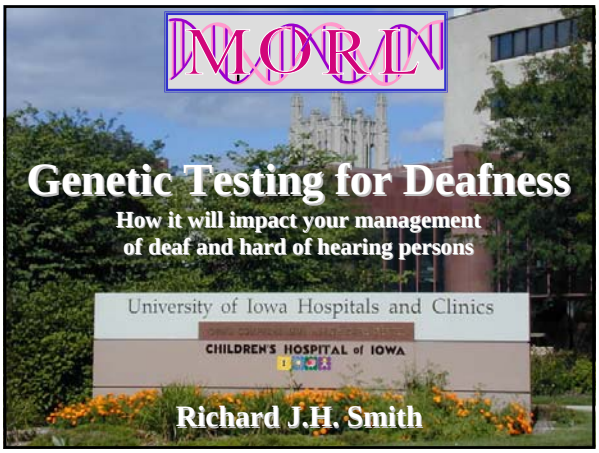
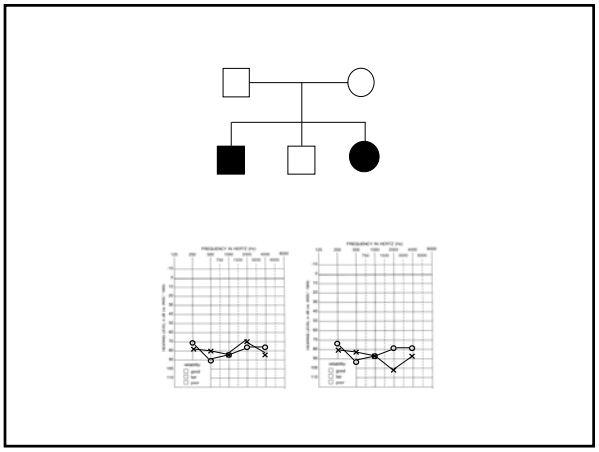


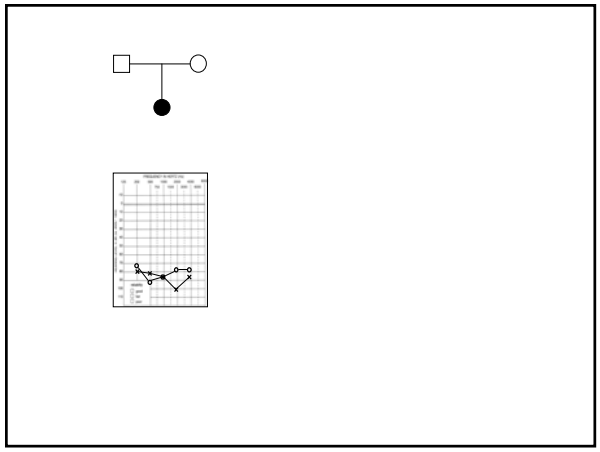
Genetic Testing for Deafness

How it will impact your management of deaf and hard of hearing persons

Today's goals are to review

- Where we are….
  - The importance of genetic testing for deafness
- Where we are going….
  - Initiatives to improve genetic testing
  - Initiatives to improve gene discovery
- Why we should get there….
  - Genetic testing and habilitation options for hearing loss



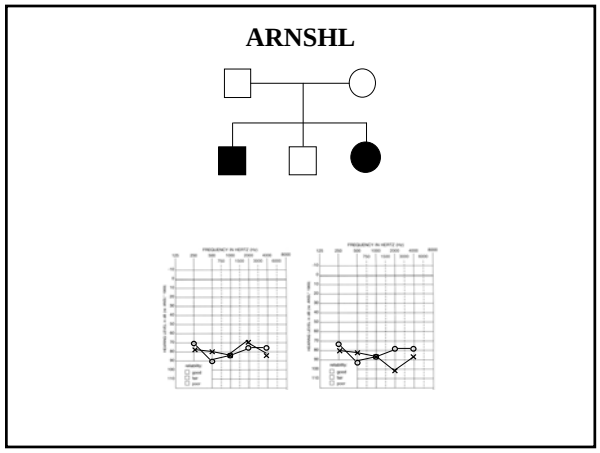


What do families want to know?

- What caused the hearing loss?
- Will the hearing loss progress?
- Is there anything else going on?
- How will my child do with hearing aids? With a CI?
- If I have other children, will they have hearing loss?
- When my child has children, will they have hearing loss?

Genetic testing can help you answer these questions.

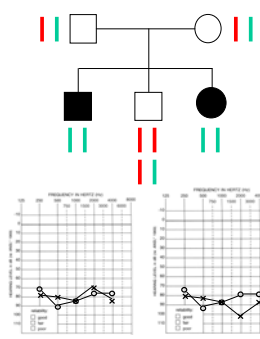
ARNSHL



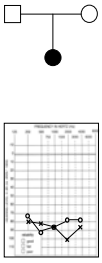
ARNSHL

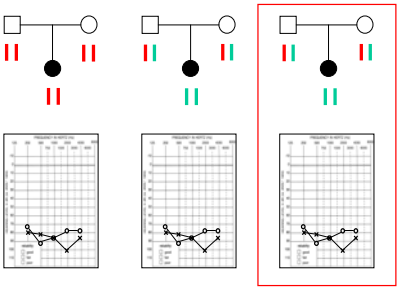
= normal copy of gene

= abnormal copy of gene



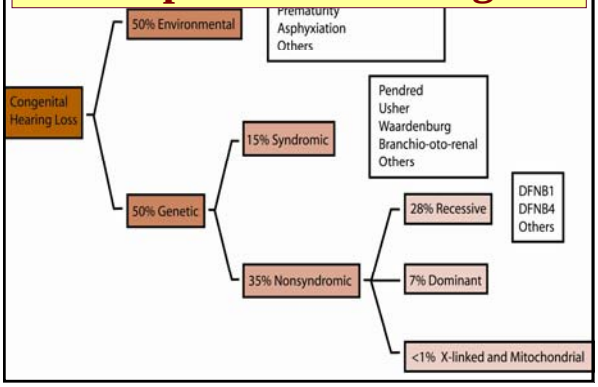
In most developed countries, mutations in *GJB2* are found in over half of cases.

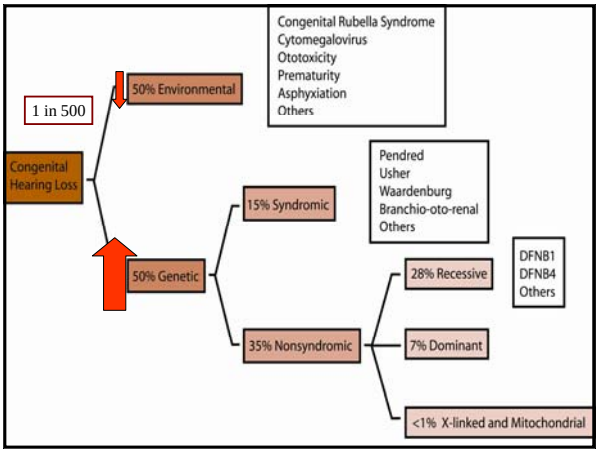




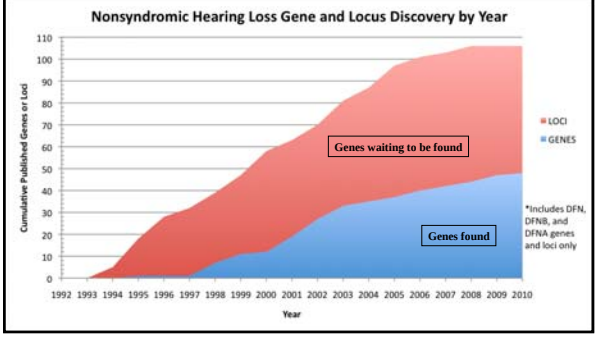
In one-third of families like this, mutations will be found in *GJB2*.
Green et al *JAMA* 281:2211-16, 1999

The scope of the challenge ….





Non-Syndromic Deafness
- extremely heterogeneous -



Where we are ….

Genes	USA Labs	Others
★ *GJB2* (DFNB1, DFNA3)	27	26
GJB6 (DFNB1)	7	4
SLC26A4 (DFNB4, PDS)	9	3
EYA1 (BOR)	1	2
TECTA (DFNB21, DFNA8/12)	1	0
OTOF (DFNB9)	3	2
KCNQ4 (DFNA2)	1	1
POU3F4 (DFN3)	2	2
WFS1 (DFNA6/14/38; DIDMOAD)	1	4
MTRNR1 (A1555G)	13	5
MTTS1 (A7445G)	5	3
MTTL1 (A3243G)	16	15

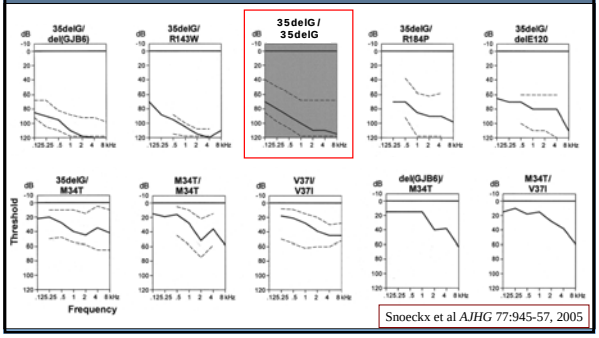
GJB2 - related deafness

- we know the phenotype -

- Prelingual deafness
- Non-syndromic
- Mild-to-profound hearing loss
- Generally not progressive (exception V37I)
- No vestibular abnormalities
- No radiographic abnormalities
- No sex bias
- In the USA, diagnosed in 1/3 of CI patients with congenital severe-to-profound deafness

GJB2 - related deafness

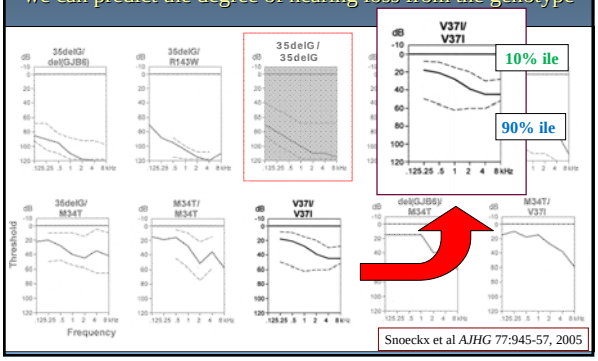
- we can predict the degree of hearing loss from the genotype -



Snoeckx et al *AJHG* 77:945-57, 2005

GJB2 - related deafness

- we can predict the degree of hearing loss from the genotype -



Snoeckx et al *AJHG* 77:945-57, 2005

GJB2 - related deafness

- we can offer meaningful genetic counseling -

Example: A hearing couple have a deaf child, want to know why and want to know the chance of having another deaf child.

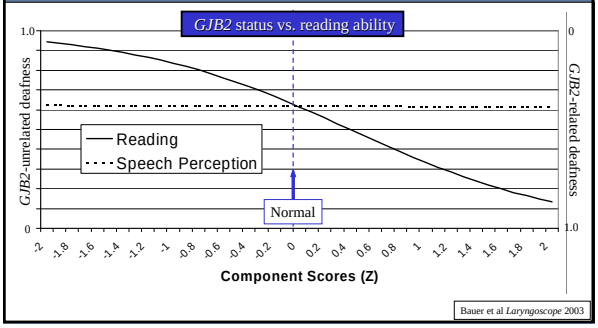
GJB2 genotypes:	deaf child	35delG/35delG
	mother	35delG/normal
	father	35delG/normal

Recurrence chance: 25%

THEORETICAL ALTERNATIVE RESULTS: If genetic testing is NOT offered the recurrence chance is 17%.

GJB2 - related deafness

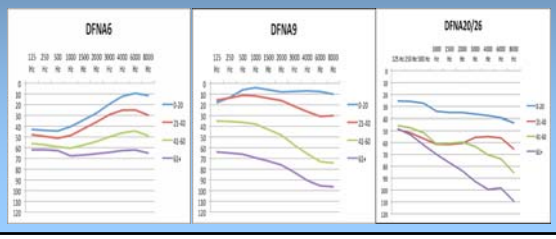
- we can predict CI performance -



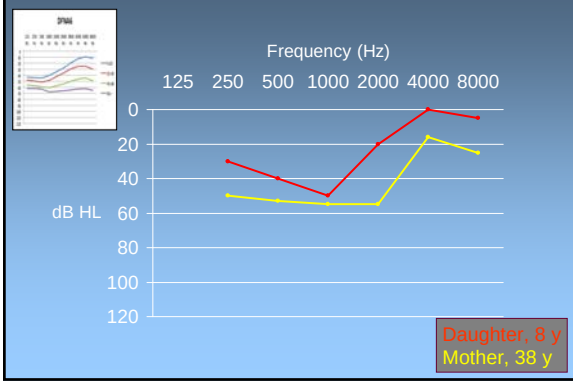
Bauer et al *Laryngoscope* 2003

ADNSHL

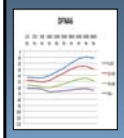
- **No single gene** accounts for the majority of ADNSHL
- Audioprofiling
  - Clinical value – provide prognostic information to patient
  - Research value – prioritize genetic testing



DFNA6 AUDIOPROFILING



DFNA6 AUDIOPROFILING

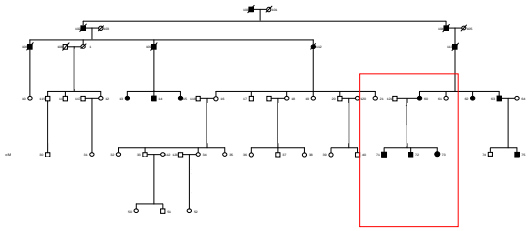


Family history + | **Family history -**

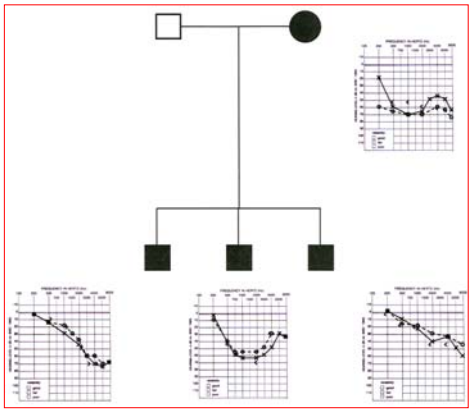
***WFS1* mutation screening**

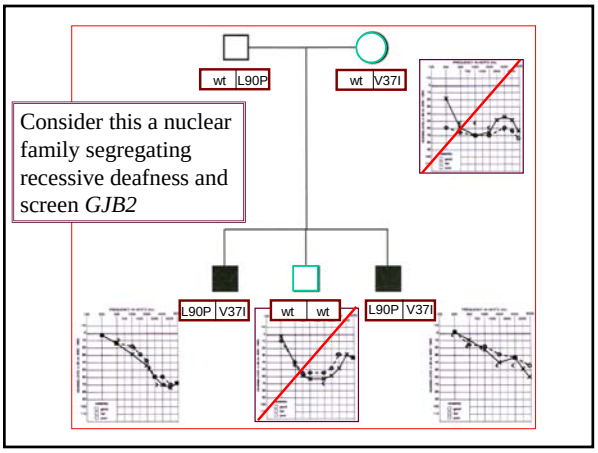
77% of families | **9% of sporadic cases**

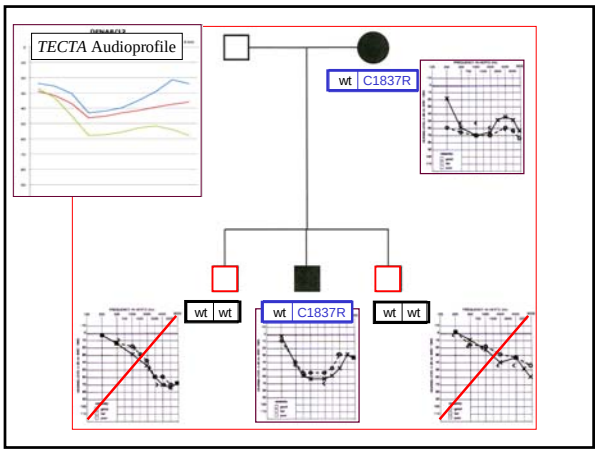
A Large Pedigree Segregating ADNSHL



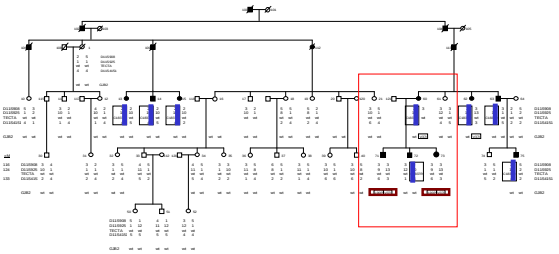
Unable to localize the genetic cause……







A Large Pedigree Segregating ADNSHL



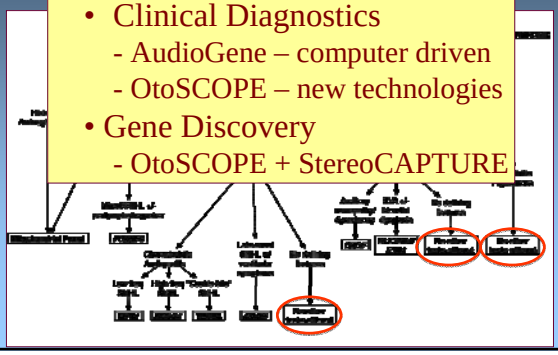
… and ARNSHL

Meyer et al *Clin Genet* 2007

DIAGNOSTICS

Where we are going ….

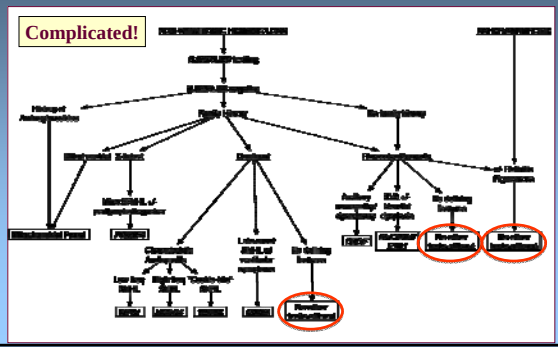
- Clinical Diagnostics
  - AudioGene – computer driven
  - OtoSCOPE – new technologies
- Gene Discovery
  - OtoSCOPE + StereoCAPTURE



DIAGNOSTICS

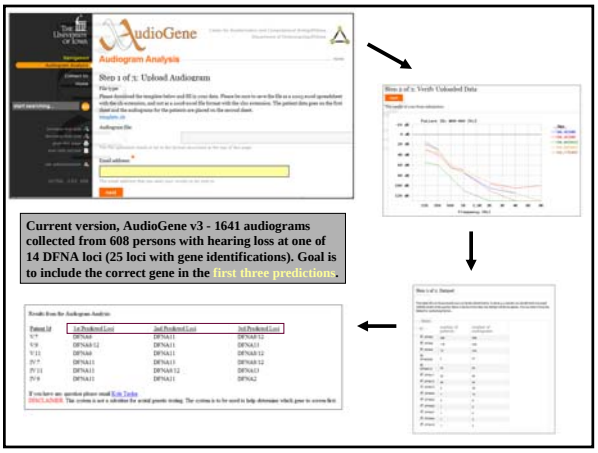
Current Algorithm

Complicated!



http://audiogene.eng.uiowa.edu/

Hildebrand et al *Laryngoscope* 2009



Current version, AudioGene v3 - 1641 audiograms collected from 608 persons with hearing loss at one of 14 DFNA loci (25 loci with gene identifications). Goal is to include the correct gene in the first three predictions.

OtoSCOPE

OtoSCOPE™

Otologic Sequence Capture of Pathogenic Exons

OtoSCOPE

(Otologic Sequence Capture of Pathogenic Exons)

- 57 genes: non-syndromic genes, microRNAs, mitochondrial mutations, Ushers

OtoSCOPE

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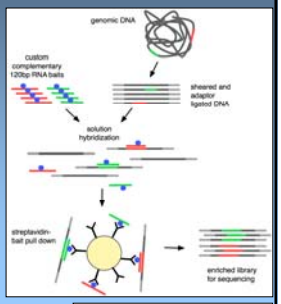
- Agilent solution-based capture and enrichment of all exons

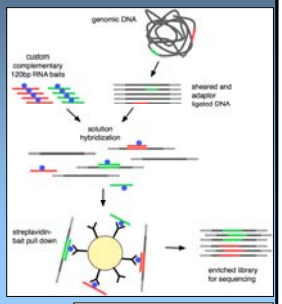


Adapted from Metzker *Nat Rev Gen* 11:31-46, 2010

OtoSCOPE

(Otologic Sequence Capture of Pathogenic Exons)

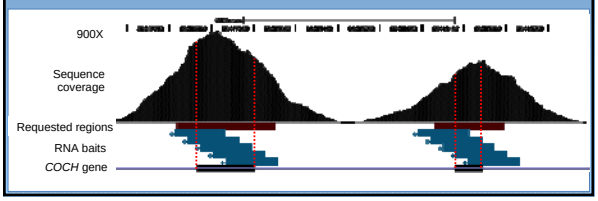
- 57 genes: non-syndromic genes, microRNAs, mitochondrial mutations, Ushers
- Agilent solution-based capture and enrichment of all exons
- NEXT GEN sequencing



Adapted from Metzker *Nat Rev Gen* 11:31-46, 2010

Sequencing Results

Sequence Output	32 million mappable reads per sample 2.2 billion mappable bases
Coverage Depth	>700X depth of coverage per base
Target Enrichment	>1,500X enrichment
% Targeted coding sequence coverage	97.8%

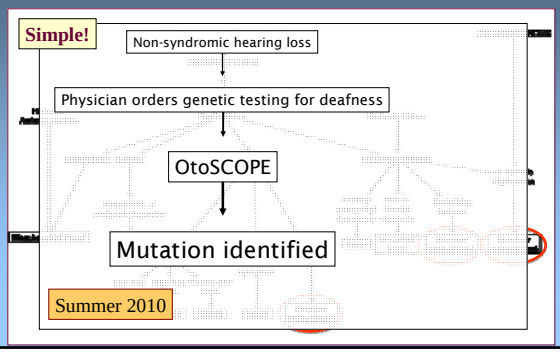


Subjects - Results

Sample	Status	Inheritance	Result
A1	Positive Control - *COCH*	dominant	Mutation in *COCH*
A2	Positive Control – *GJB2*	recessive	Mutation in *GJB2*
A3	Biological replicate of #2	recessive	Mutation in *GJB2* – High reproducibility
A4	Negative Control	-	No significant variations found
A5	Unknown	recessive	Mutation in *STRC* (only 4th identified)
A6	Unknown	recessive	2 novel candidate mutations
A7	Unknown	dominant	4 novel candidate mutations
A8	Unknown	dominant	Mutation in *KCNQ4*

- Genotyped 424 SNPs in 8 samples (average of 53 per sample) using Sanger sequencing and compared results
  - 0 False Positives; > 99.9% Specificity
  - 1 False Negative; > 99.6% Sensitivity
- OtoSCOPE results compare favorably with Sanger sequencing

DIAGNOSTICS
Future Algorithm



Why we should get there ....

- Novel therapies for deafness
  - Genotype-specific therapies
    - Dependent on the specific (personal) type of hearing loss
    - Mechanism-specific therapies
      - Modify gene expression
        - » **RNAi**
        - » Medical therapy

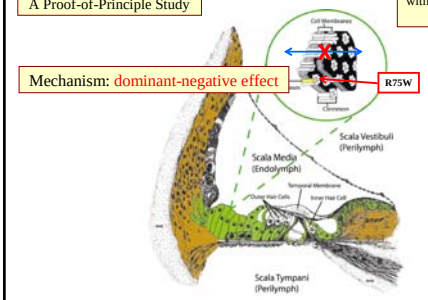
Prevention of hearing loss vs. correction of hearing loss

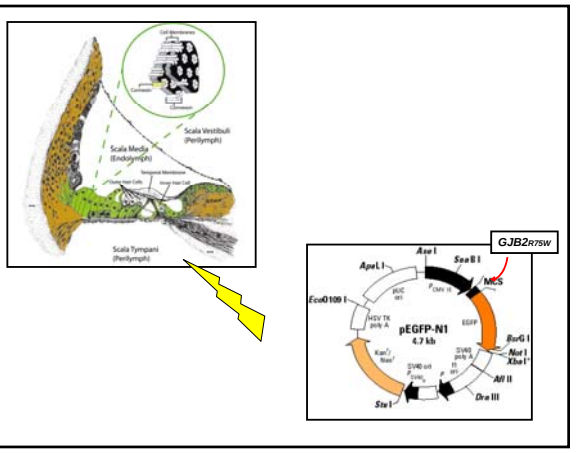
RNAi

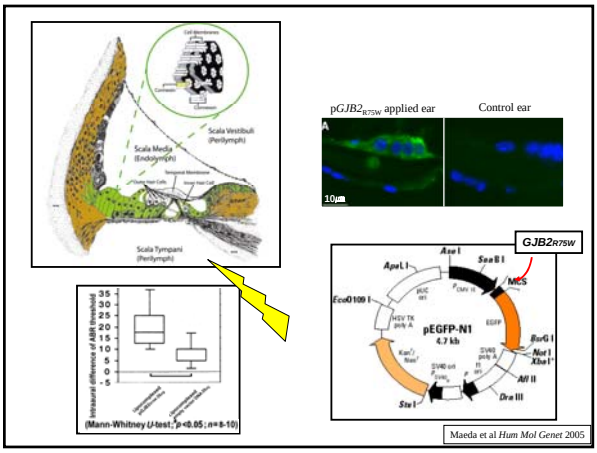
DFNA3 – *GJB2*
A Proof-of-Principle Study

Specific mutations that interfere with function of the remaining normal copy of *GJB2*

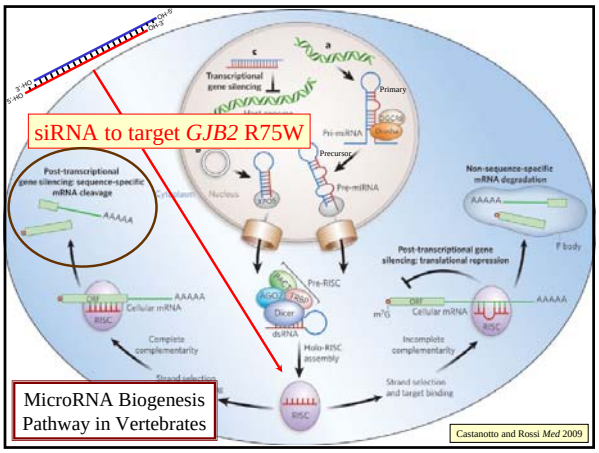
Mechanism: dominant-negative effect

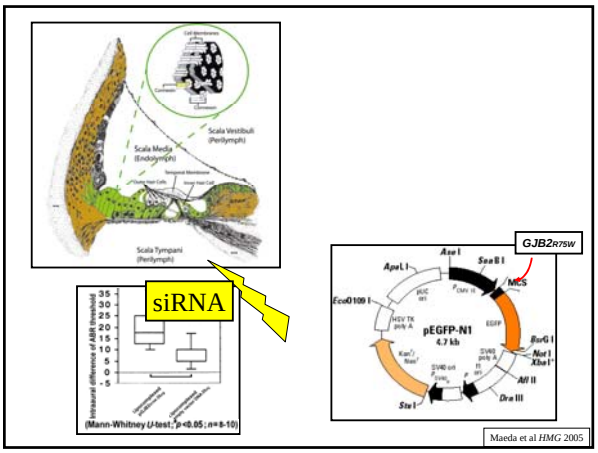


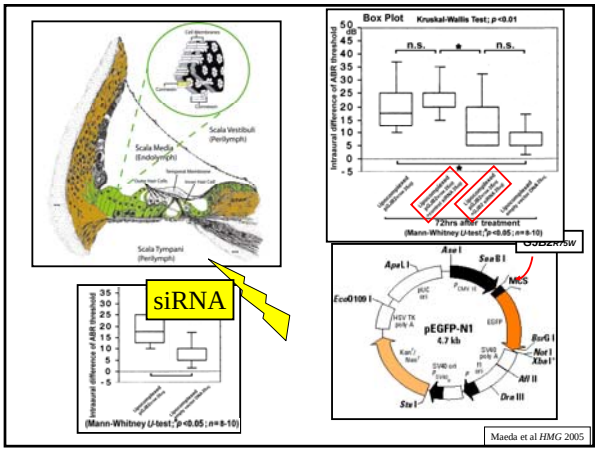


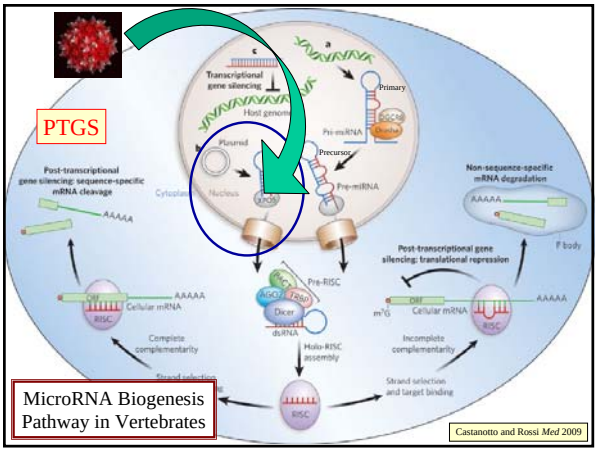


Maeda et al *Hum Mol Genet* 2005





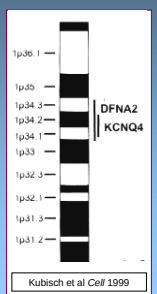




DFNA2 – *KCNQ4*

Dutch DFNA2 family, 1929
Ensink et al *Eur Arch Otorhinolaryngol* 1999

- DFNA2 - *KCNQ4* and *GJB3*
- *KCNQ4* - member of the KCNQ family of voltage-gated K+ channels
  - 4 KCNQ4 subunits aggregate to form functional channel
  - Mutant allele - only 1/16 of channels functional
  - One of the more frequently encountered ADNSHL loci

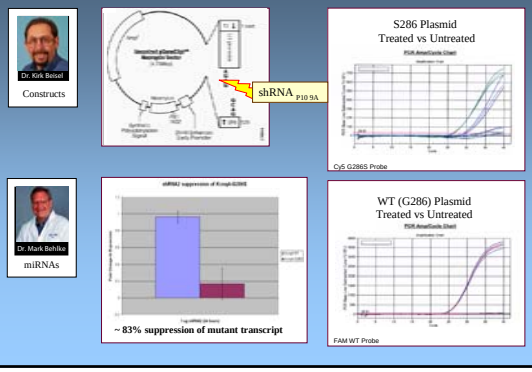


Kubisch et al *Cell* 1999

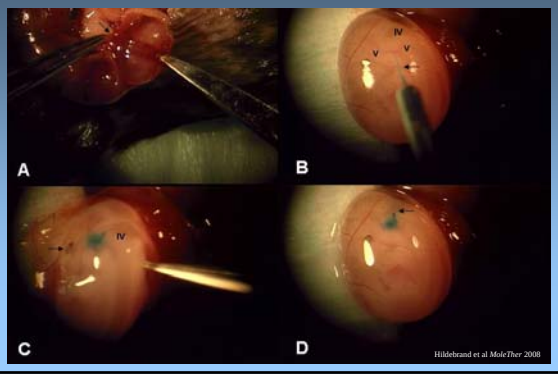
What do we need?

- Animal model
- Construct to suppress mutant allele
- Vector to deliver the construct to the appropriate cells
- Method to introduce the vector
- A lot of luck

shRNA - GeneClip Hairpin Cloning



Mouse *Exo Utero* Surgery



Mouse Operating Room

Conclusions

Today's goals were to review

- Where we are….
  - Genetic testing is important to deaf and hard-of-hearing persons and their families
  - Genetic testing may be valuable as a part of a CI evaluation
- Where we are going….
  - Several initiatives are ongoing to improve clinical diagnostics including AudioGene and OtoSCOPE
  - Hair bundle twist off > OtoSCOPE + StereoCAPTURE > mouse mutant = improved insight into biology of hearing and deafness
- Why we should get there….
  - Genotype-specific therapies will be developed to stop expression of dominant-negative alleles using RNAi