I**) and 12 months (**G, J**). **E'-G':** Box plot showing the DPOAE amplitude at 32 kHz. **H'-J':** Histogram showing the DPOAE threshold at 32 kHz. **A-C, E-J:** data are expressed as mean ±95% CI, one-way ANOVA test followed by Dunn's test: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.005, **** P ≤ 0.0001; **H'-J'** data are expressed as mean ± SEM, t-test: **P ≤ 0.01, **** P ≤ 0.0001. **Note:** *Iqgap1*$^{-/-}$ mice show hearing loss in the high and low frequencies at 1 month of age that progresses by 6 months of age to all frequencies.

Altered auditory pathway in IQGAP1-deficient mice

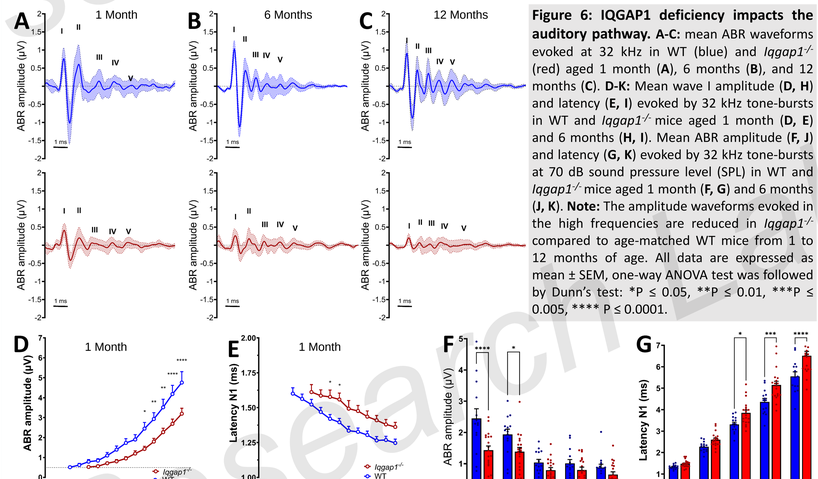

Figure 6: IQGAP1 deficiency impacts the auditory pathway. A-C: mean ABR waveforms evoked at 32 kHz in WT (blue) and *Iqgap1*$^{-/-}$ (red) aged 1 month (**A**), 6 months (**B**), and 12 months (**C**). **D-K:** Mean wave I amplitude (**D, H**) and latency (**E, I**) evoked by 32 kHz tone-bursts in WT and *Iqgap1*$^{-/-}$ mice aged 1 month (**D, E**) and 6 months (**H, I**). Mean ABR amplitude (**F, J**) and latency (**G, K**) evoked by 32 kHz tone-bursts at 70 dB sound pressure level (SPL) in WT and *Iqgap1*$^{-/-}$ mice aged 1 month (**F, G**) and 6 months (**J, K**). **Note:** The amplitude waveforms evoked in the high frequencies are reduced in *Iqgap1*$^{-/-}$ compared to age-matched WT mice from 1 to 12 months of age. All data are expressed as mean ± SEM, one-way ANOVA test was followed by Dunn's test: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.005, **** P ≤ 0.0001.

IQGAP1 deficiency induces severe OHC loss in the apex

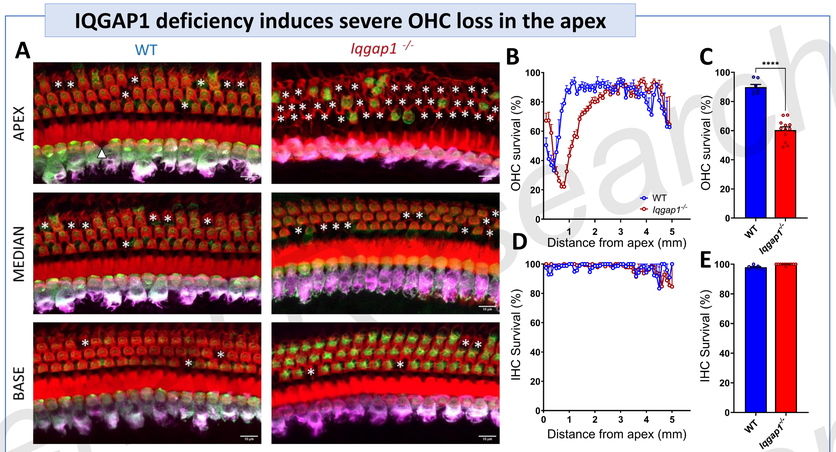

Figure 7: IQGAP1 deficiency induces severe OHC loss in the apex. A: Representative confocal images at different cochlear localizations – Apex, Median and Base – from WT (Right) and *Iqgap1*$^{-/-}$ (Left) mice aged 12 months immunolabeled with VGLUT3 (magenta), phalloidin rhodamin (red) and Myosin7α (green). Missing OHC (white asterisk), missing IHC (arrow). Scale bar = 10 µm. **B, D:** Cytocochleograms representing percentage survival of OHC (**B**) or IHC (**D**) across the tonotopic axis at 12 months of age in WT (blue), and *Iqgap1*$^{-/-}$ (red). **C, E:** Histogram showing the percentage survival of OHC (**C**) or IHC (**E**) at the apex. All data are expressed as mean ± SEM, t-test: **** P ≤ 0.0001.

Conclusion:

These results demonstrate an essential role for IQGAP1 in cochlear physiology and support its requirement for the maintenance of hearing function and OHC survival.

Acknowledgements:

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