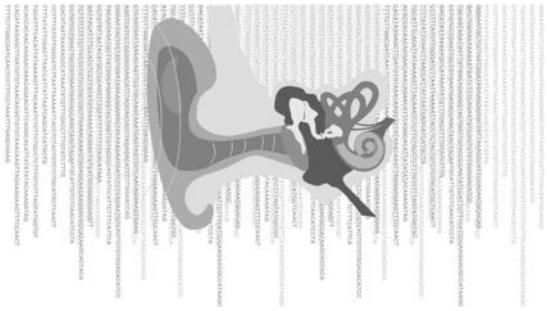
- If you are viewing this course as a recorded course after the live webinar, you can use the scroll bar at the bottom of the player window to pause and navigate the course.
- This handout is for reference only. Non-essential images have been removed for your convenience. Any links included in the handout are current at the time of the live webinar, but are subject to change and may not be current at a later date.

Epigenetics of the Auditory System: Implications for Hearing and Deafness,

Presented in partnership with American Auditory Society



Prof. Karen B. Avraham, Ph.D.
Department of Human Molecular Genetics & Biochemistry, Sackler Faculty of Medicine
Sagol School of Neuroscience
Drs. Sarah and Felix Dumont Chair for Research of Hearing Disorders

Learning Outcomes

After this course, participants will be able to:

- Define genetics and genomics and the relevance to hereditary deafness.
- Define the main epigenetic processes.
- Describe how to identify genetic variants associated with deafness.

Time Magazine

Genetics of hearing loss

- Why is gene discovery important for hearing & deafness?
- What are direct applications of genetic diagnosis today?

Epigenetics of hearing loss

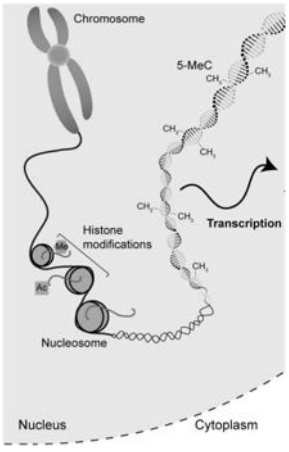
- How does regulation of genes define genetic pathways?
- Will epigenetics make a difference for deafness in therapy?

Some essential definitions

- Genetics: study of heredity
- Epigenetics: study of changes caused by modification of gene expression
- Genome: complete set of genes or genetic material present in a cell or organism
- Genomics: branch of molecular biology concerned with the structure, function, evolution, and mapping of genomes

> "Genetics is a way of thinking.
> Genomics is a set of tools."
> Mary-Claire King, Science 331:1026

- Each chromosome contains DNA double helix.
- Packaged by proteins, including histones H2A, H2B, H3 and H4.
- Chromatin: DNA-protein complex.
- Nucleosome: basic component of chromatin - DNA complex and core histones.



Seminars in Cell & Developmental Biology 65 (2017) 69–79
Contents lists available at ScienceDirect
Seminars in Cell & Developmental Biology
journal homepage: www.elsevier.com/locate/semcdb

Review

Insights into inner ear-specific gene regulation: Epigenetics and non-coding RNAs in inner ear development and regeneration

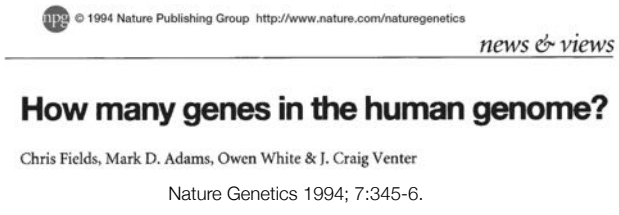
Angelika Doetzlhofer[a,*], Karen B. Avraham[b]

The Human Genome Project

- Identify all the genes in human DNA.
- Determine the sequence of the 3 billion bases that make up human DNA.
- Address the ethical, legal, and social issues (ELSI) that may arise from the project.

How many genes are there in the human genome?

In 2000 estimate was at 100,000-150,000
~20,000 is more accurate



news & views

How many genes in the human genome?

Chris Fields, Mark D. Adams, Owen White & J. Craig Venter

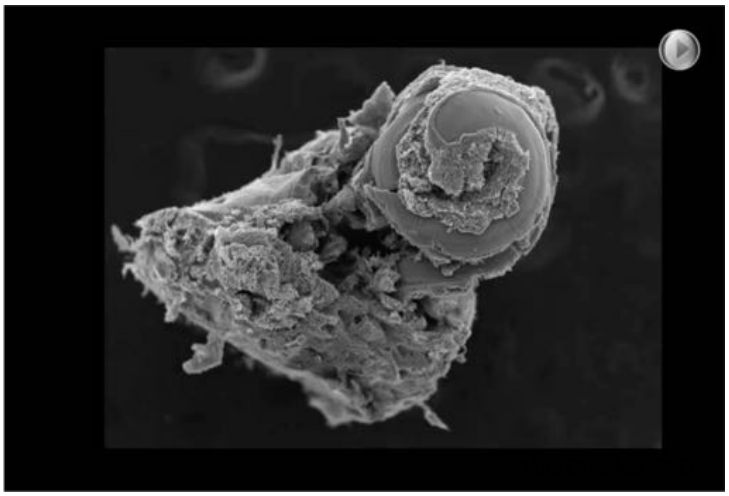
Nature Genetics 1994; 7:345-6.

Era of personalized / precision genomic medicine

To enable clinicians to quickly, efficiently and accurately predict the most appropriate course of action for a patient

Diagnostics
Sequencing
⟹ List of variants ⟹
Pharmacogenetics
Gene therapy
Stem cell therapy

Gene name	amino acid substitution	DNA mutation
USH2A	T3635I	G->G,A
USH2A	I2169T	A->G,A
CDH23	V2640F (V2635F)	G->T
USH1C	E451D	C->G
MYH9	R1466W	G->A
CELSR1	N1884S	T->C
DIAPH1		C->G
GPR98	L103P (Y515H)	T->C
GPR98	A2034T	G->A
GPR98	I150V	A->G,A
GPR98	P1036P	G->A
NR4A3		C->T
TMC1	E81K	G->G,A
TMC1	M486T	T->C,T



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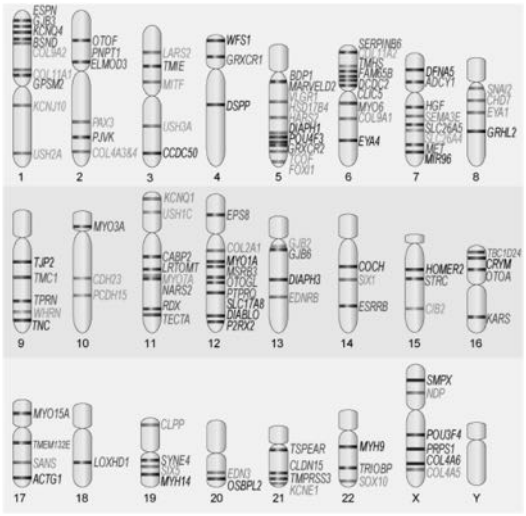
Genetics of hearing loss

- Approx 100 genes are known to be involved in deafness
- First genes 'mapped' in 1992 and 1994: DFNA1 and DFNB1
- Genes 'cloned' in 1997: DIAPH1 and GJB2
- Techniques:
- Linkage analysis
- High-throughput sequencing

Neuron
Review **Hearing Impairment: A Panoply of Genes and Functions**

Dror & Avraham 2010



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First gene for dominant non-syndromic deafness

PNAS Proceedings of the National Academy of Sciences of the United States of America

The gene for an inherited form of deafness maps to chromosome 5q31

PEDRO E. LEON*, HENRIETTE RAVENTOS*, ERIC LYNCH†, JAN MORROW†, AND MARY-CLAIRE KING† 1992

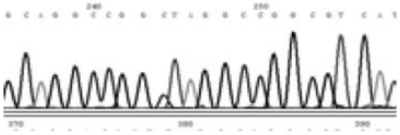
Science

Nonsyndromic Deafness DFNA1 Associated with Mutation of a Human Homolog of the *Drosophila* Gene *diaphanous*

Eric D. Lynch,* Ming K. Lee, Jan E. Morrow, Piri L. Welcsh, Pedro E. León, Mary-Claire King 1997

CONTINUED

Gene discovery for deafness: 2010-present



Sanger sequencing

'read' ~1000 bp at a time
For discovery of mutation in a single gene

Next-generation sequencing
Deep sequencing
Massively Parallel Sequencing

'read' millions of bp at a time

For discovery of variant, search through entire genome

The $1000 genome

"I'd rather spend my money on my genome than on a Bentley or an airplane." Biotechnology entrepreneur and millionaire Dan Stoicescu

2007 Watson, $1 million
2007 Knome, $350,000
2009 Complete Genomics, $5000 for bulk
2010 Illumina, consumers, doctor's note, $50,000
2011 23&Me, $999 Exome
2014 Illumina HiSeqX Ten Sequencer, $1000
2016 Illumina HiSeq Five System
HiSeq 3000/4000 Systems
2018 NextSeq 550 System, PacBio & more

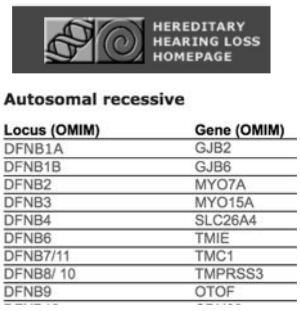
AMERICAN ACADEMY OF OTOLARYNGOLOGY–HEAD AND NECK SURGERY FOUNDATION

Massively Parallel Sequencing for Genetic Diagnosis of Hearing Loss: The New Standard of Care

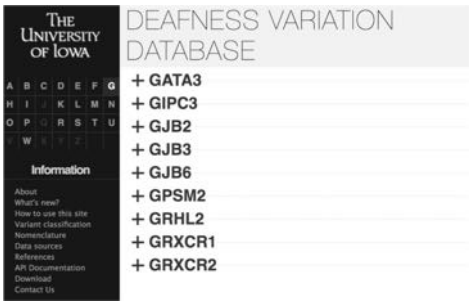
A. Eliot Shearer, MD, PhD[1], and Richard J. H. Smith, MD[1,2,3]

Otolaryngol Head Neck Surg. 153, 175-182 (2015)

How do you find information about known genes?

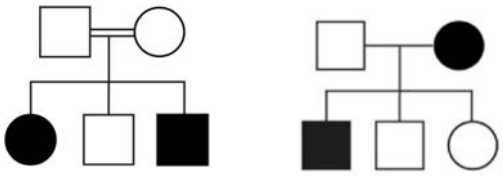


http://hereditaryhearingloss.org/



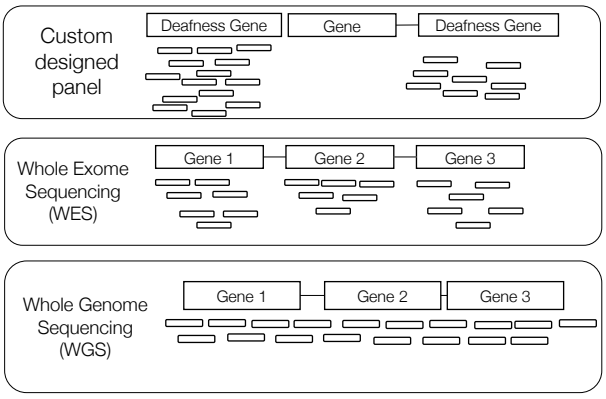
http://deafnessvariationdatabase.org/

Hearing impaired individual arrives to the clinic: What's next?



- Family and medical history
- Rule out non-genetic causes (CMV, exposure to noise, ototoxic drugs)
- Determine mode of inheritance
- Screen for common genes
- Screen for genes associated with ethnic background
- Perform deafness gene panel and high-throughput sequencing
- Perform whole exome sequencing
- Perform whole genome sequencing

High-throughput sequencing



news@JAMA

Geneticists Recommend Disclosing "Incidental" Findings for Certain Disorders

BY BRIDGET M. KUEHN ON MARCH 21, 2013

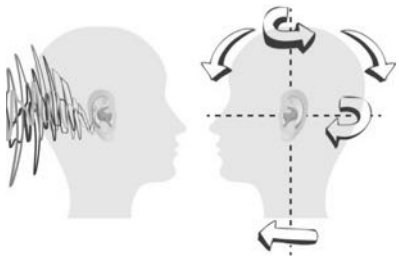
ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

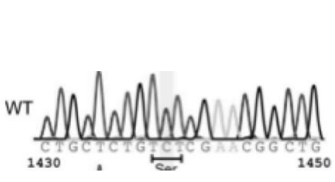
Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

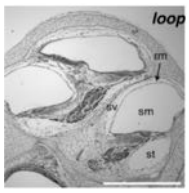
Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

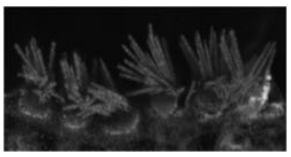
The role of models in deafness gene discovery

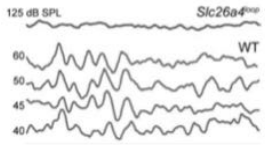
- Similarities in genomes
- Structure & function of inner ear
- Finding genes
- Understanding mechanism







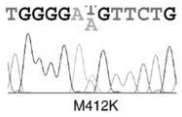




Beethoven, a mouse model for dominant, progressive hearing loss DFNA36

nature genetics

Sarah Vreugde[1]*, Alexandra Erven[2]*, Corné J. Kros[3], Walter Marcotti[3], Helmut Fuchs[4], Kiyoto Kurima[5], Edward R. Wilcox[5], Thomas B. Friedman[5], Andrew J. Griffith[5], Rudi Balling[4], Martin Hrabé de Angelis[4], Karen B. Avraham[1] & Karen P. Steel[2]



Dominant and recessive deafness caused by mutations of a novel gene, *TMC1*, required for cochlear hair-cell function

Kiyoto Kurima[1], Linda M. Peters[2], Yandan Yang[1], Saima Riazuddin[2], Zubair M. Ahmed[2,3], Sadaf Naz[2], Deidre Arnaud[4], Stacy Drury[4], Jianhong Mo[2], Tomoko Makishima[1], Manju Ghosh[5], P.S.N. Menon[5], Dilip Deshmukh[6], Carole Oddoux[7], Harry Ostrer[7], Shaheen Khan[3], Sheikh Riazuddin[3], Prescott L. Deininger[8], Lori L. Hampton[9], Susan L. Sullivan[10], James F. Battey, Jr.[9], Bronya J.B. Keats[4], Edward R. Wilcox[2], Thomas B. Friedman[2] & Andrew J. Griffith[1,11]

OPEN ACCESS Freely available online

PLOS ONE

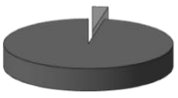
A Novel DFNA36 Mutation in *TMC1* Orthologous to the Beethoven (*Bth*) Mouse Associated with Autosomal Dominant Hearing Loss in a Chinese Family

Yali Zhao[1,2], Dayong Wang[1], Liang Zong[1], Feifan Zhao[1], Liping Guan[3], Peng Zhang[3], Wei Shi[1], Lan Lan[1], Hongyang Wang[1], Qian Li[1], Bing Han[1], Ling Yang[4], Xin Jin[3,5], Jian Wang[3], Jun Wang[3], Qiuju Wang[1]*

Epigenetics: beyond the sequence

- Epigenetic changes are genetic changes that are heritable that do not depend on DNA sequence changes. Leave A, C, T, G intact.
- Inherited changes in gene expression through the modification of DNA and chromatin structure but not the DNA sequence.

Key epigenetic modifications

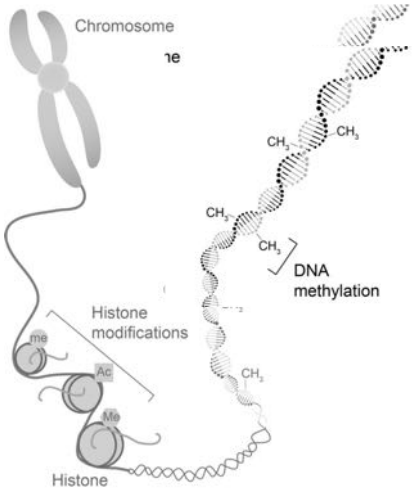


- Majority of the genome is not expressed
- Less than 3% of the expressed RNA in mammals is protein-coding RNA

DNA methylation
- Methyl group added to DNA nucleotide

Histone proteins and modifications
- Compact DNA and regulate chromatin

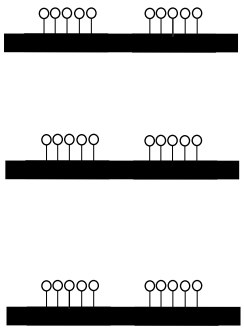
Chromatin modifying and DNA binding proteins
- Bind DNA, adjust chromatin conformation, modify histones



Methylation

- Methyl group tags DNA
- Activates or represses genes
- Covalent binding of methyl group to a C nucleotide

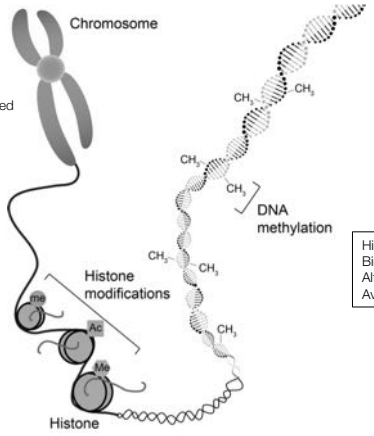
Methylation tissue specificity



- Zygote stage genome stripped of DNA methylation
- Gradually accumulates as move along cell differentiation
- Differential methylation in tissues
- Contributes to tissue specific expression

Histones and histone modification (PTM)

Histones
Proteins
DNA winds around histones
Enables DNA to be compacted
For gene regulation



Euchromatin
Less condensed

Histone modification
Binding of epigenetic factors to histone 'tails'
Alters extent to which DNA is wrapped around histones
Availability of gene in DNA to be activated

Heterochromatin
Highly condensed
represses gene transcription

Histone modifications

Mark distinct regulatory elements

- Histone 3 lysine 4 tri-methylation (H3K4me3): positively correlated to the TSS of actively transcribed genes
- Histone 3 lysine 27 acetylation (H3K27ac): active state of promoters and enhancer elements
- Histone 3 lysine 27 tri-methylation (H3K27me3): mark genes found in a poised expression state, mainly in stem or progenitor cells

Epigenomic landscapes of primary human tissues and cells

111 epigenomes

Roadmap Epigenomics Consortium et al. Nature 518 (2015)

Epigenetic regulation of *Atoh1* guides hair cell development in the mammalian cochlea

Zlatka P. Stojanova[1], Tao Kwan[1] and Neil Segil[1,2,*] 2015

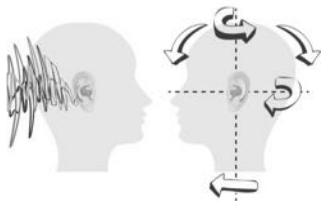
Development

- Sensory hair cell differentiation depends on regulated expression of Atoh1
- In mammals, if hair cells die they do not regenerate, leading to permanent deafness.
- In non-mammalian vertebrates regeneration occurs through upregulation of Atoh1 in the surviving supporting cells that surround hair cells, leading to functional recovery.

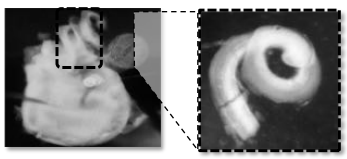
Dynamic changes in the histone modifications
H3K4me3/H3K27me3, H3K9ac and H3K9me3
reveal progression from poised, to active, to repressive marks

Blueprint of the auditory and vestibular systems

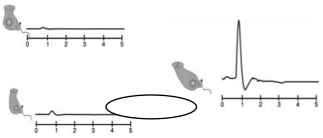
How does regulation of gene expression govern the pathways that determine inner ear function and how do alterations in regulation, on a genetic and epigenetic level, contribute to the pathology of deafness?



Epigenetics of the inner ear



Sensory epithelium
E16.5, P0, P22



Transcriptome mRNA miRNA lncRNA	Nucleosome occupancy profiling	Histone Post-Translational Modification (PTM) profiles	DNA Methylation profiles
RNA-Seq	ATAC-Seq	cChIP-Seq	Whole genome bisulfite sequencing

Overlay data to generate complete picture of the epigenome

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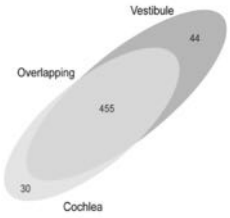
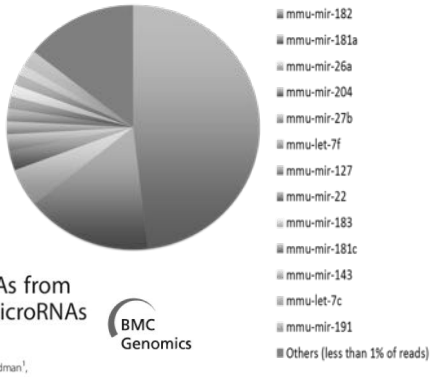
microRNAs in the inner ear





Next-generation sequencing of small RNAs from inner ear sensory epithelium identifies microRNAs and defines regulatory pathways

BMC Genomics

Anya Rudnicki[1†], Ofer Isakov[2†], Kathy Ushakov[1], Shaked Shivatzki[1], Inbal Weiss[1,3], Lilach M Friedman[1], Noam Shomron[2] and Karen B Avraham[1*]

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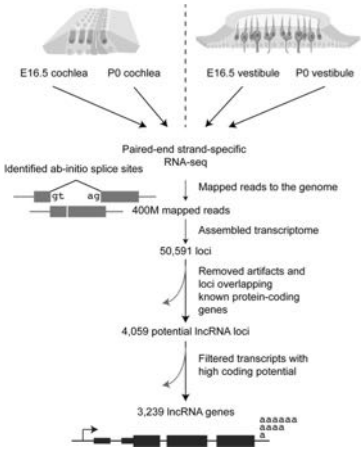
Long non-coding RNAs in the inner ear



SCIENTIFIC REPORTS

Genome-wide identification and expression profiling of long non-coding RNAs in auditory and vestibular systems

Kathy Ushakov[1], Tal Koffler-Brill[1], Aviv Rom[1], Kobi Perl[1,2], Igor Ulitsky[1] & Karen B. Avraham[1]

- Large and diverse class of transcribed RNA
- >200 nt
- Do not encode proteins
- Regulators of gene expression
- Changes chromatin structure, modification, protein structure

CONTINUED

Therapy: the future in deafness

- Different forms of deafness – link to methylation patterns
- Polymorphisms on DNA level in methylated regions
- Entry points for modifications

Neuron
Article Neuron 75, 283–293, July 26, 2012

Restoration of Hearing in the VGLUT3 Knockout Mouse Using Virally Mediated Gene Therapy

Omar Akil,[1] Rebecca P. Seal,[3] Kevin Burke,[1] Chuansong Wang,[4] Aurash Alemi,[1] Matthew During,[4] Robert H. Edwards,[2] and Lawrence R. Lustig[1,*]

Rescue of hearing and vestibular function by antisense oligonucleotides in a mouse model of human deafness

nature medicine
MARCH 2013

Jennifer J Lentz[1,6], Francine M Jodelka[2,6], Anthony J Hinrich[2,6], Kate E McCaffrey[2], Hamilton E Farris[1], Matthew J Spalitta[1], Nicolas G Bazan[3], Dominik M Duelli[4], Frank Rigo[5] & Michelle L Hastings[2]

***Tmc* gene therapy restores auditory function in deaf mice**

Charles Askew,[1,2] Cylia Rochat,[3] Bifeng Pan,[1] Yukako Asai,[1] Hena Ahmed,[1] Erin Child,[1] Bernard L. Schneider,[3] Patrick Aebischer,[3] Jeffrey R. Holt[1]*

www.ScienceTranslationalMedicine.org 8 July 2015

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CRISPR-Cas9 genome editing in deafness

nature
International journal of science

Treatment of autosomal dominant hearing loss by *in vivo* delivery of genome editing agents

Xue Gao[1,2,3†*], Yong Tao[4,5†*], Veronica Lamas[4], Mingqian Huang[4], Wei-Hsi Yeh[1,2,3,6], Bifeng Pan[7], Yu-Juan Hu[4,5], Johnny H. Hu[1,2,3], David B. Thompson[1,2], Yilai Shu[4,8], Yamin Li[9], Hongyang Wang[4,10], Shiming Yang[10], Qiaobing Xu[9], Daniel B. Polley[4], M. Charles Liberman[4], Wei-Jia Kong[5], Jeffrey R. Holt[7], Zheng-Yi Chen[4]§ & David R. Liu[1,2,3]§

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TEL AVIV UNIVERSITY

Prof. Karen B. Avraham, Ph.D.
Department of Human Molecular Genetics & Biochemistry, Sackler Faculty of Medicine
Sagol School of Neuroscience
Drs. Sarah and Felix Dumont Chair for Research of Hearing Disorders
karena@tauex.tau.ac.il

i-CORE Israeli Centers of Research Excellence
ISRAEL SCIENCE FOUNDATION
ACTION ON HEARING LOSS
BSF United States–Israel Binational Science Foundation
HFSP
NIH NIDCD

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- Koffler, T., Ushakov, K. and Avraham, K.B. (2015) Genetics of hearing loss – syndromic. Otolaryngol Clin North Am. 48:1041-1061.
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