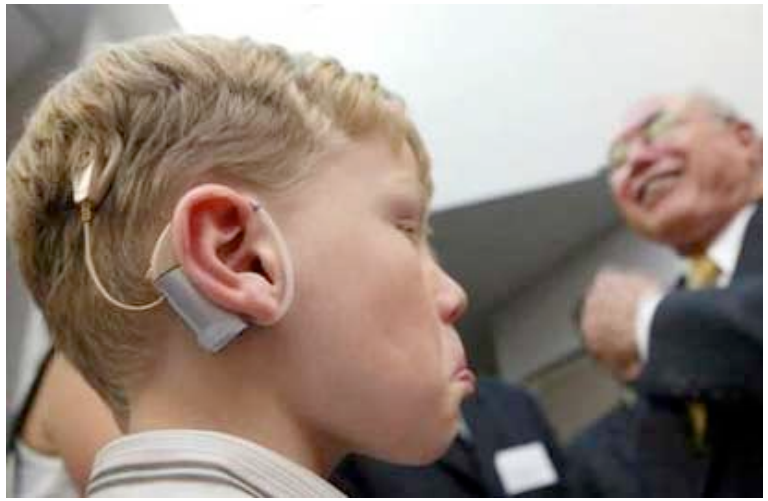
Nonsyndromic Deafness

A genetic silence.

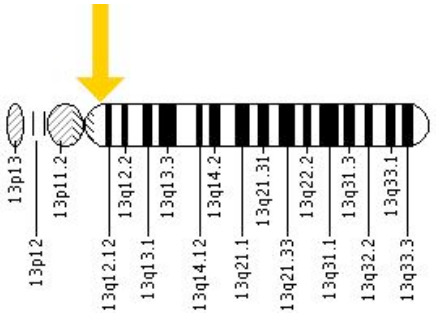
Introduction/ Symptoms

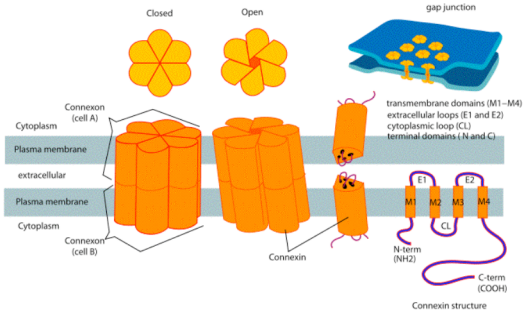
- **Nonsyndromic deafness is defined as: *"hearing loss that is not associated with other signs and symptoms"*.**
- **Thus the only Symptom is hearing loss.**
- **Deafness can manifest at any age; from being congenital -- present at birth--to developing late into old age.**
- **Deafness can be bilateral or unilateral; affecting both ears or one ear, respectively.**
- **Hearing loss ranges from mild to profound (complete or almost complete deafness).**
- **The condition can progress with age or may remain stable.**
- **Deafness may be more or less focused on specific pitches (frequencies) of sound.**



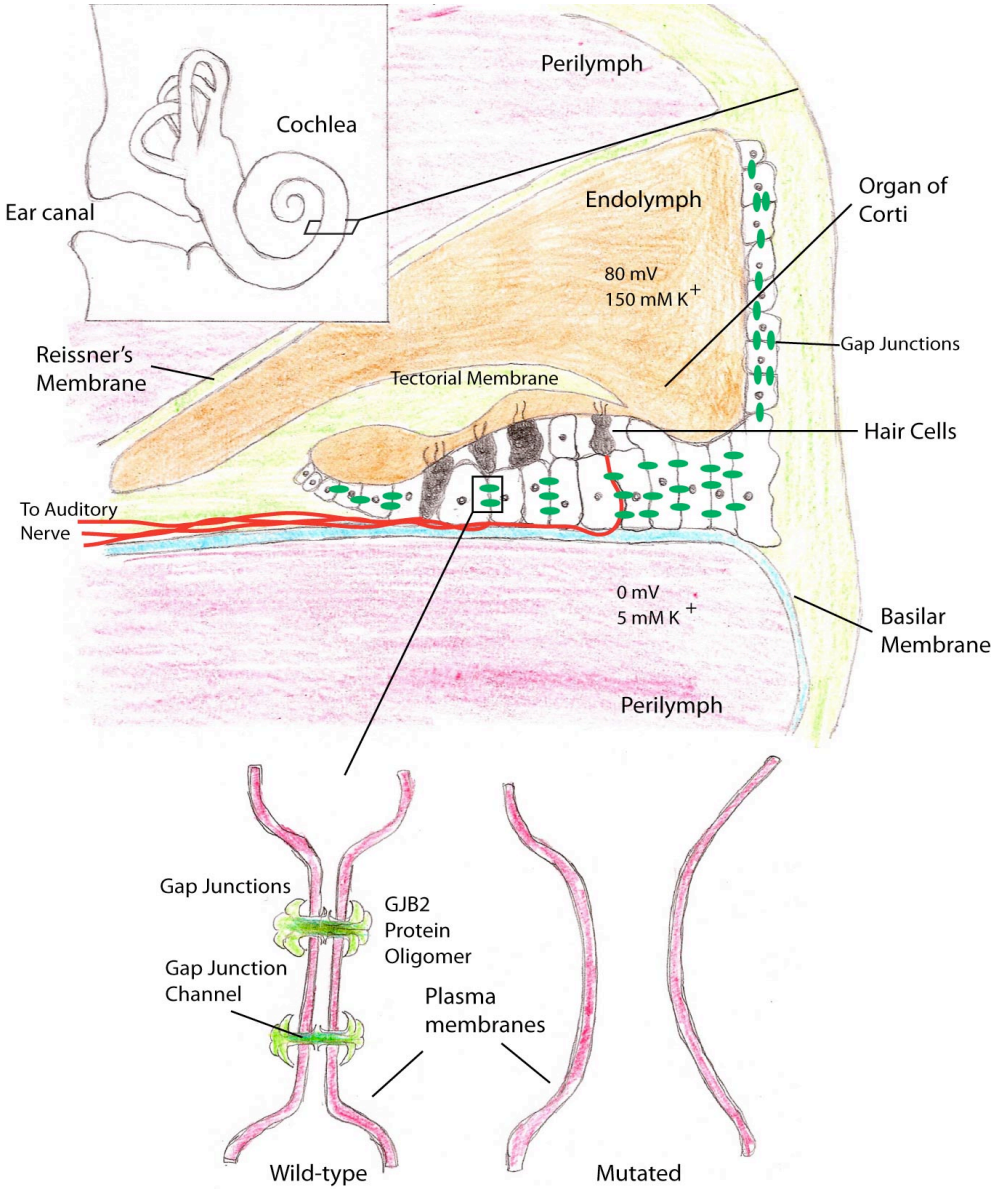
Genetics

- Hundreds of defects on more than 30 genes, across different chromosomes, have been identified by researches for causing Nonsyndromic deafness.
- Due to this diversity of genotypes, Nonsyndromic deafness can have one of many inheritance patterns: 75-80% autosomal recessive (DFNB), 20-25% autosomal dominant (DFNA), 1-2% X-Linked (DFNX), <1% mitochondrial.
- **GJB2/ Connexin 26** defects are the focus of my presentation, GJB2 associated defects are mostly, though not exclusively, autosomal recessive (can be dominant).
- The Gap Junction Beta-2 gene is located on the long (q) arm of chromosome 13, between positions 11 and 12: base pair 20,761,601 to base pair 20,767,113 (5512 bp long).
- The wild type GJB2 proteins form Gap Junction Channels, connecting cells. The junctions allow for the transfer and flow of ions, amino acids, and signaling molecules between cells.
- 6 GJB2 proteins join to form a transmembrane hemichannel--from inside the cell to outside--this hemichannel binds to an identical hemi channel of another cell, forming the Gap Junction Channel between two cells.
- Several mutations on GJB2, deletions, substitutions or point mutations, can render the protein use less, either the hemichannel does not form properly, it is unable dock into the membrane, or it is otherwise defective.
- In order for hearing to occur, there has to be a certain concentration of nutrients in the endolymph--a fluid that fills the cochlea--if the GJB2 channels are defective, the endolyph does not have its proper nutrient content, and cells responsible for hearing are unable to function and they eventually may die.



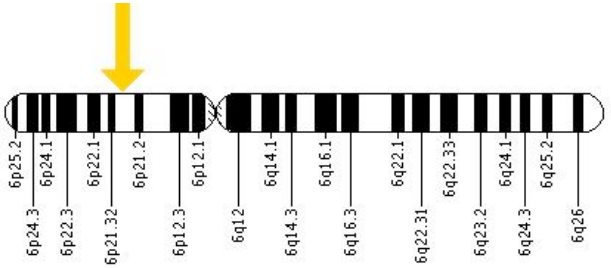


Location of Defective GBJ2

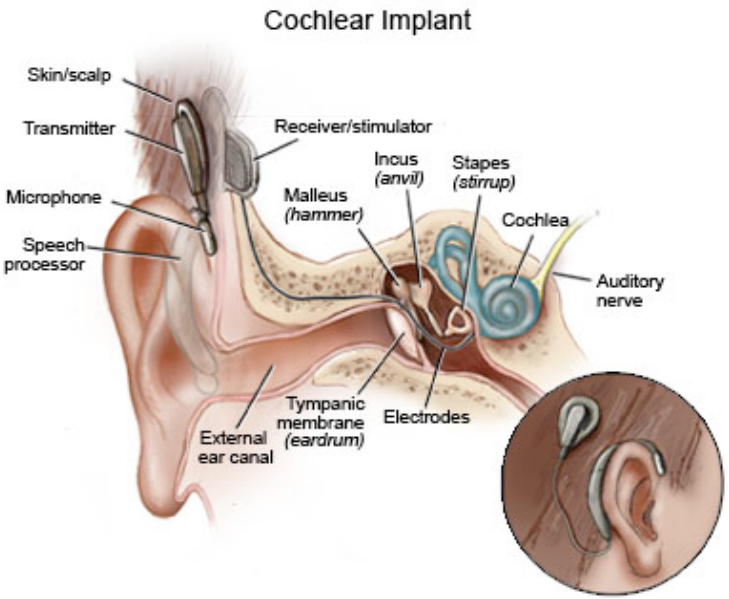


Genetics (continued)

- Another defect that can cause nonsyndromic deafness is COL11A2
- It is located on chromosome 6, at position 21.3
- The gene codes for Type XI Collagen. Collagen XI is a connective tissue found in the muscles, joints and skin.
- It part of the endolymph, and has other functions in the inner ear.
- Defects in COL11A2 cause defective collagen fibrils to form, inhibiting the proteins normal function.
- Two families so far have been identified as having nonsyndromic deafness related to point mutations on the gene.
- The defect is inherited with the autosomal dominant inheritance pattern.



Current Therapy



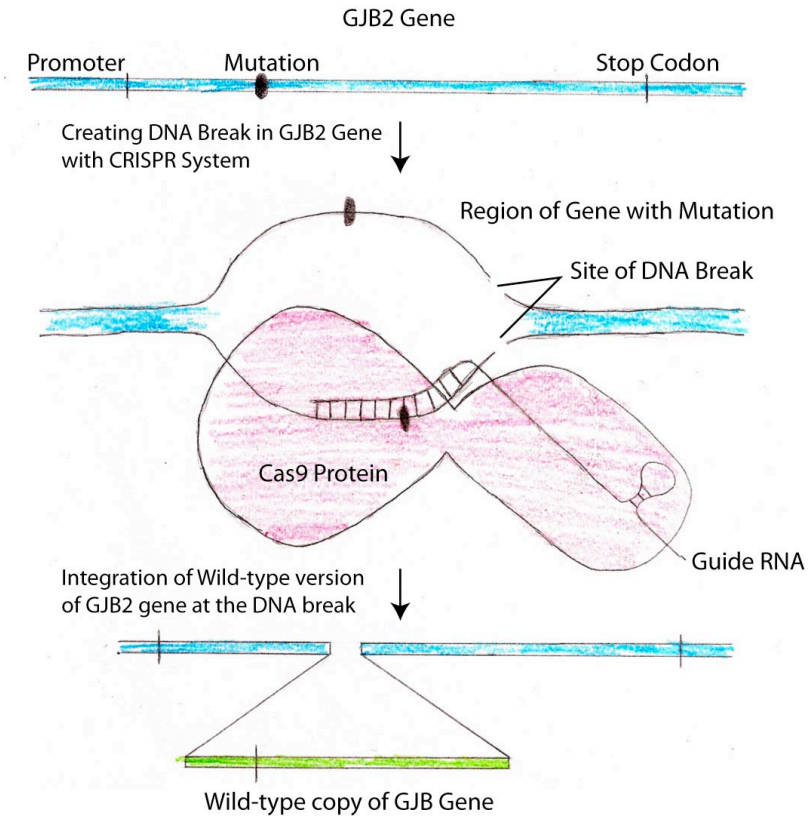
- There is no current cure for nonsyndromic deafness.
- In the case of unilateral, or mild cases hearing aids can be used to amplify existing hearing.
- In more profound cases Cochlear Implants are used.
- The implants receive sound from outside the ear, convert this sound into electrical impulses, and transmit these impulses through wire directly to the auditory nerve; thus bypassing defective tissues.
- Limitations: implants provide users with some auditory perception, but the quality of it is far worse than normally heard sound.
- Wearers can only follow conversations in quiet situations, with one person speaking close to the receiver at a given time.
- There are many users who apparently do not benefit significantly from the implants.
- Listening to music or hearing in wet situations is impossible with the implants at the moment.

Current Research

- Reasearchers at the Istituto Veneto di Medicina Molecolare, Padova, Italy have done preliminary research to correct the GJB2 gene in mice.
- They first deleted the natural gene using a gene knockout system, producing deaf mice.
- Then they expressed the wild-type version of GJB2 using a bovine adenovirus vector in cells derived from the cochlea of the deaf mice.
- These cells showed restoration of the GJB2 gap junctions.
- In the future the scientists hope to test if this technique will be able to restore ear function.
- LIMITATIONS: This concept has not yet been tested in live animals.
- The gene therapy system is primitive; rather than correcting a defective gene the researchers simply added the wild-type variant.

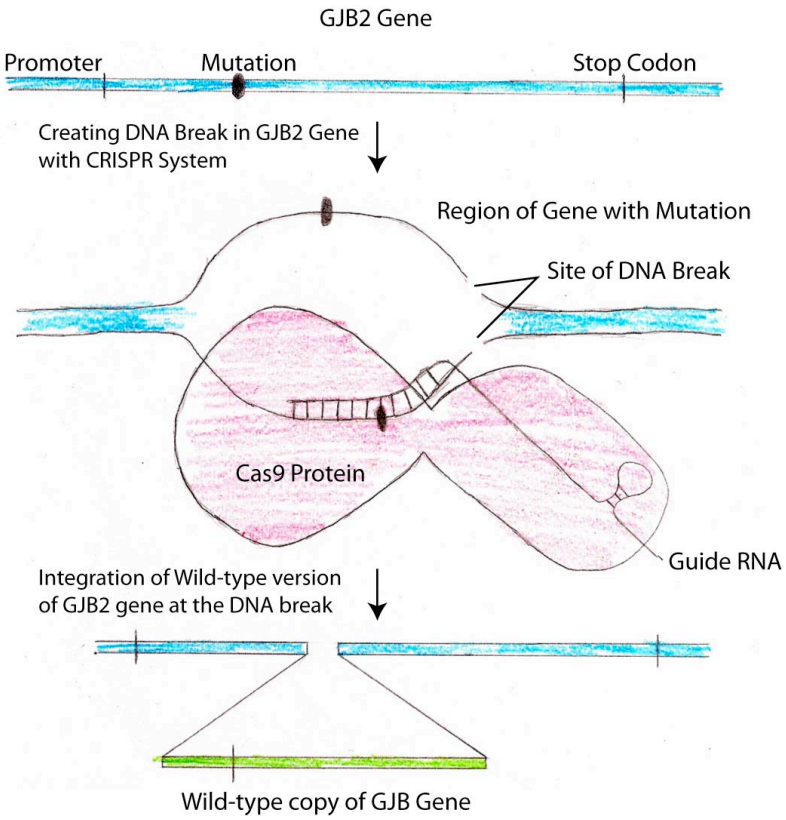
Proposed Cure

- **My approach is to replace the defective gene with the correct wild-type version.**
- **I will simultaneously knockout the defective gene, and introduce the wild-type.**
- **I will use the recently developed CRISPR (Clustered Regularly Spaced Interspersed Repeats) gene editing system.**
- **Bacteria have evolved an adaptation in which they store bits of bacteriophage DNA from viruses that they been infected by in the past.**
- **These bits are stored among palidromic repeats and are used to inactivate complimentary DNA of new infections.**
- **I will use this a protein called Cas 9 (CRSPR Associated 9) derived from this system, along with a guide RNA complimentary to the defective sequence.**



Proposed Cure (continued)

- The guide RNA directs the complex to the defective gene.
- Cas9 is an endonuclease, it will continuously cut the DNA at the target location inactivating it.
- We will simultaneously introduce the wild-type gene.
- The DNA repair path way in the cell constantly repairs DNA breaks by ligation.
- Sometimes it will insert the wild-type DNA at the location of the cleavage.
- Since the wild-type DNA is not the target of the Cas 9-Guide RNA complex, the repair becomes permanent.
- All three components of the cure: DNA encoding Cas 9, Guide RNA and the wild-type sequence, will be introduced simultaneously using retroviral vectors targeted to surface proteins of cochlear cells.
- Retroviral vectors can be engineered to be benign reducing risks of infection.



Limitations of Proposal

- This approach is completely theoretical and success is not at all certain.
- It is unclear at what stage of development the cure should be administered.
- Cohclear cells may have suffered irreversible damage even after gap junctions are restored.
- The approach will have to be tailored for each of hundreds of mutations.

Citations

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