GJB2 gene therapy-response of two pre-clinical mouse models of the most frequent form of human deafness, DFNB1

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INTRODUCTION

More than 1.5 billion people worldwide live with hearing loss (HL). Sensorineural HL affects one new-born in 700-1000 and approximately one child or young adult in 500 before the age of 20.

GJB2-related autosomal recessive non syndromic HL (also referred as DFNB1) is the most common genetic cause of congenital sensorineural HL in many world populations, frequently accounting up to half cases.

GJB2 encodes connexin-26 (Cx26) gap-junction channel protein that plays a key role in ions and metabolites homeostasis necessary to cochlear development and sensory hair cells function and survival.

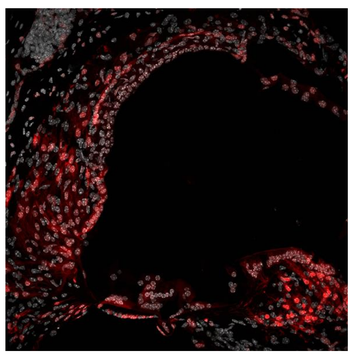
In the present study, we developed experimental mouse models for GJB2 human deafness forms and assessed our recombinant adeno-associated virus (AAV) GJB2 gene therapy (GT) approach.

METHODS

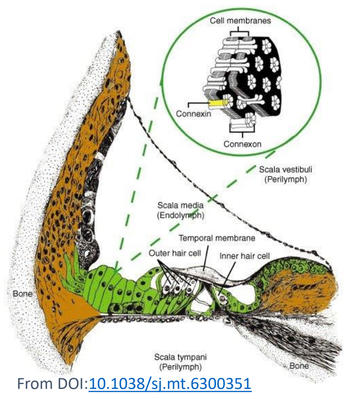
Conditional KO mouse models development :

To circumvent embryonic lethality caused by Gjb2 inactivation, conditional knockouts were generated.

The same Cre-recombinant mice which displays a large cochlear spatial expression were used in both models.



Tomato expression in Cre-recombinant mice bred with stop flox/flox tdTomato mice offspring (cochlea cryosection at 1 month)



From DOI:10.1038/sj.mt.6300351

Cx26 expression in the nonsensory epithelium and in the connective tissue cell system

We developed :

- ***Gjb2*$^{cKO2/cKO2}$ model**

resulting in a biallelic *Gjb2* inactivation mimics the most common form of DFNB1.

Gjb2$^{cKO2/cKO2}$ mice display **profound** congenital deafness (100dB at 1month) and cochlear morphological defect.

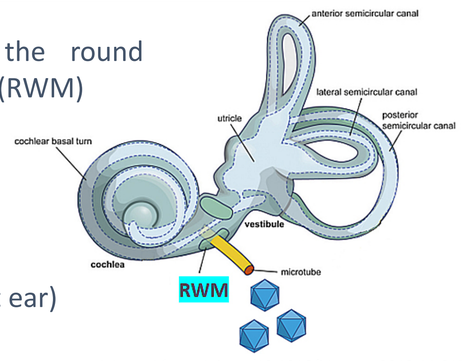
- ***Gjb2*$^{cKO2/Hmut}$ model**

compound heterozygote, expressing a human missense pathogenic variant, that corresponds to a frequent human GJB2 mutation and *Gjb2* inactivation on the other allele.

Gjb2$^{cKO2/Hmut}$ mice display **severe** congenital deafness (80-90dB at 1 month, increasing over time) and altered cochlear structure

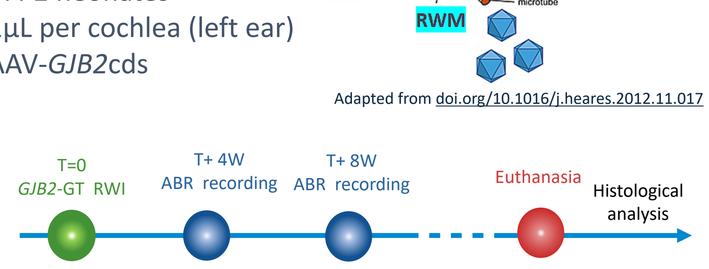
Inner ear surgical approach :

Injection through the round window membrane (RWM)



In P2 neonates
1µL per cochlea (left ear)
AAV-*GJB2*cds

Adapted from doi.org/10.1016/j.heares.2012.11.017



CONCLUSION

P2 inner ear delivery of *GJB2*-GT improves audition likely by preserving cochlear structure and by impeding sensory hair cells and supporting cells loss in both preclinical models of human pathogenic GJB2 variants.

RESULTS

1. GJB2-GT improves audiologic performance in both *Gjb2*$^{cKO2/cKO2}$ and *Gjb2*$^{cKO2/Hmut}$ mice

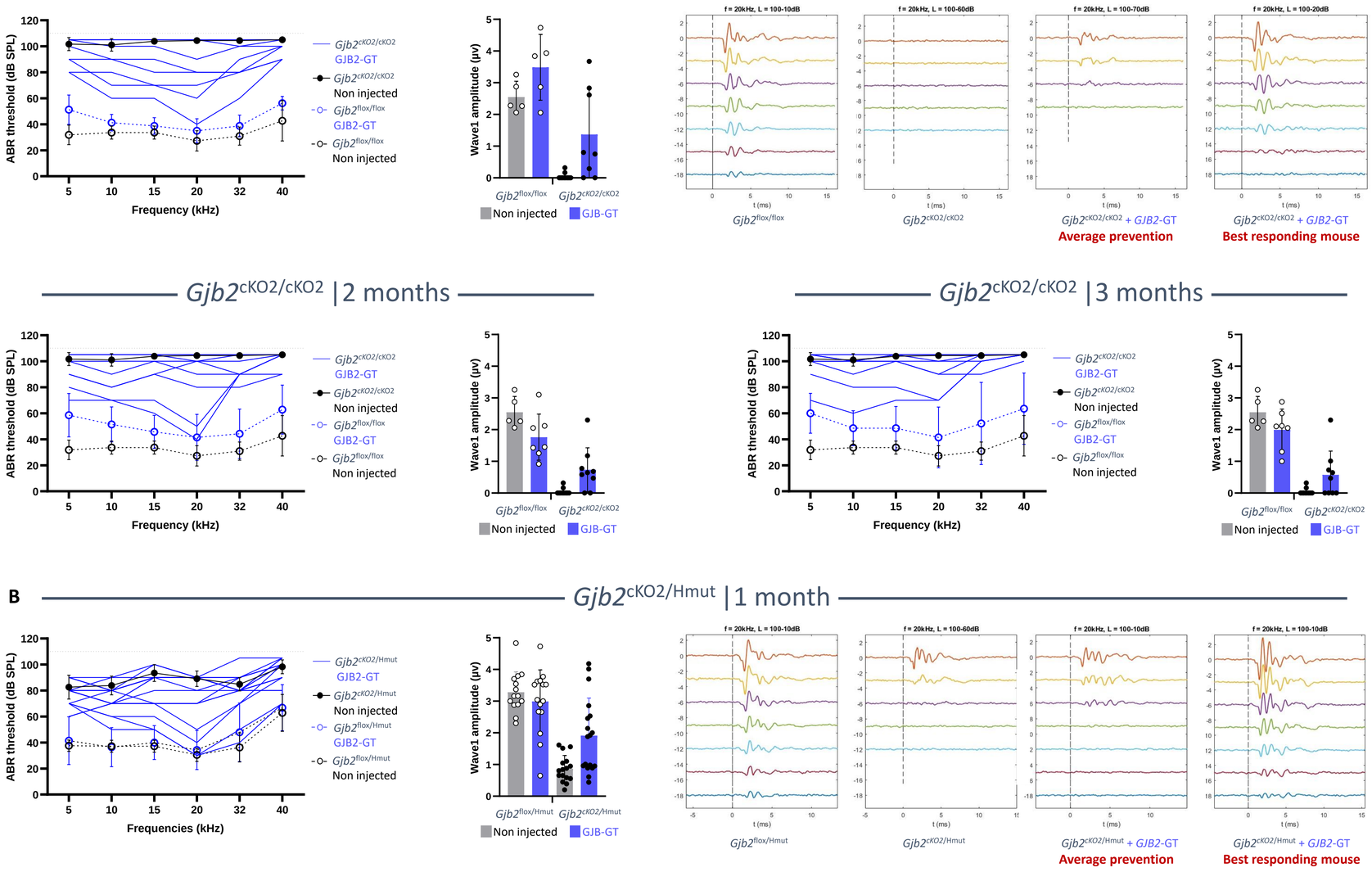


Figure 1: Auditory brainstem response assessments were performed monthly on *Gjb2*$^{flox/flox}$ and *Gjb2*$^{cKO2/cKO2}$ (**A**) or *Gjb2*$^{cKO2/Hmut}$ (**B**) mice (black curves and grey histograms) and on P2 GJB2-GT *Gjb2*$^{flox/flox}$ and *Gjb2*$^{cKO2/cKO2}$ or *Gjb2*$^{cKO2/Hmut}$ injected mice (blue curves and histograms). Curves represent ABR thresholds mean ± SD; non-injected *Gjb2*$^{flox/flox}$ mice (black dashed curve, open black circle); non-injected *Gjb2*$^{cKO2/cKO2}$ mice (black curve, closed black circle); GJB2-GT *Gjb2*$^{flox/flox}$ injected mice (blue dashed curve, open blue circle). Blue curves with no symbols represent ABR thresholds for each GJB2-GT *Gjb2*$^{cKO2/cKO2}$ injected mice. 110dB SPL dotted line indicates ABR measure limit. Histograms represent wave 1 amplitudes at 15kHz mean ± SD. Symbols represent each mouse (*Gjb2*$^{flox/flox}$ open circles; *Gjb2*$^{cKO2/cKO2}$ or *Gjb2*$^{cKO2/Hmut}$ mice: closed circles). Representative examples of ABR traces at 20kHz at 1 month.

➞ ABR thresholds and wave 1 amplitude improvement in both profound hearing-impaired *Gjb2*$^{cKO2/cKO2}$ and severe hearing-impaired *Gjb2*$^{cKO2/Hmut}$

50% efficiency 1 month post surgery, best responding mice display full wave 1 amplitude restoration

➞ Therapeutic effect is preserved 2 months post surgery, up to 3 months for best responding mice

2. GJB2-GT preserves cochlear development in both *Gjb2*$^{cKO2/cKO2}$ and *Gjb2*$^{cKO2/Hmut}$ mice

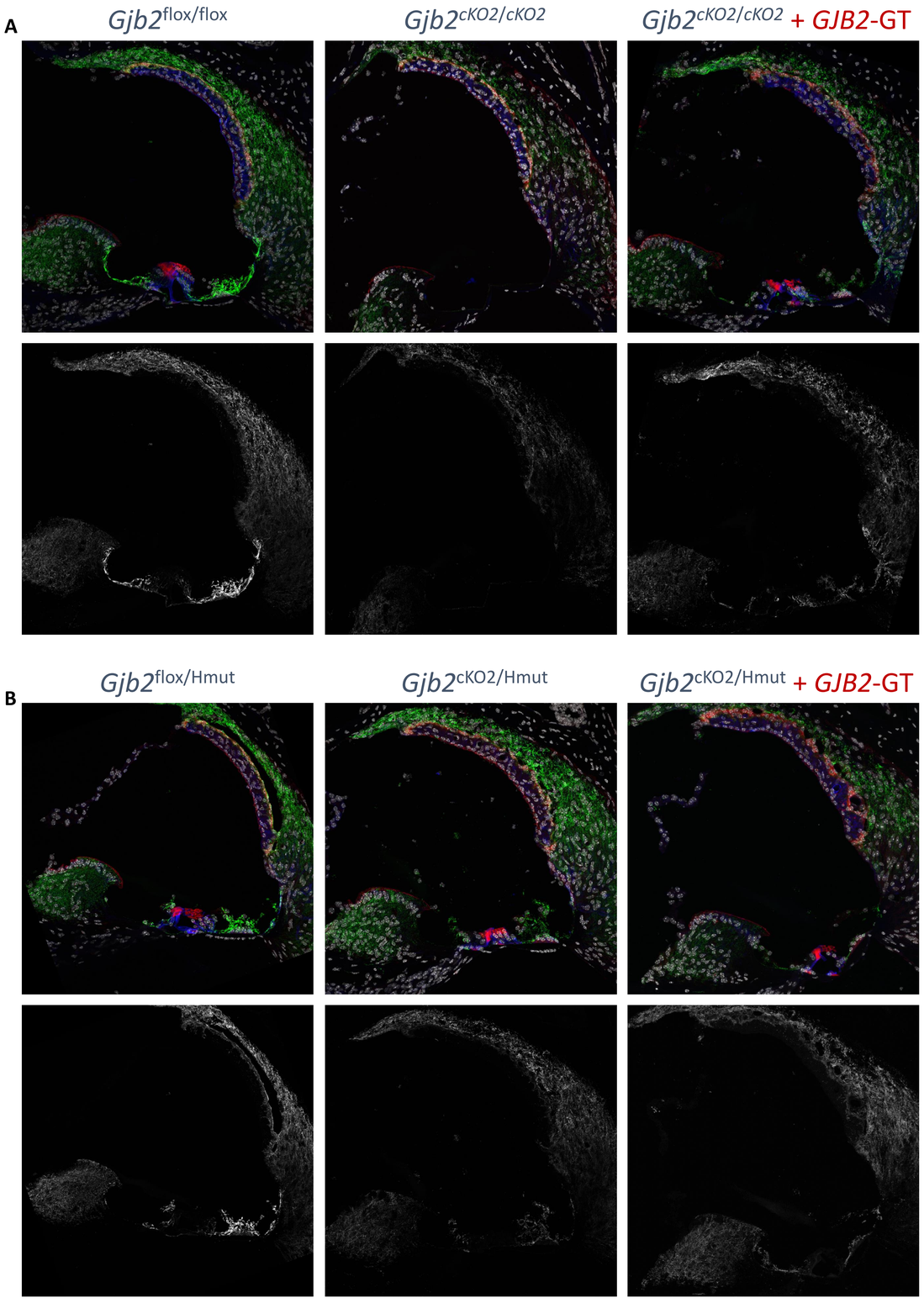


Figure 2: **A.** Immunostaning were performed on cryosections of Gjb2$^{flox/flox}$ and Gjb2$^{cKO2/cKO2}$ at 1 month and 3 months for *Gjb2*$^{cKO2/cKO2}$ *GJB2*-GT mice.

Upper panel : Dapi in white, Phalloidin in red, Tubulin in blue and Cx26 in green. Lower panel : Cx26 in white

In *Gjb2*$^{cKO2/cKO2}$ mice:

- **Strong Cx26 expression decrease in type 1 and 2 fibrocytes**
- **Complete loss of the neurosensory epithelium**

In GJB2-GT Gjb2$^{cKO2/cKO2}$ responding mice:

➞ The neurosensory epithelium loss is prevented

➞ Expression of Gjb2 in all Cx26 positive cells is detected

➞ The organ of Corti morphology is comparable to that of Gjb2$^{flox/flox}$ mice

B. Immunostaning were performed on cryosections of Gjb2$^{flox/Hmut}$ and Gjb2$^{cKO2/Hmut}$ mice at 1 month and at 6 months for *Gjb2*$^{cKO2/Hmut}$ *GJB2*-GT injected mice. Upper panel : Dapi in white, Phalloidin in red, Tubulin in blue and Cx26 in green. Lower panel : Cx26 in white

In *Gjb2*$^{cKO2/Hmut}$ mice:

- **Organ of Corti is collapsed**
- **Cx26 expression is reduced in all Cx26 positive cells**

In GJB2-GT Gjb2$^{cKO2/Hmut}$ responding mice:

➞ Organ of Corti morphology is preserved

➞ Expression of Gjb2 in all Cx26 positive cells is detected, higher than in non injected Gjb2$^{cKO2/Hmut}$ mice

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