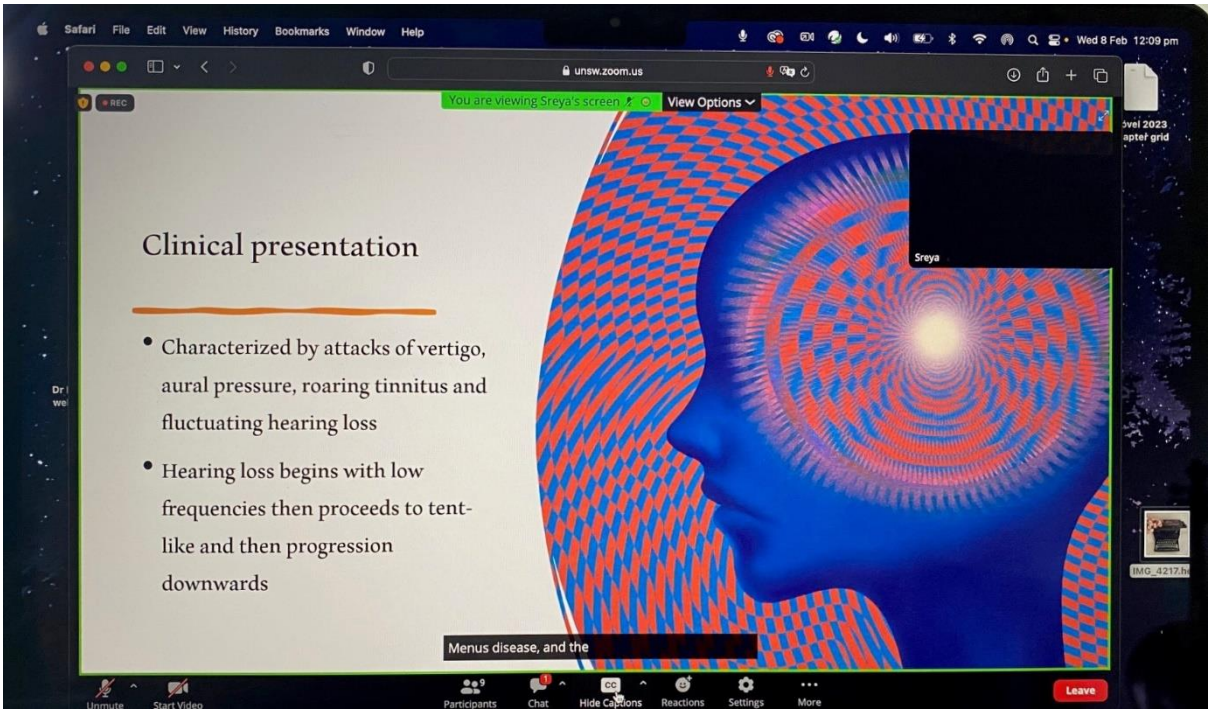
<u>Prof Jeffrey Harris UNSW Meniere's Presentation – 8 February 2023</u>

New insights on the etiopathogenesis of Meniere's Disease Zoom Session

Gary was the host:

- Stated that Meniere's is one of the most profound and disabling conditions of the inner ear
- UNSW is doing some outstanding research



Next speaker:

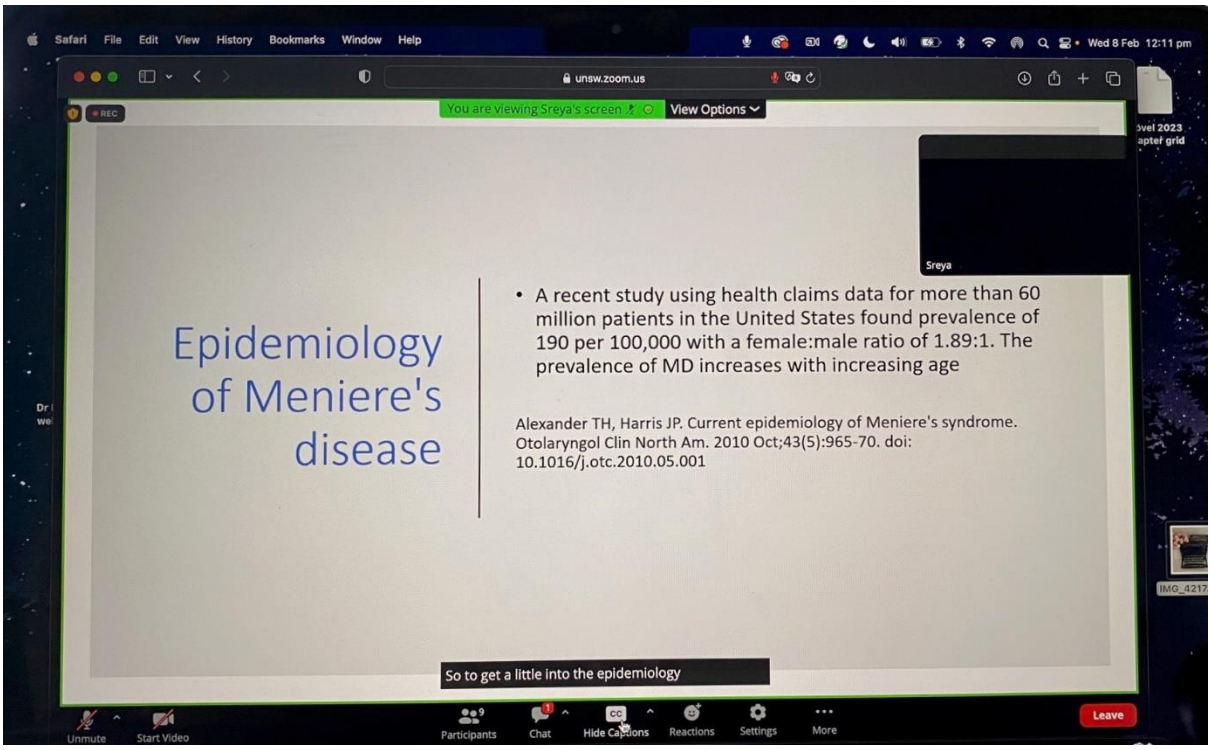
Prof Jeffrey Harris

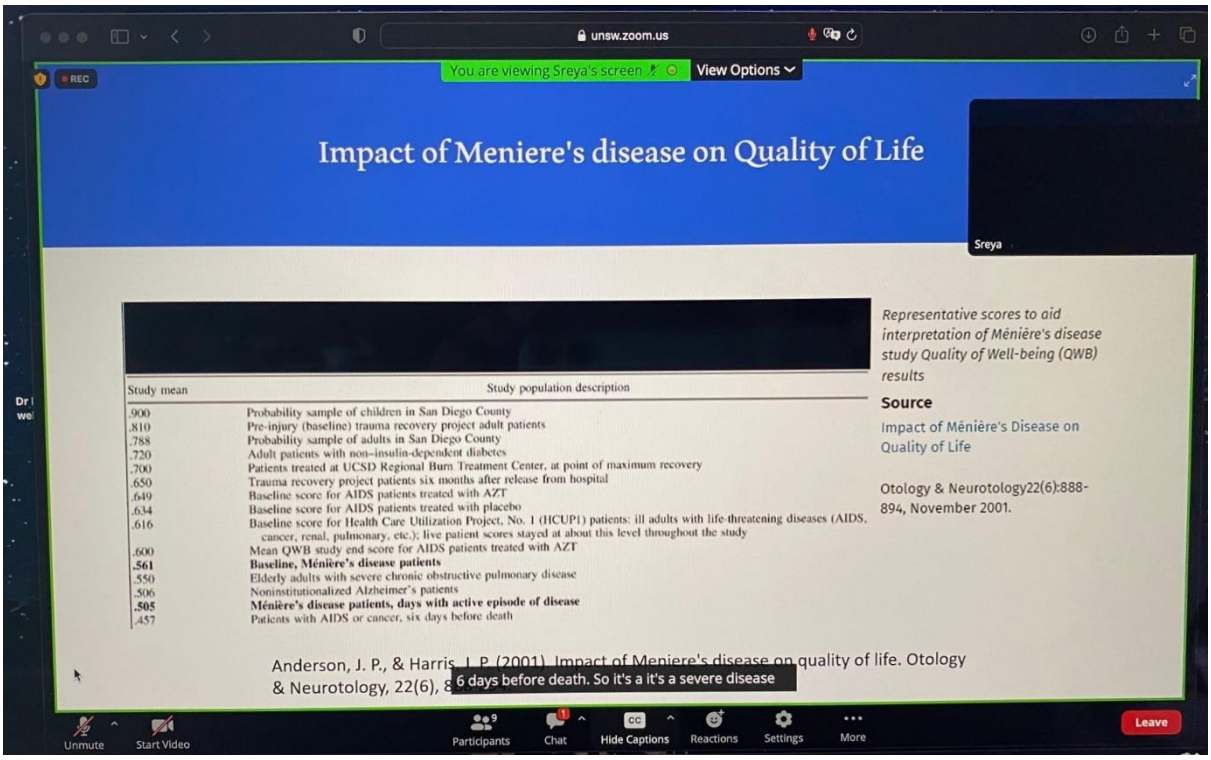
Bio –
Jeffrey P Harris, MD, PhD, is Distinguished Professor and Chief of the Department of Otolaryngology / Head and Neck Surgery at the University of California, San Diego and Staff Surgeon at the VA Healthcare System. He is the Past President of the American Otological Society and the Association for Research in Otolaryngology. During his career he has focused his practice on microsurgery of the ear and skull base. He has written and lectured extensively on autoimmune inner ear disease, Meniere's disease and otosclerosis. He is co-founder of Otonomy, Inc and serves as a consultant. He has authored five textbooks and over 250 articles.

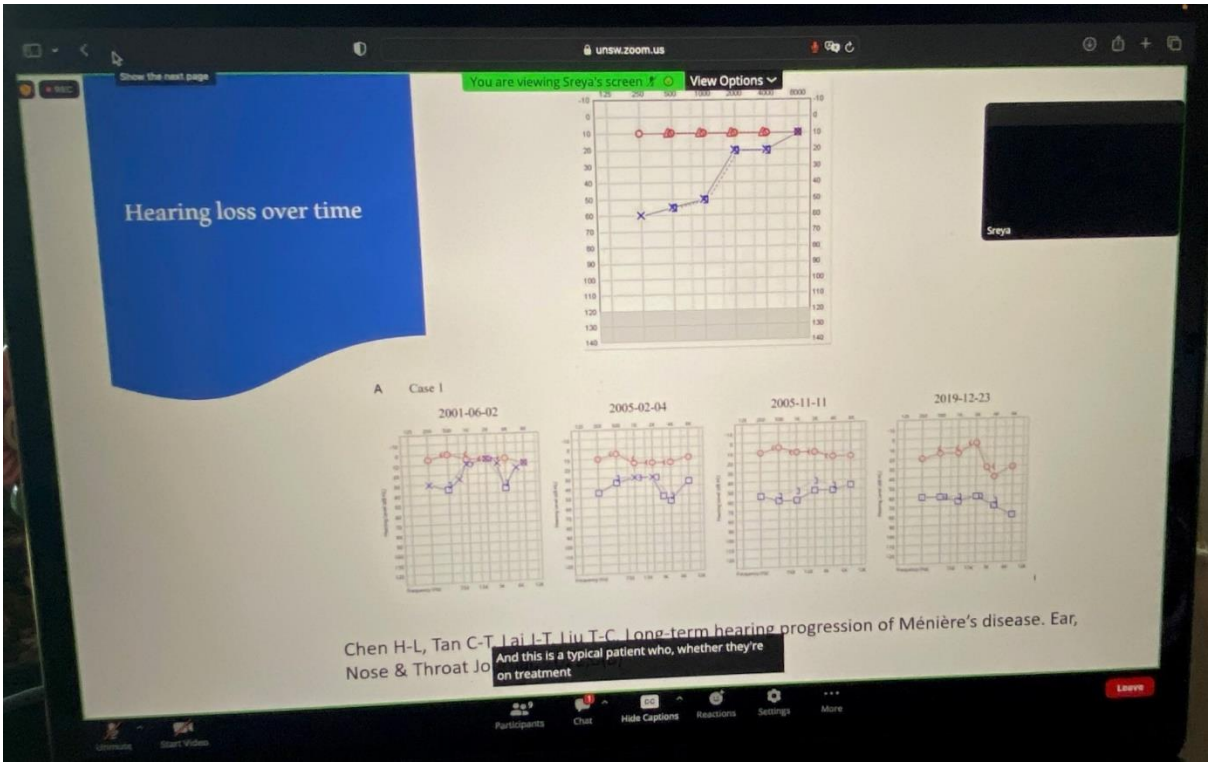
In talking on Zoom:

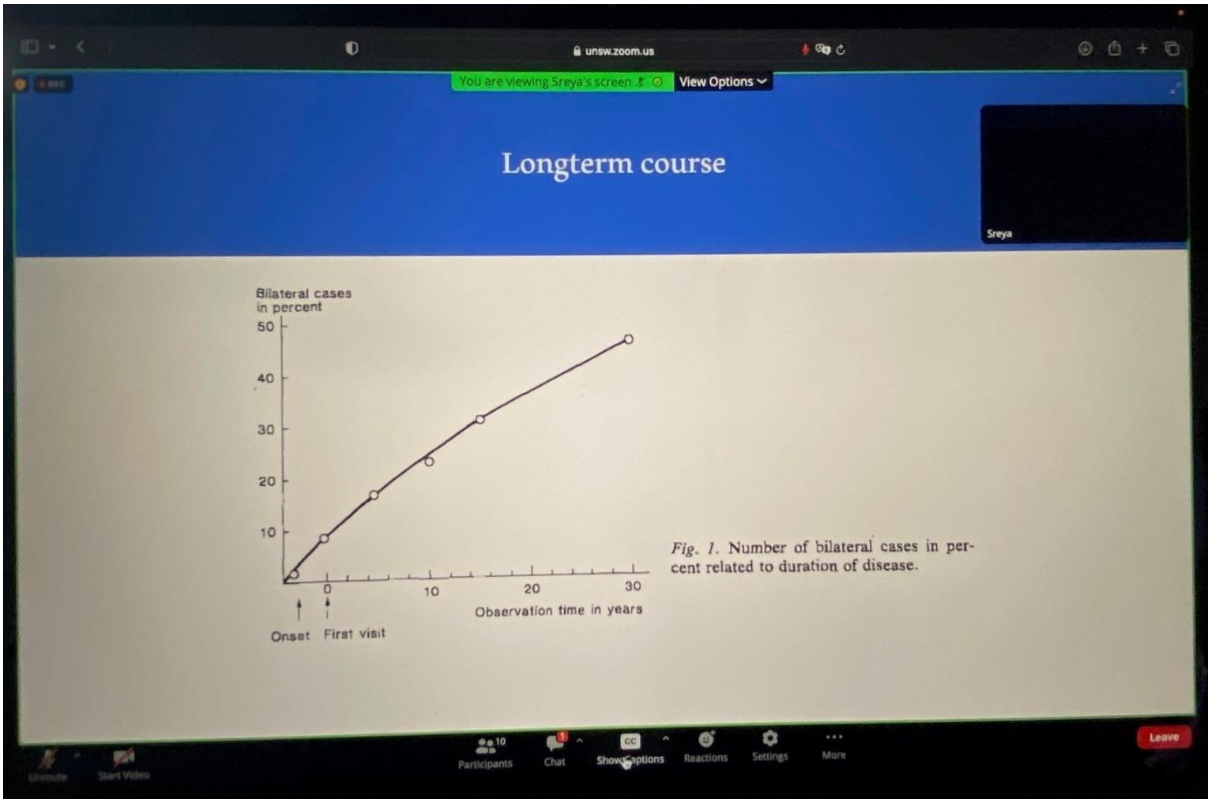
- Has no answers about MD, yet, but can share what they think is going on.
- MD was named 161 years ago

- They don’t know what starts MD, or what is the tipping point.
- You can have MD with or without vertigo, and then is it migraine or the inner ear?
- Stated that when a person is in an active meniere’s episode, it is like someone who is 6 days before death.
- If you have gone bi-lateral in the first 8 years of having MD, you probably won’t go bi-lateral









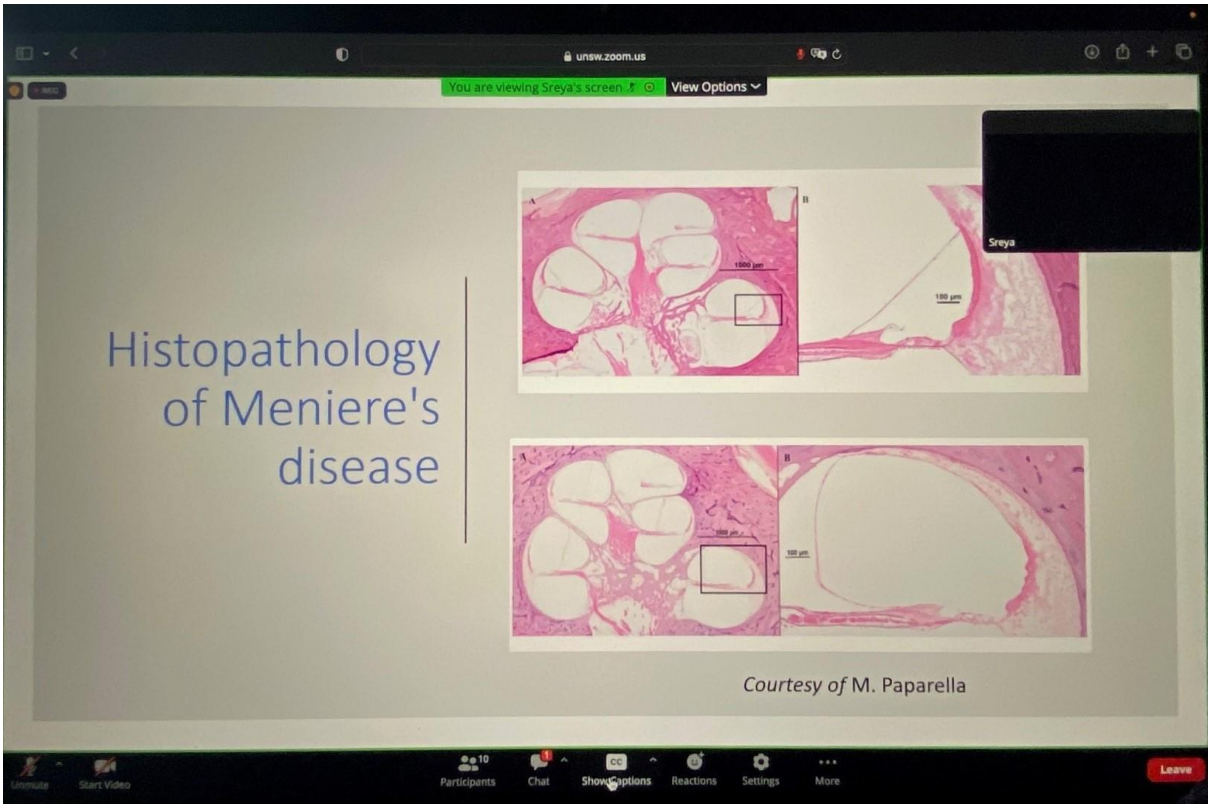
Histopathology of MD

- Too much endolymphatic fluid. But why?
- MD is either too much secretion, or too little reabsorption
- Why do MD ears have too much endolymph fluid?
- One hypothesis is that they have decreased resorption
- Rupture theory causes attacks of vertigo, but it doesn't explain things
- Too much fluid the endolymphatic sac takes on is of importance

- Polygenetic traits often have an environmental trigger
- Endolymphatic sac surgery has been found not to be effective. If the pa-tient had jour-neyed with the MD, they would be a the same place after 8 years as having the surgery
- Gene mutations
- Multipfactorial causes of MD (same slide Dr Daniel Brown showed)
- Injuries to the ear can cause MD
- ION channel is Prof Jeffrey Harris's interest – the APT2B2 calcium channel came up in Rick Friedman's study

Prof Jeffrey Harris recommends as initial treatment when you are diagnosed:

- Diuretic
- Low salt
- Steroid shots to the inner ear



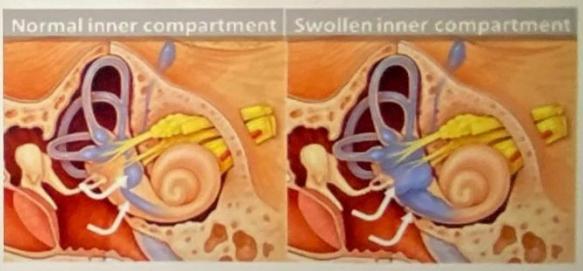
unsw.zoom.us

You are viewing Sreya's screen | View Options

All Meniere's cases have endolymphatic hydrops bu… all ears with endolymphatic hydrops have Meniere'…

Sreya

- Rauch SD, Merchant SN, Thedinger BA. Meniere's Syndrome and Endolymphatic Hydrops: Double-Blind Temporal Bone Study. *Annals of Otology, Rhinology & Laryngology*. 1989;98(11):873-883



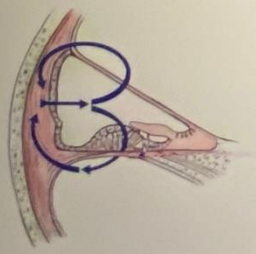
unsw.zoom.us

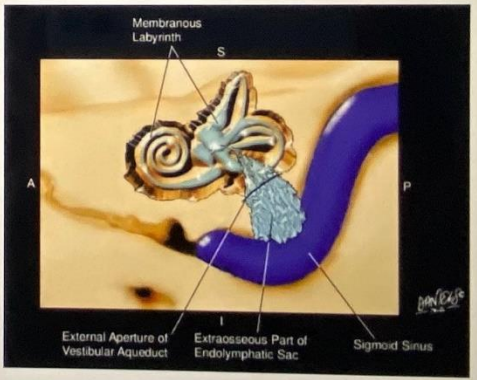
You are viewing Sreya's screen | View Options

Fluid circulation in the inner ear- In Meniere's too m… secretion or too little reabsorption?

Sreya

- Radial flow
- Longitudinal flow





unsw.zoom.us

You are viewing Sreya's screen View Options

Sreya

Why do MD ears have too much endolymph?

- One hypothesis is that the endolymphatic sac malfunctions and there is decreased resorption

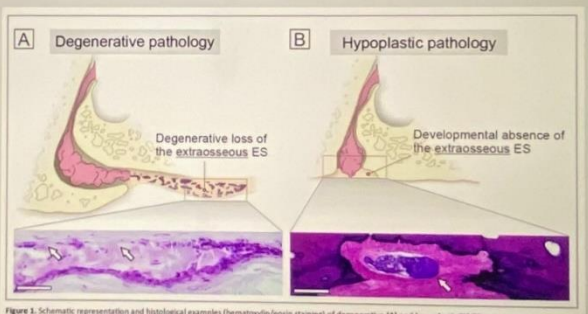


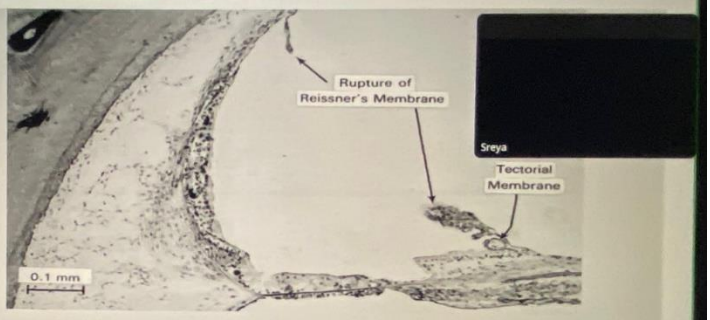
Figure 1. Schematic representation and histological examples (hematoxylin/eosin staining) of degenerative (A) and hypoplastic (B) ES pathologies, as present in cases with idiopathic endolymphatic hydrops. *Adapted from (37), according to the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), under which the original article is distributed.*

unsw.zoom.us

You are viewing Sreya's screen View Options

Sreya

Histopathology of Meniere's disease "rupture theory"



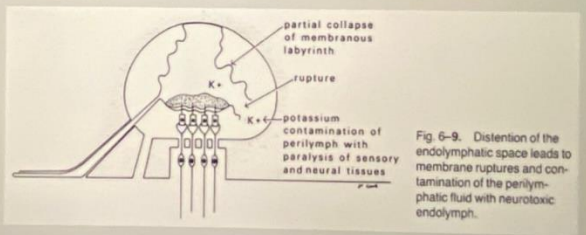
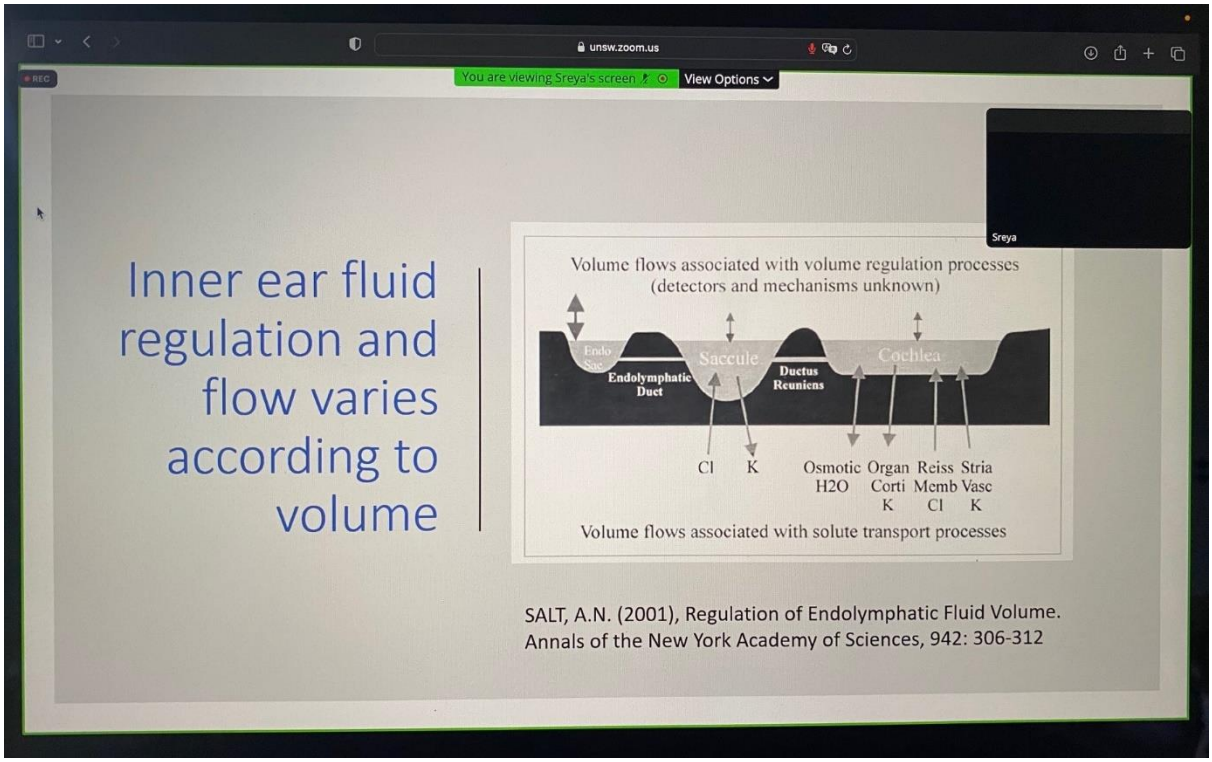
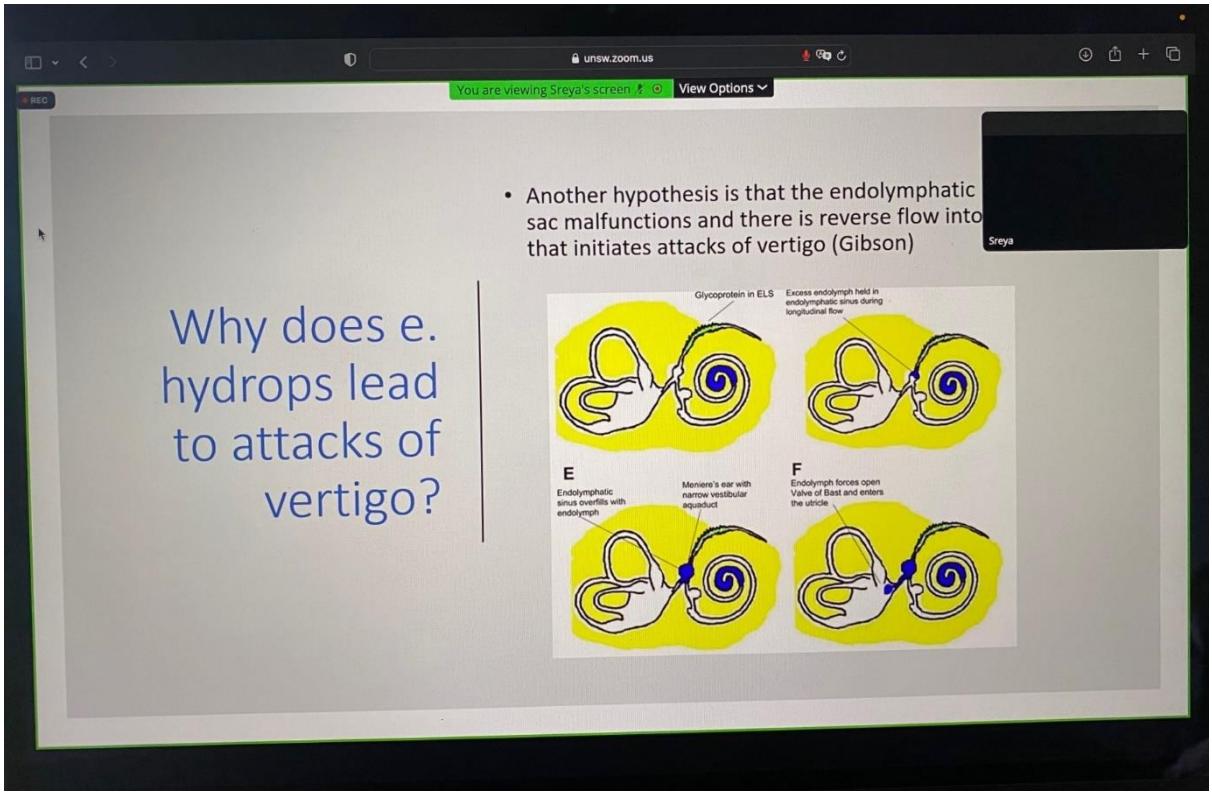


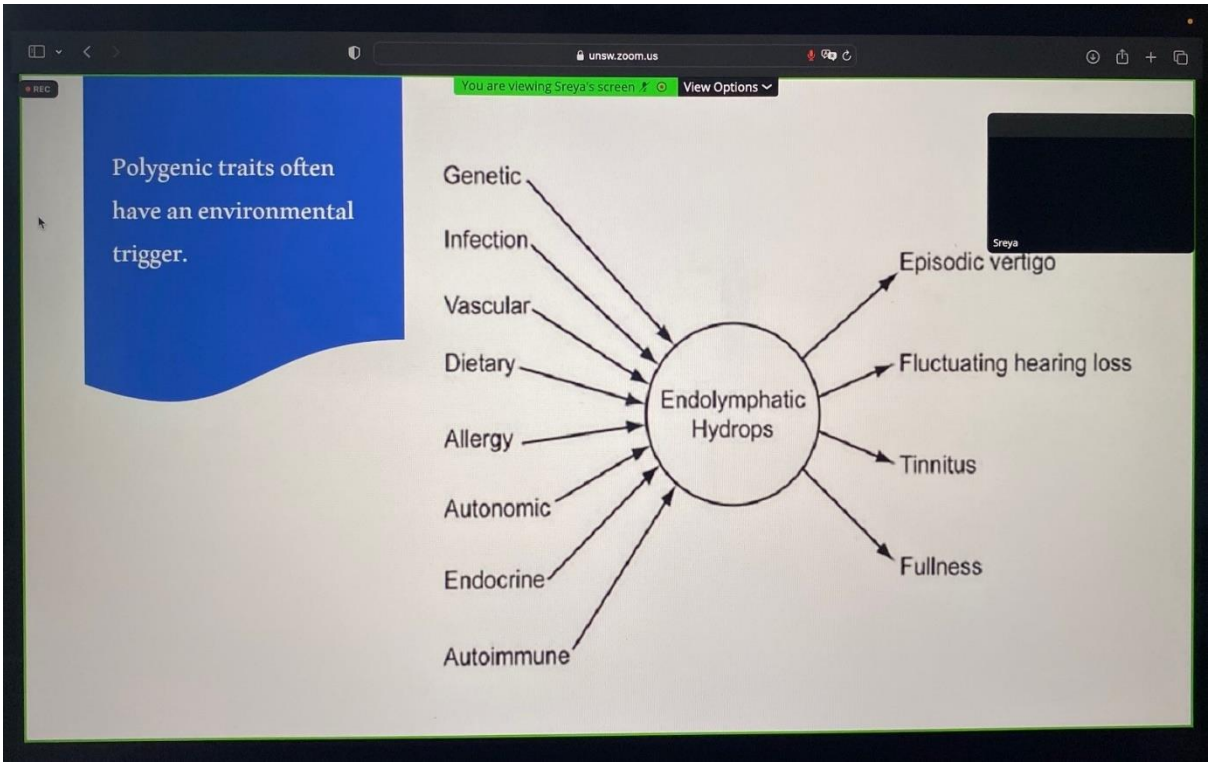
Fig. 6–9. Distention of the endolymphatic space leads to membrane ruptures and contamination of the perilymphatic fluid with neurotoxic endolymph.

After: H.F. Schuknecht

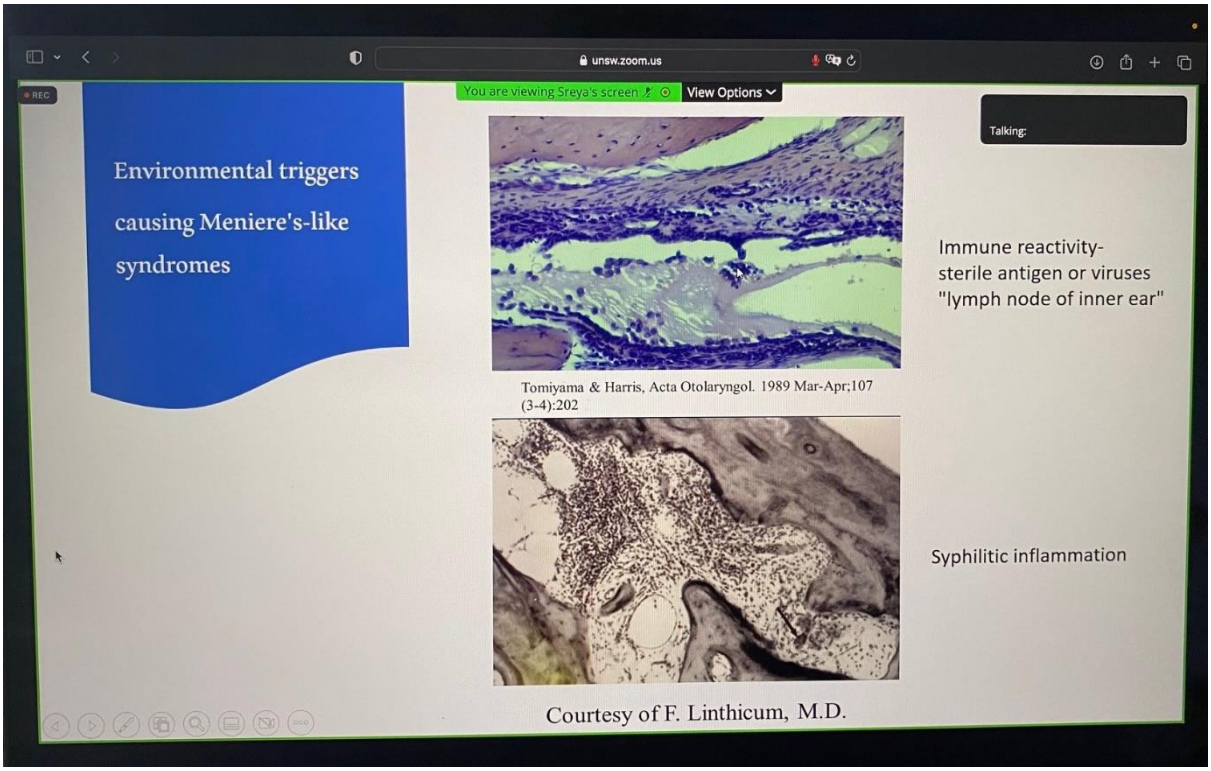


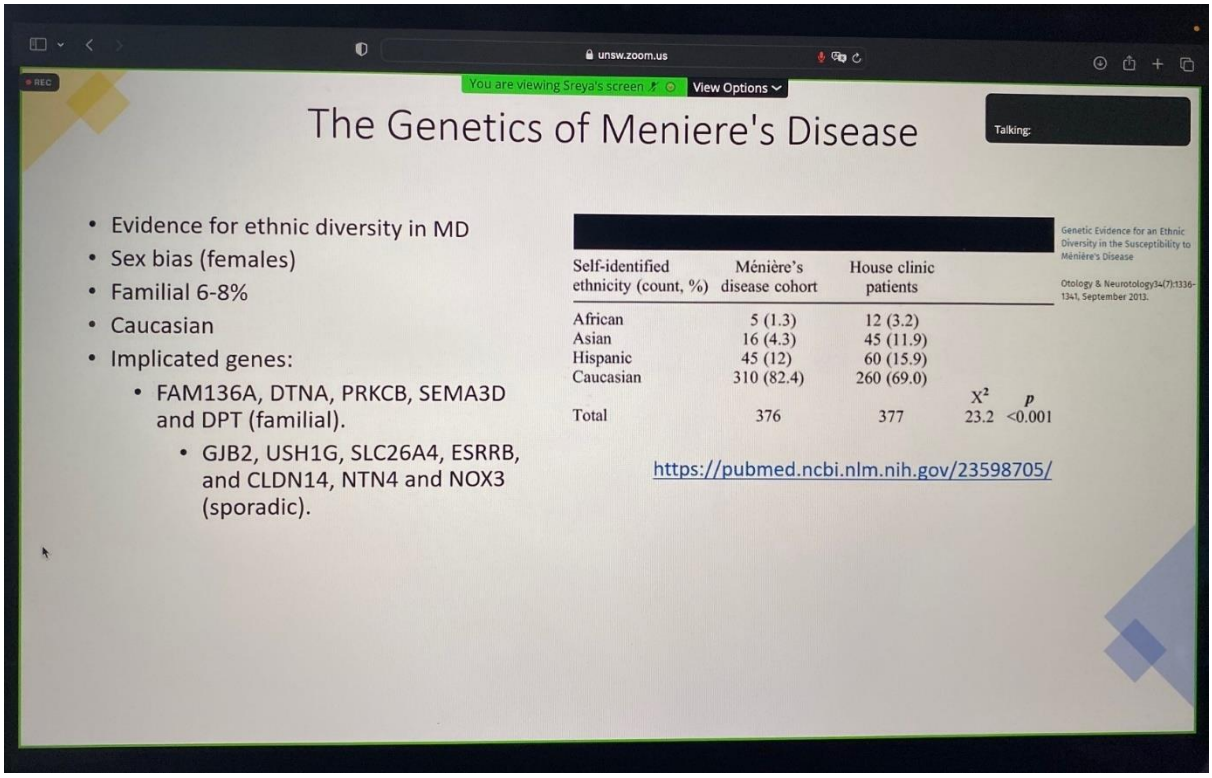


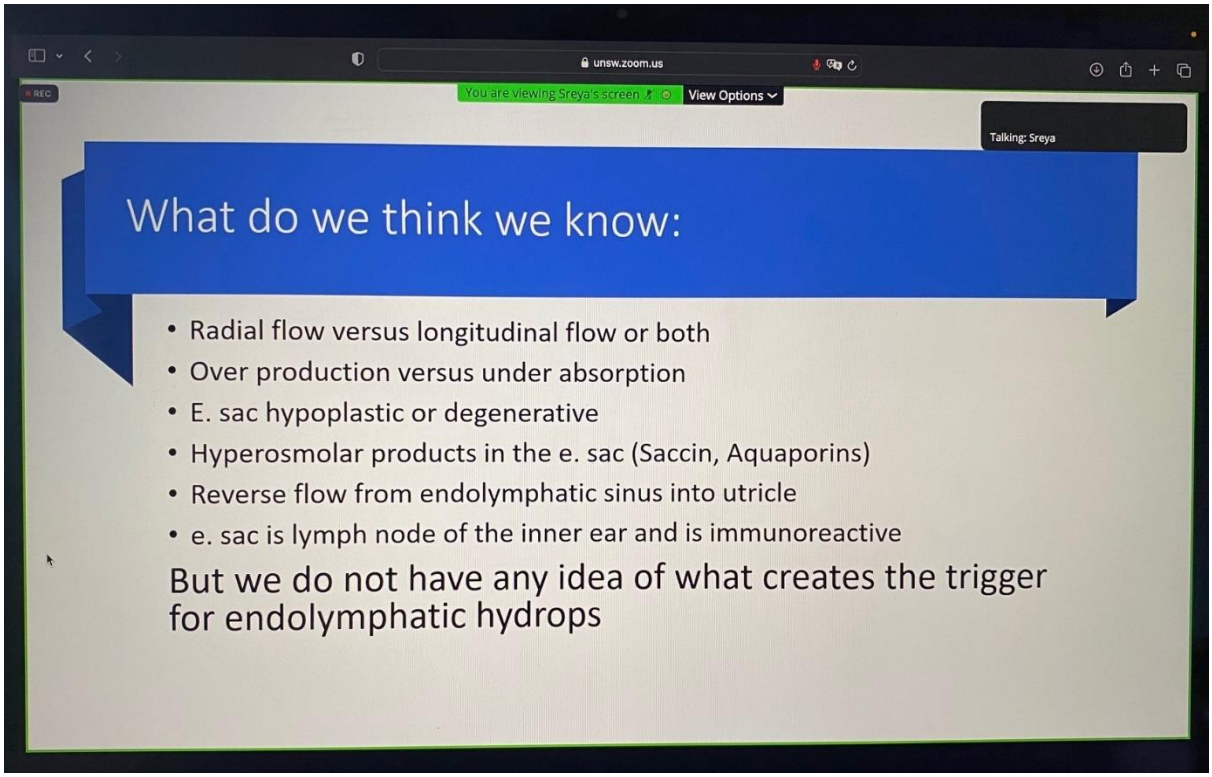
(This is Prof Bill Gibson's research)

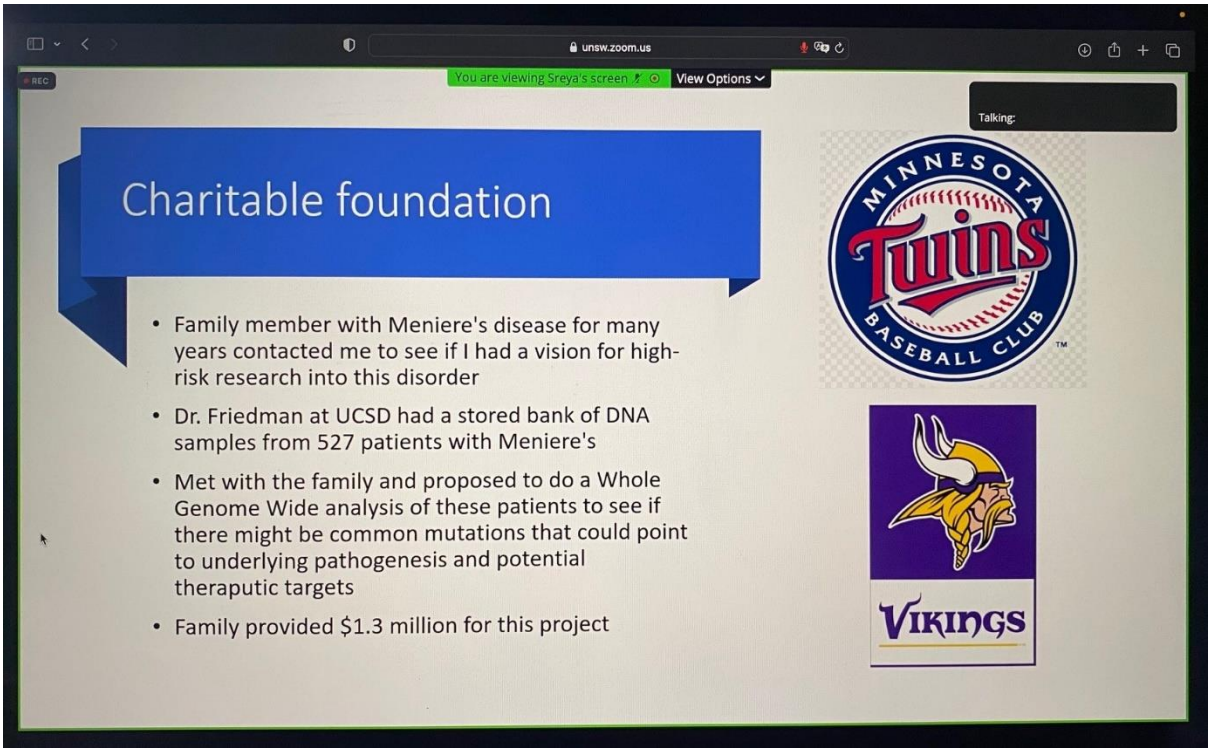


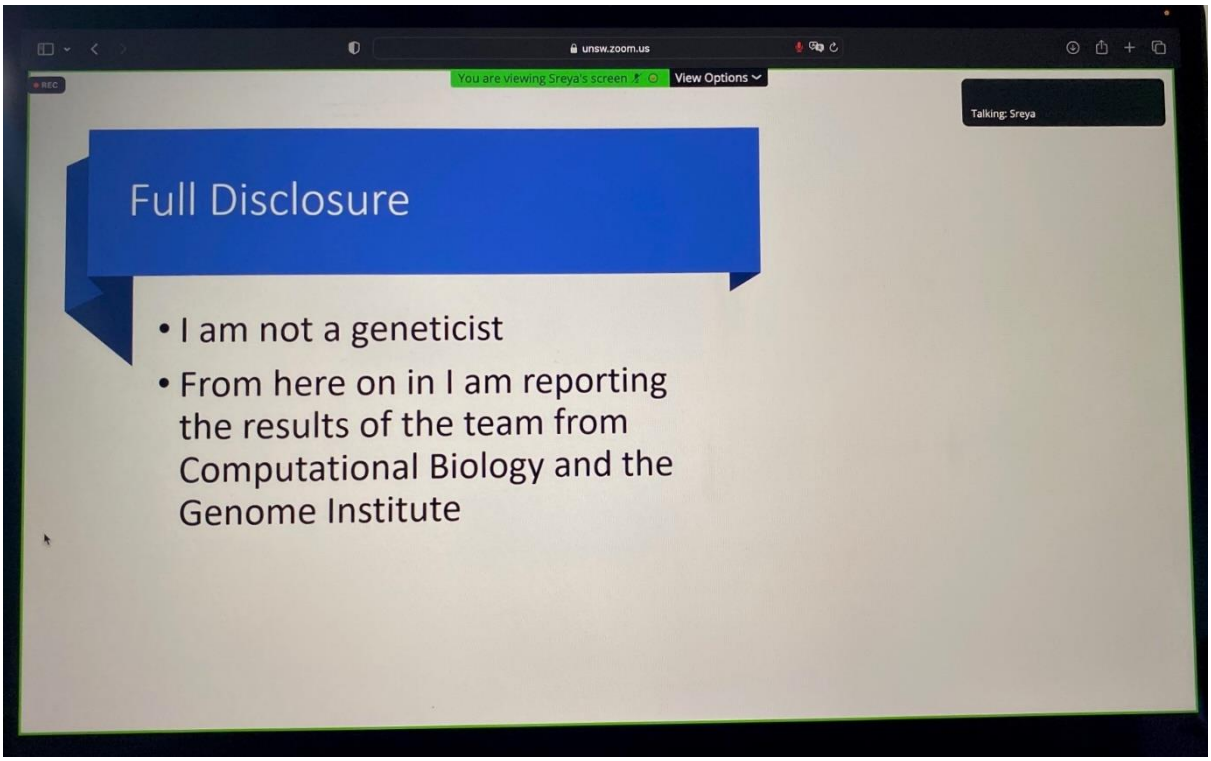
(This is Dr Daniel Brown's)




unsw.zoom.us
You are viewing Sreya's screen
View Options
Talking:
The Genetics of Meniere's Disease
• Evidence for ethnic diversity in MD
• Sex bias (females)
• Familial 6-8%
• Caucasian
• Implicated genes:
• FAM136A, DTNA, PRKCB, SEMA3D and DPT (familial).
• GJB2, USH1G, SLC26A4, ESRRB, and CLDN14, NTN4 and NOX3 (sporadic).
Genetic Evidence for an Ethnic Diversity in the Susceptibility to Ménière's Disease
Otology & Neurotology34(7):1336-1341, September 2013.
Self-identified ethnicity (count, %) | Ménière's disease cohort | House clinic patients
African 5 (1.3) 12 (3.2)
Asian 16 (4.3) 45 (11.9)
Hispanic 45 (12) 60 (15.9)
Caucasian 310 (82.4) 260 (69.0)
Total 376 377 X² 23.2 p <0.001
https://pubmed.ncbi.nlm.nih.gov/23598705/


unsw.zoom.us
You are viewing Sreya's screen
View Options
Talking: Sreya
What do we think we know:
• Radial flow versus longitudinal flow or both
• Over production versus under absorption
• E. sac hypoplastic or degenerative
• Hyperosmolar products in the e. sac (Saccin, Aquaporins)
• Reverse flow from endolymphatic sinus into utricle
• e. sac is lymph node of the inner ear and is immunoreactive
But we do not have any idea of what creates the trigger for endolymphatic hydrops





Next speaker (Zoom to the USA):

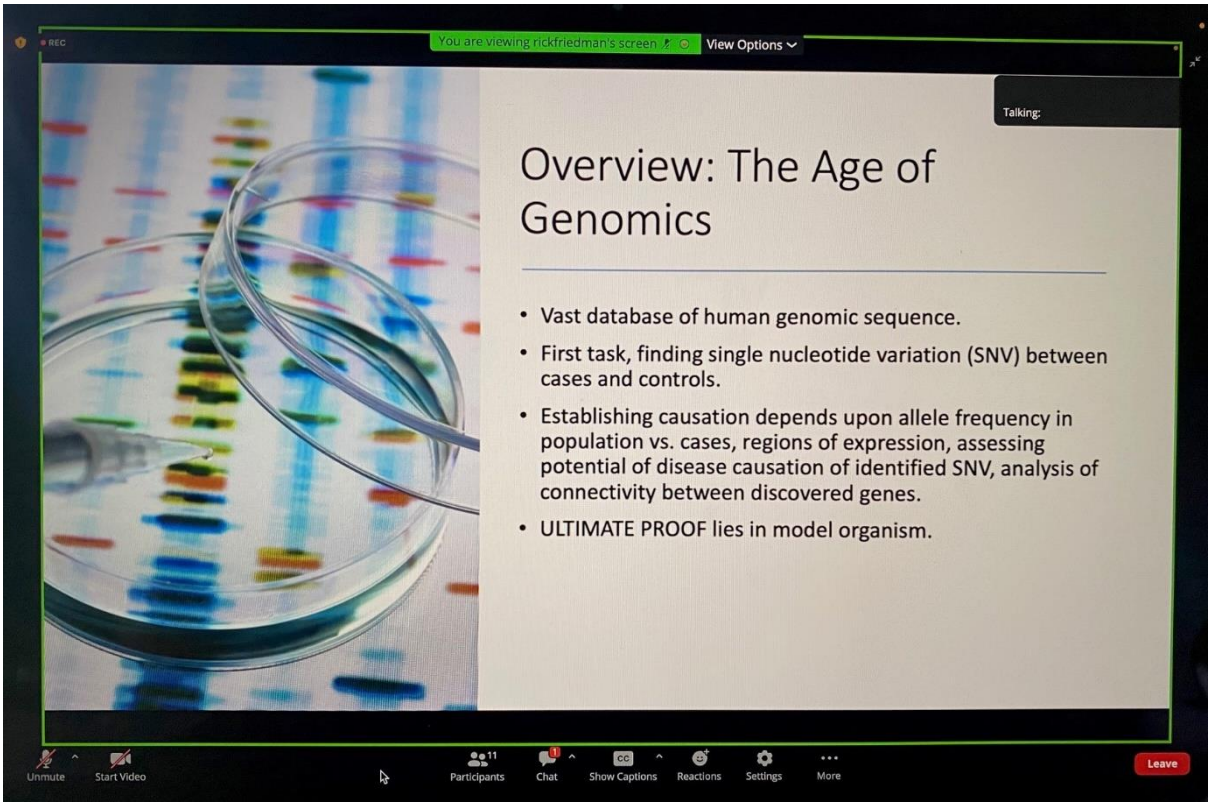
Rick Friedman
Bio:

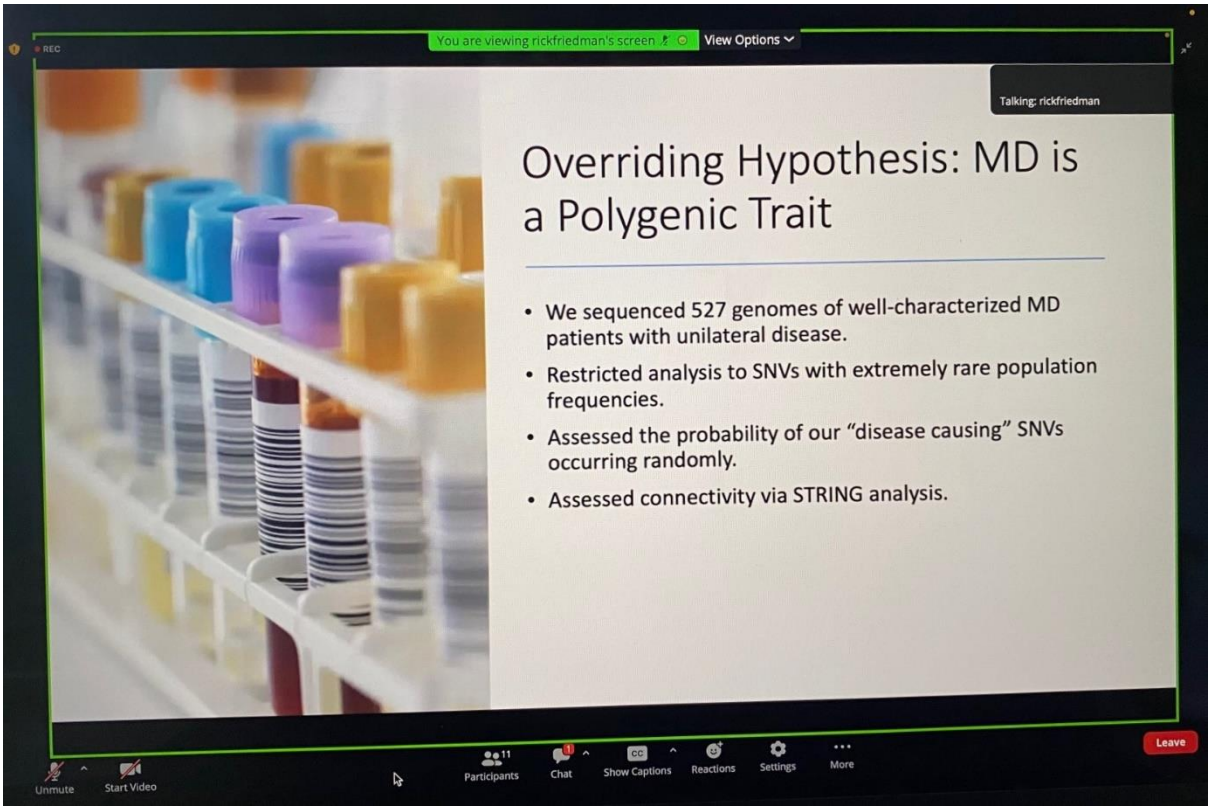
Dr. Rick Friedman received his Medical Degree in 1988 and completed his Doctoral Degree in 1994 at the University of California, San Diego. While earning his P.h.D., Friedman served as a resident in the Division of

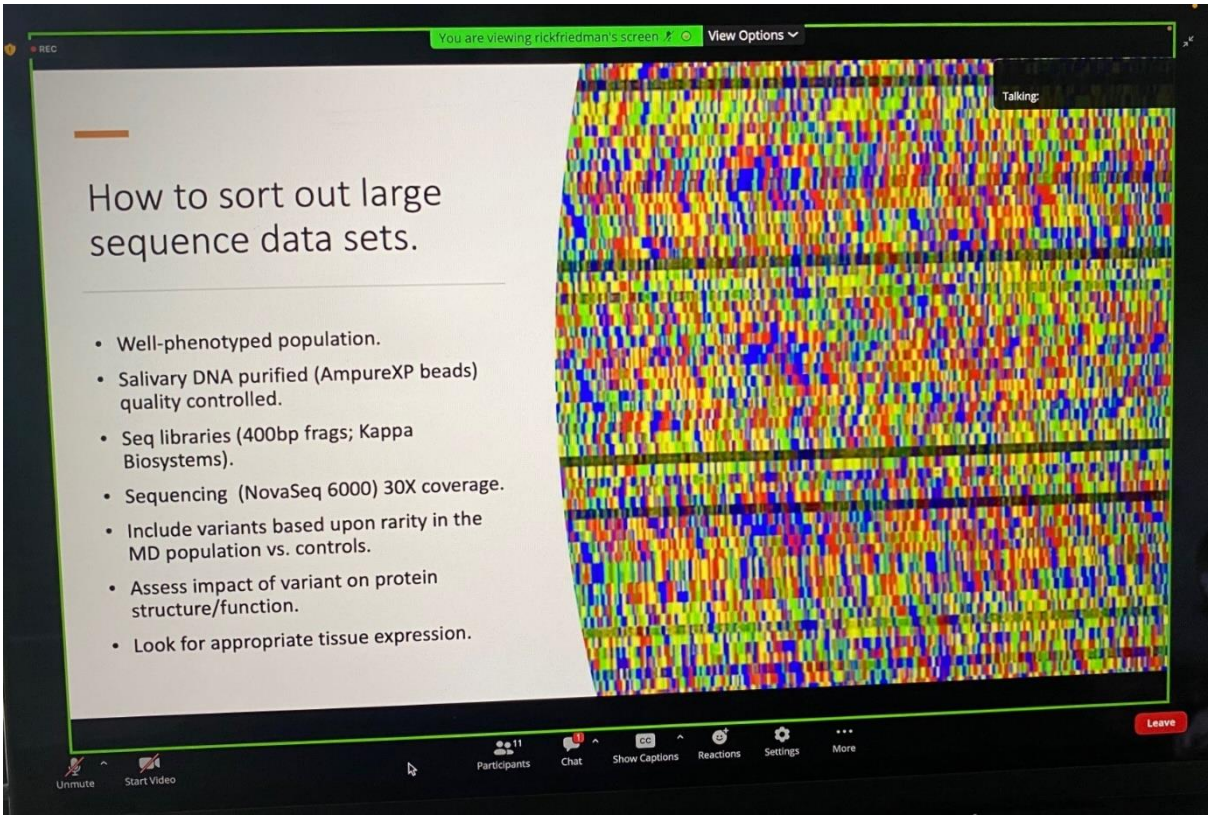
Otolaryngology at the UCSD Medical Center. In 1995, Dr. Friedman came to the House Clinic as a clinical fellow and then joined the group as an Associate. Dr. Friedman also serves as research section chief of Hereditary Ear Disorders in the House Ear Institute's Department of Cell and Molecular Biology. The focus of his laboratory is the identification and analysis of genes and networks contributing to complex traits involving hearing and balance in humans and mice [Presbycusis (ARHI), Meniere's Disease (MD), and Noise-Induced Hearing Loss (NIHL)]. We identified and association between ARHI and SNP variation within the GRM7 gene. We have collected the largest sample to date of individuals with Meniere's disease and seek to identify associated genes and pathways. We are incorporating a systems-based genetic approach consisting of the integration of natural genetic variation in inbred strains and molecular phenotypes (cochlear eQTL) to study ARHI and NIHL in mice. In collaboration with A. Jake Lusis, Ph.D. we are adapting the Hybrid Mouse Diversity Panel as a resource for studying complex traits in the mouse (ARHI, NIHL, and vestibular function).

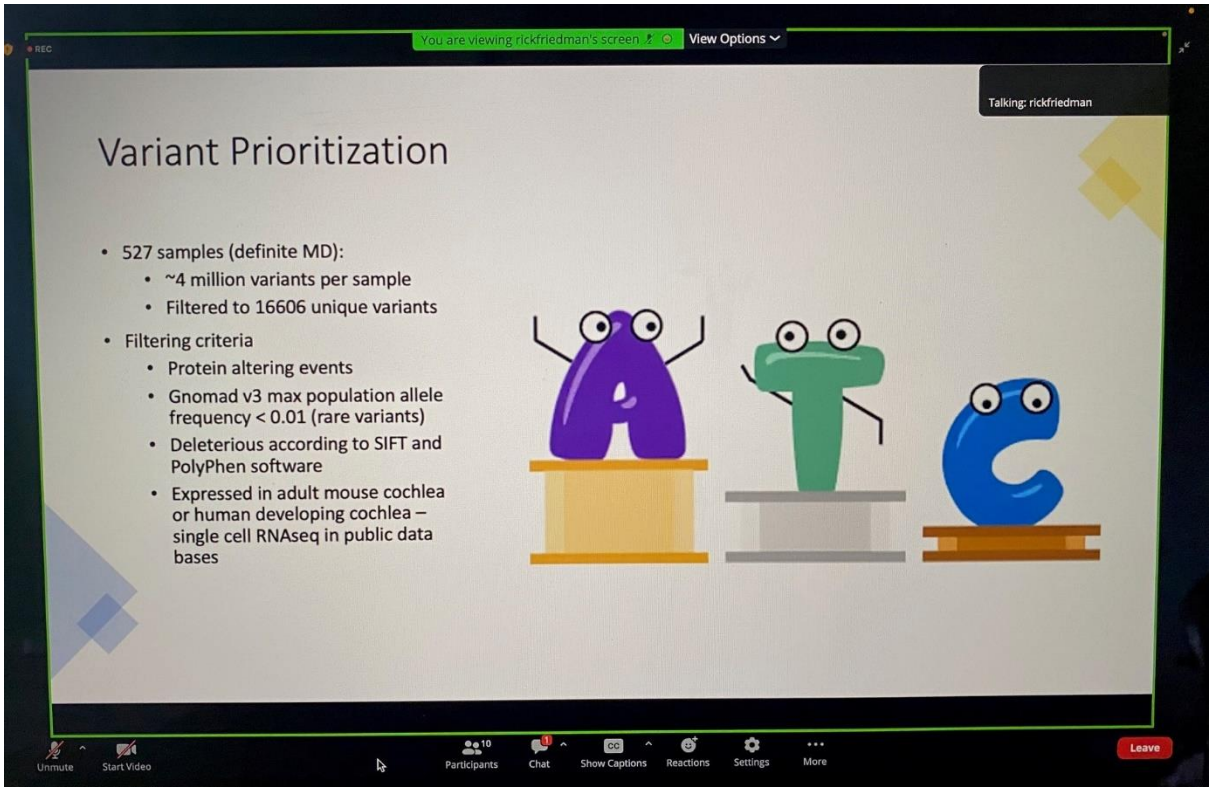
Rick Friedman, in talking on Zoom:

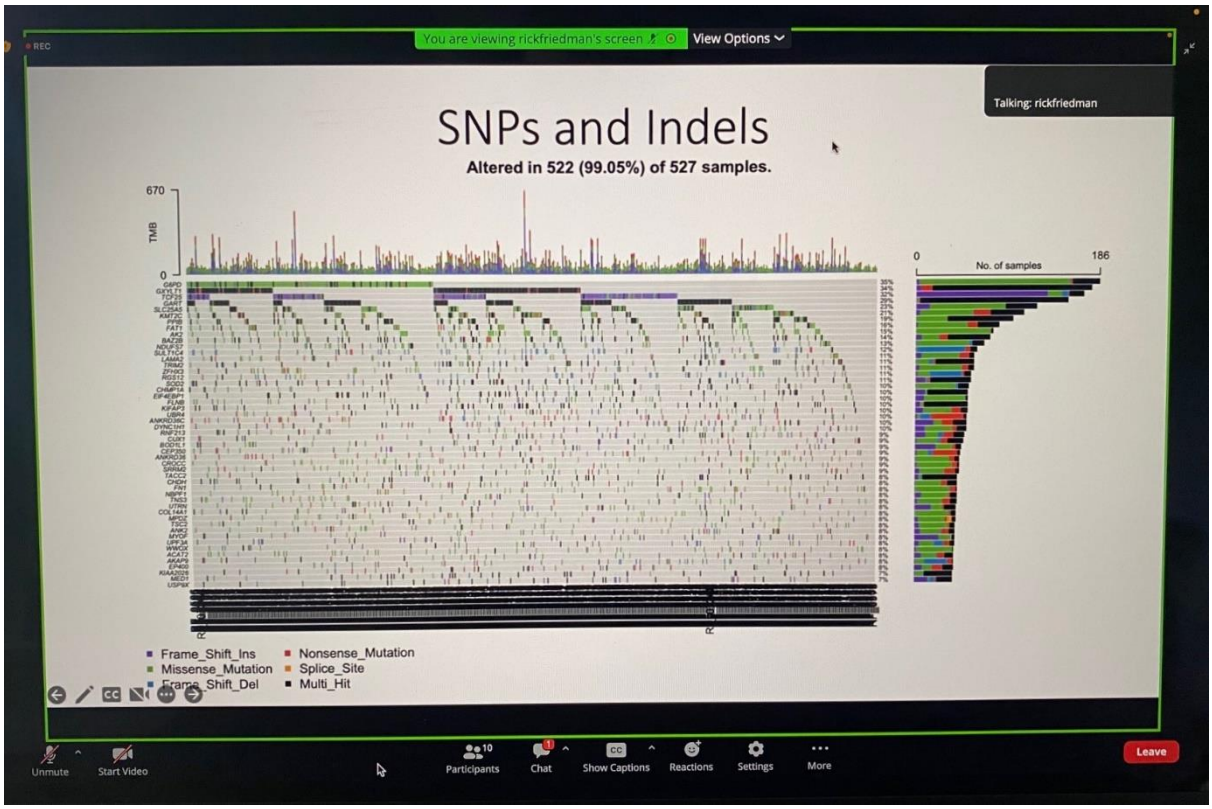
- Is mapping the genome of MD – analysis of the whole human genome
- 21 genes explain 95.3% of cases
- Defect in the ion transport protein
- CRISPR for direct targeted treatment for MD
- Changes in genetic structure
- Retinal cells (eyes) mimic hair cells in the inner ear

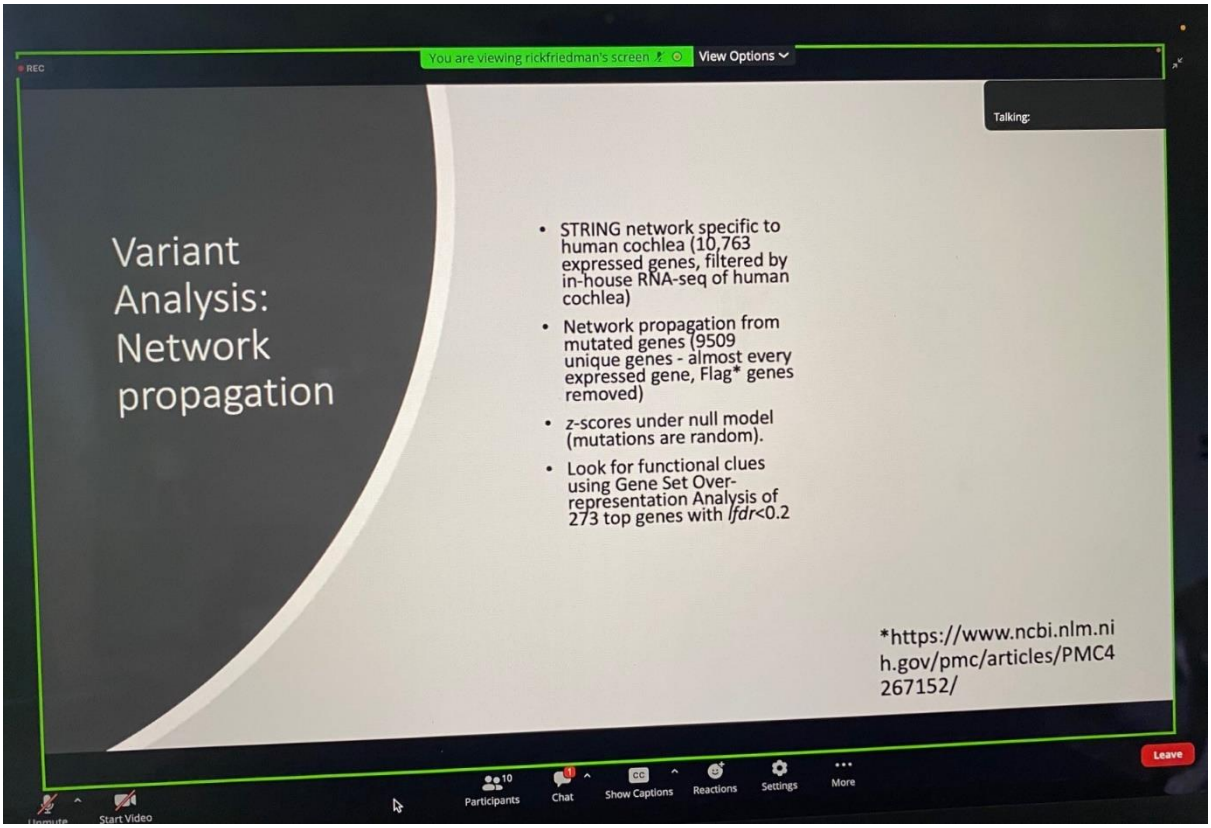


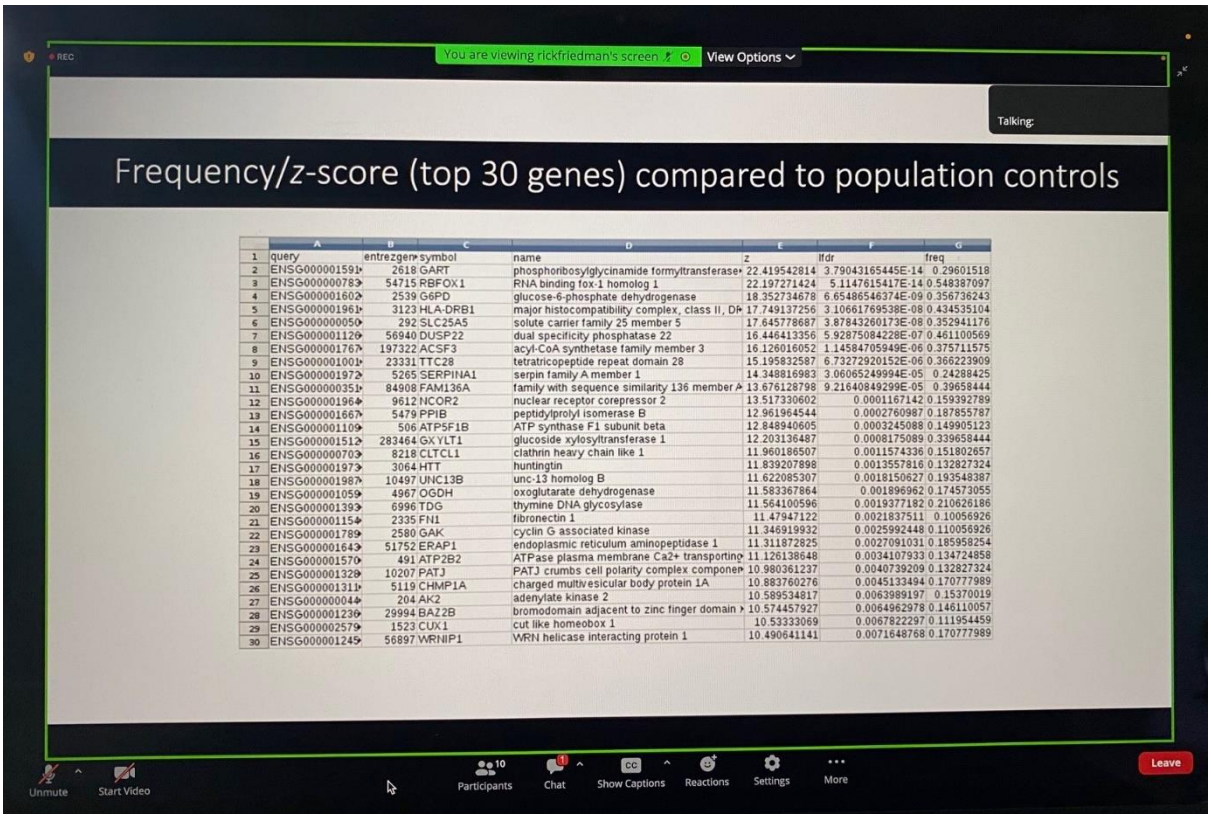

You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
Overriding Hypothesis: MD is a Polygenic Trait
• We sequenced 527 genomes of well-characterized MD patients with unilateral disease.
• Restricted analysis to SNVs with extremely rare population frequencies.
• Assessed the probability of our "disease causing" SNVs occurring randomly.
• Assessed connectivity via STRING analysis.
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave


You are viewing rickfriedman's screen
View Options
Talking:
How to sort out large sequence data sets.
• Well-phenotyped population.
• Salivary DNA purified (AmpureXP beads) quality controlled.
• Seq libraries (400bp frags; Kappa Biosystems).
• Sequencing (NovaSeq 6000) 30X coverage.
• Include variants based upon rarity in the MD population vs. controls.
• Assess impact of variant on protein structure/function.
• Look for appropriate tissue expression.
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave

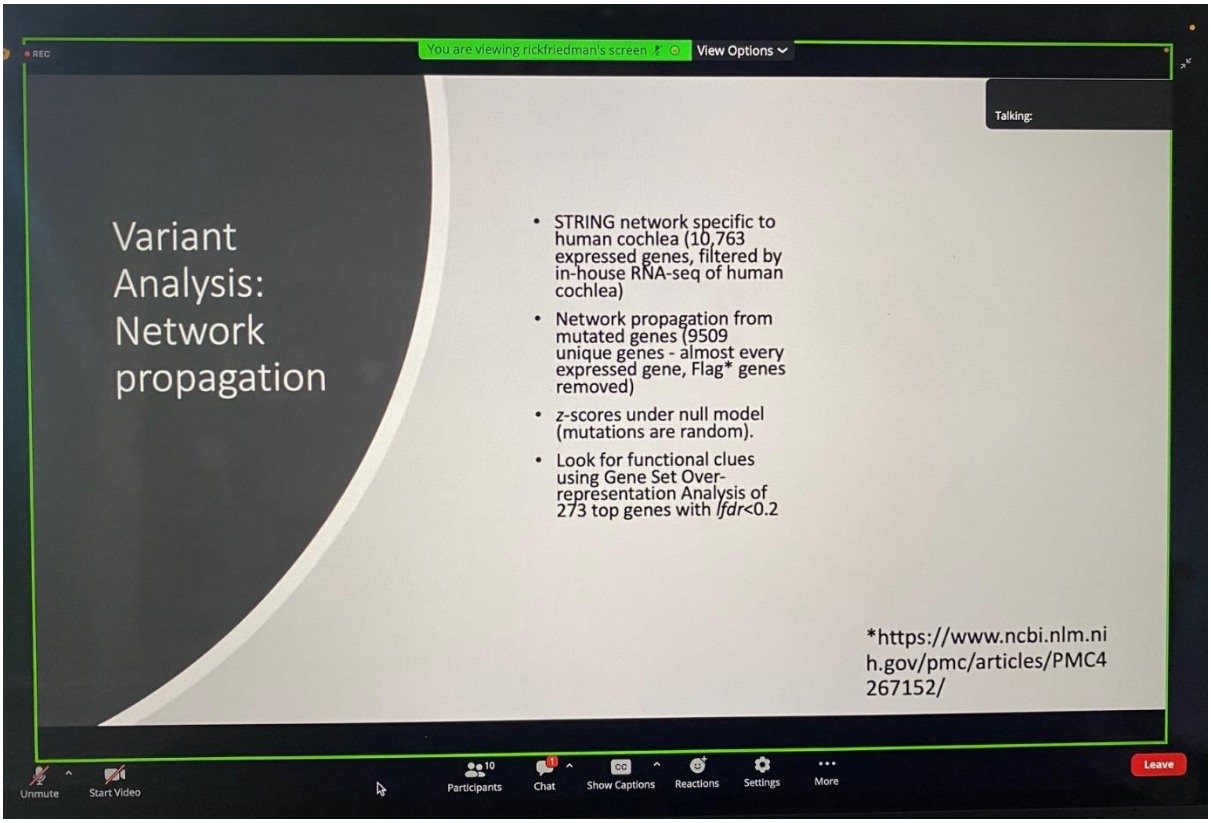

You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
Variant Prioritization
• 527 samples (definite MD):
• ~4 million variants per sample
• Filtered to 16606 unique variants
• Filtering criteria
• Protein altering events
• Gnomad v3 max population allele frequency < 0.01 (rare variants)
• Deleterious according to SIFT and PolyPhen software
• Expressed in adult mouse cochlea or human developing cochlea – single cell RNAseq in public data bases
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave

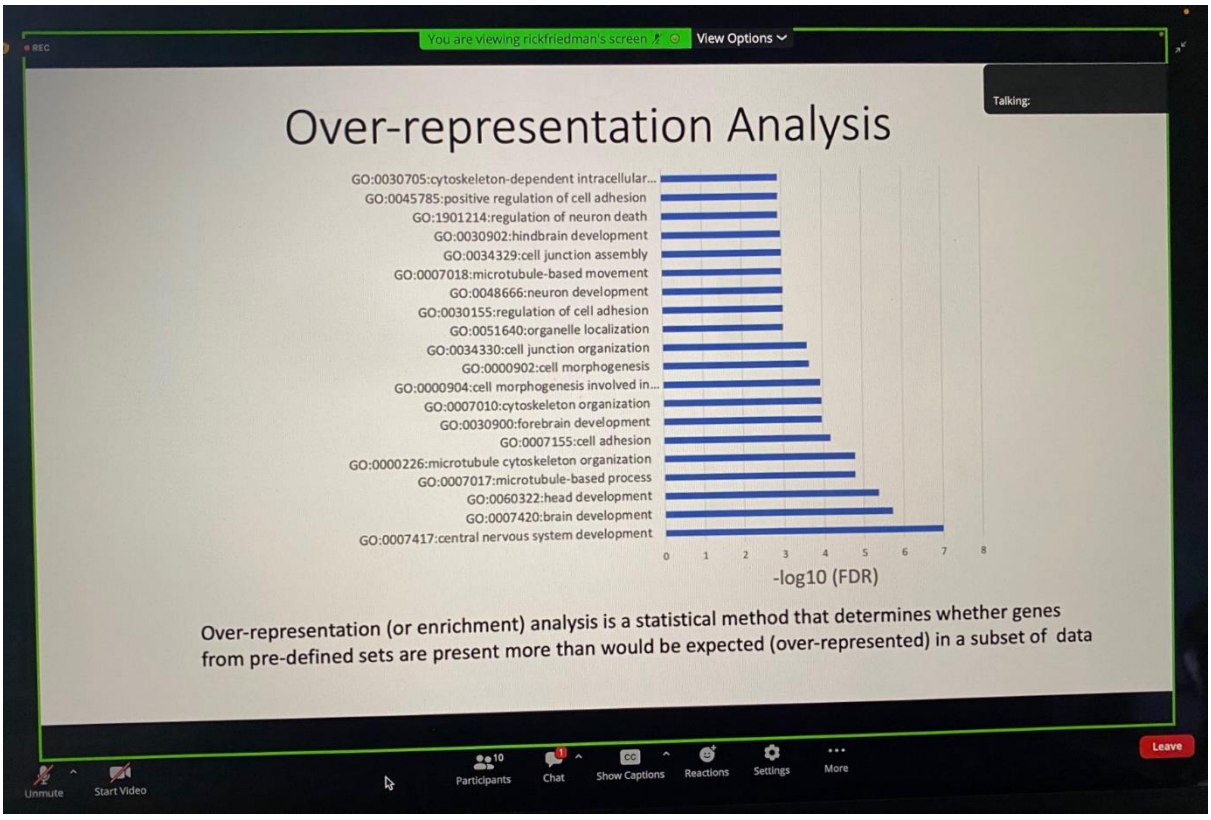

You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
SNPs and Indels
Altered in 522 (99.05%) of 527 samples.
TMB
670
0
No. of samples
0
186
Frame_Shift_Ins
Missense_Mutation
Frame_Shift_Del
Nonsense_Mutation
Splice_Site
Multi_Hit
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave

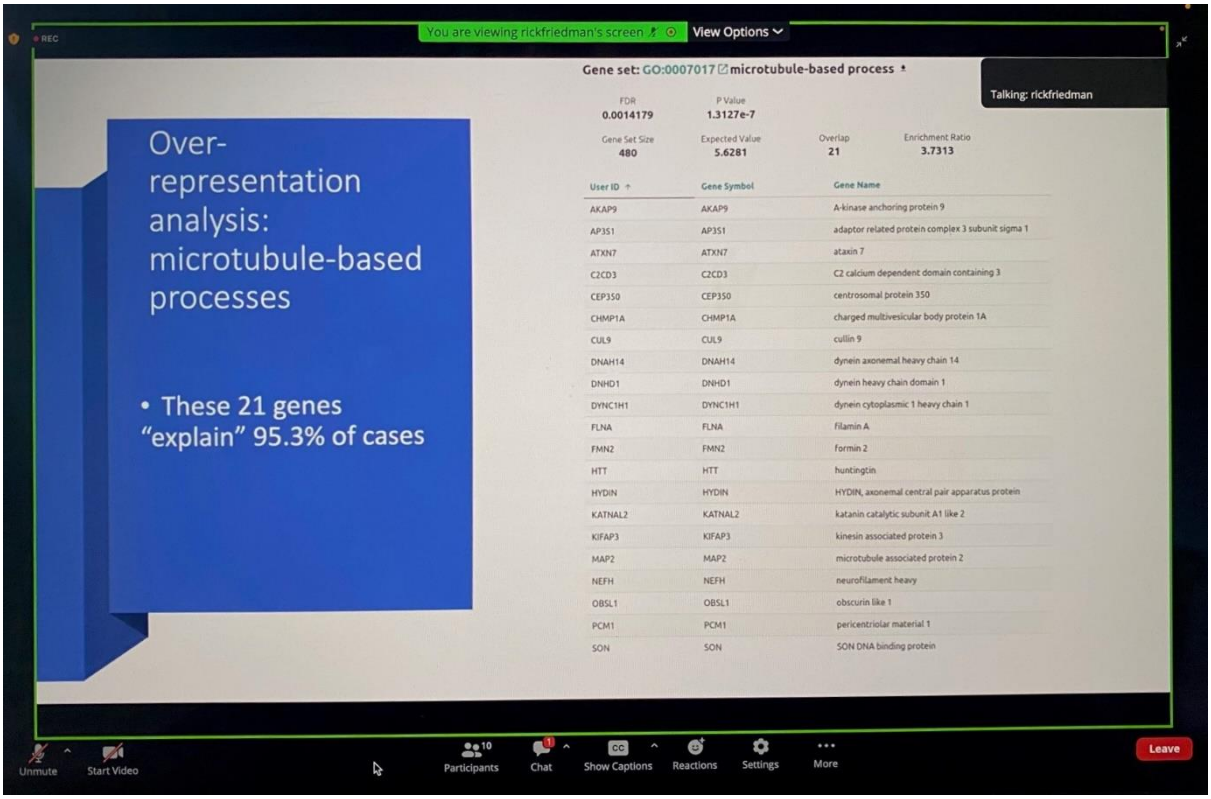


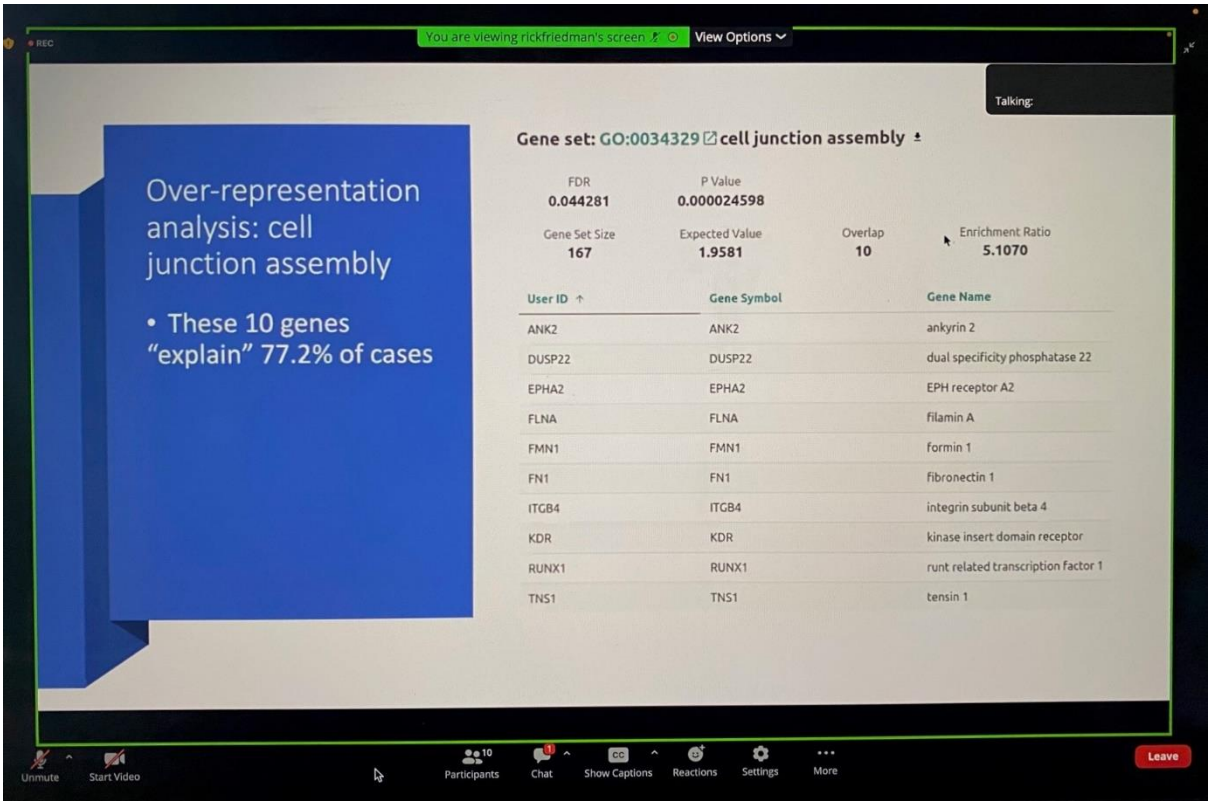


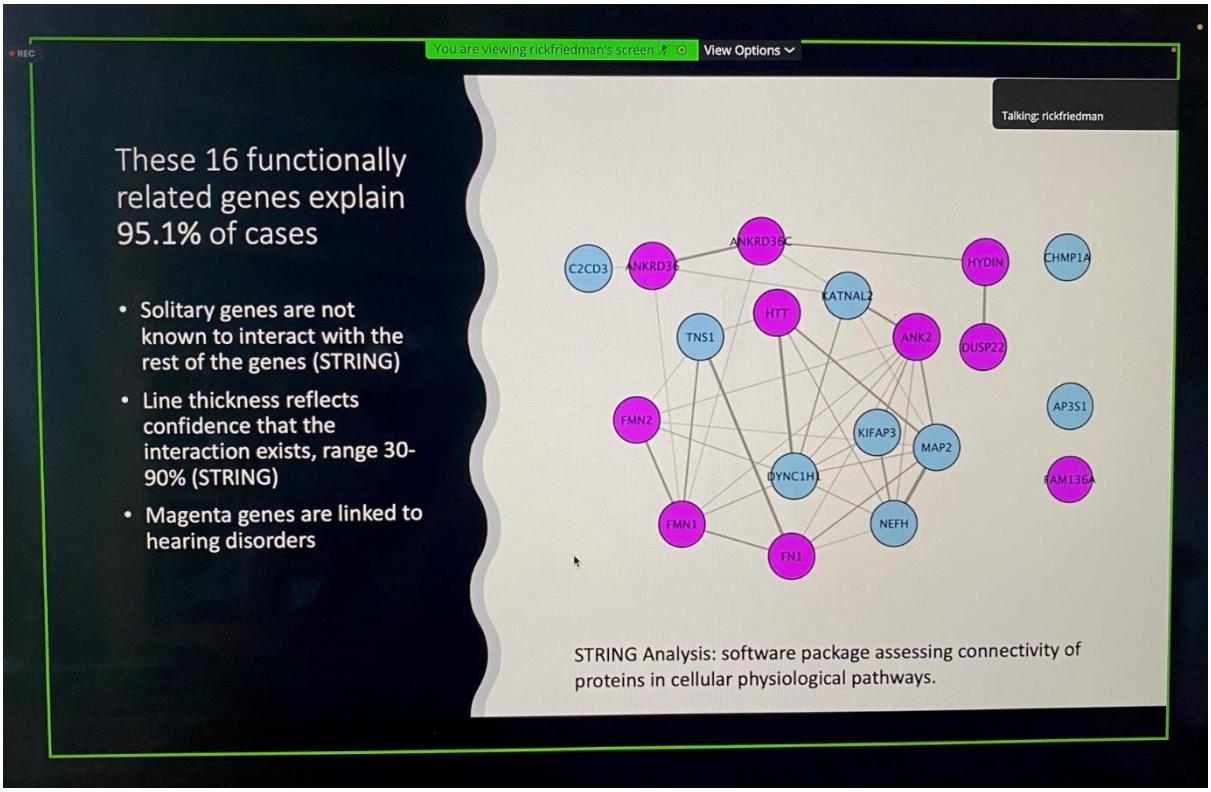
	A	B	C	D	E	F	G
1	query	entrezgen	symbol	name	z	lfdr	freq
2	ENSG00000159	2618	GART	phosphoribosylglycinamide formyltransferase	22.419542814	3.79043165445E-14	0.29601518
3	ENSG00000078	54715	RBFOX1	RNA binding fox-1 homolog 1	22.197271424	5.1147615417E-14	0.548387097
4	ENSG00000160	2539	G6PD	glucose-6-phosphate dehydrogenase	18.352734678	6.65486546374E-09	0.356736243
5	ENSG00000196	3123	HLA-DRB1	major histocompatibility complex, class II, DR	17.749137256	3.10661769538E-08	0.434535104
6	ENSG00000005	292	SLC25A5	solute carrier family 25 member 5	17.645778687	3.87843260173E-08	0.352941176
7	ENSG00000112	56940	DUSP22	dual specificity phosphatase 22	16.446413356	5.92875084228E-07	0.461100569
8	ENSG00000176	197322	ACSF3	acyl-CoA synthetase family member 3	16.126016052	1.14584705949E-06	0.375711575
9	ENSG00000100	23331	TTC28	tetratricopeptide repeat domain 28	15.195832587	6.73272920152E-06	0.366223909
10	ENSG00000197	5265	SERPINA1	serpin family A member 1	14.348816983	3.06065249994E-05	0.24288425
11	ENSG00000035	84908	FAM136A	family with sequence similarity 136 member A	13.676128798	9.21640849299E-05	0.39658444
12	ENSG00000196	9612	NCOR2	nuclear receptor corepressor 2	13.517330602	0.0001167142	0.159392789
13	ENSG00000166	5479	PPIB	peptidylprolyl isomerase B	12.961964544	0.0002760987	0.187855787
14	ENSG00000110	506	ATP5F1B	ATP synthase F1 subunit beta	12.848940605	0.0003245088	0.149905123
15	ENSG00000151	283464	GXYLT1	glucoside xylosyltransferase 1	12.203136487	0.0008175089	0.339658444
16	ENSG00000070	8218	CLTCL1	clathrin heavy chain like 1	11.960186507	0.0011574336	0.151802657
17	ENSG00000197	3064	HTT	huntingtin	11.839207898	0.0013557816	0.132827324
18	ENSG00000198	10497	UNC13B	unc-13 homolog B	11.622085307	0.0018150627	0.193548387
19	ENSG00000105	4967	OGDH	oxoglutarate dehydrogenase	11.583367864	0.001896962	0.174573055
20	ENSG00000139	6996	TDG	thymine DNA glycosylase	11.564100596	0.0019377182	0.210626186
21	ENSG00000115	2335	FN1	fibronectin 1	11.47947122	0.0021837511	0.10056926
22	ENSG00000178	2580	GAK	cyclin G associated kinase	11.346919932	0.0025992448	0.110056926
23	ENSG00000164	51752	ERAP1	endoplasmic reticulum aminopeptidase 1	11.311872825	0.0027091031	0.185958254
24	ENSG00000157	491	ATP2B2	ATPase plasma membrane Ca2+ transporting	11.126138648	0.0034107933	0.134724858
25	ENSG00000132	10207	PATJ	PATJ crumbs cell polarity complex componen	10.980361237	0.0040739209	0.132827324
26	ENSG00000131	5119	CHMP1A	charged multivesicular body protein 1A	10.883760276	0.0045133494	0.170777989
27	ENSG00000004	204	AK2	adenylate kinase 2	10.589534817	0.0063989197	0.15370019
28	ENSG00000123	29994	BAZ2B	bromodomain adjacent to zinc finger domain	10.574457927	0.0064962978	0.146110057
29	ENSG00000257	1523	CUX1	cut like homeobox 1	10.53333069	0.0067822297	0.111954459
30	ENSG00000124	56897	WRNIP1	WRN helicase interacting protein 1	10.490641141	0.0071648768	0.170777989

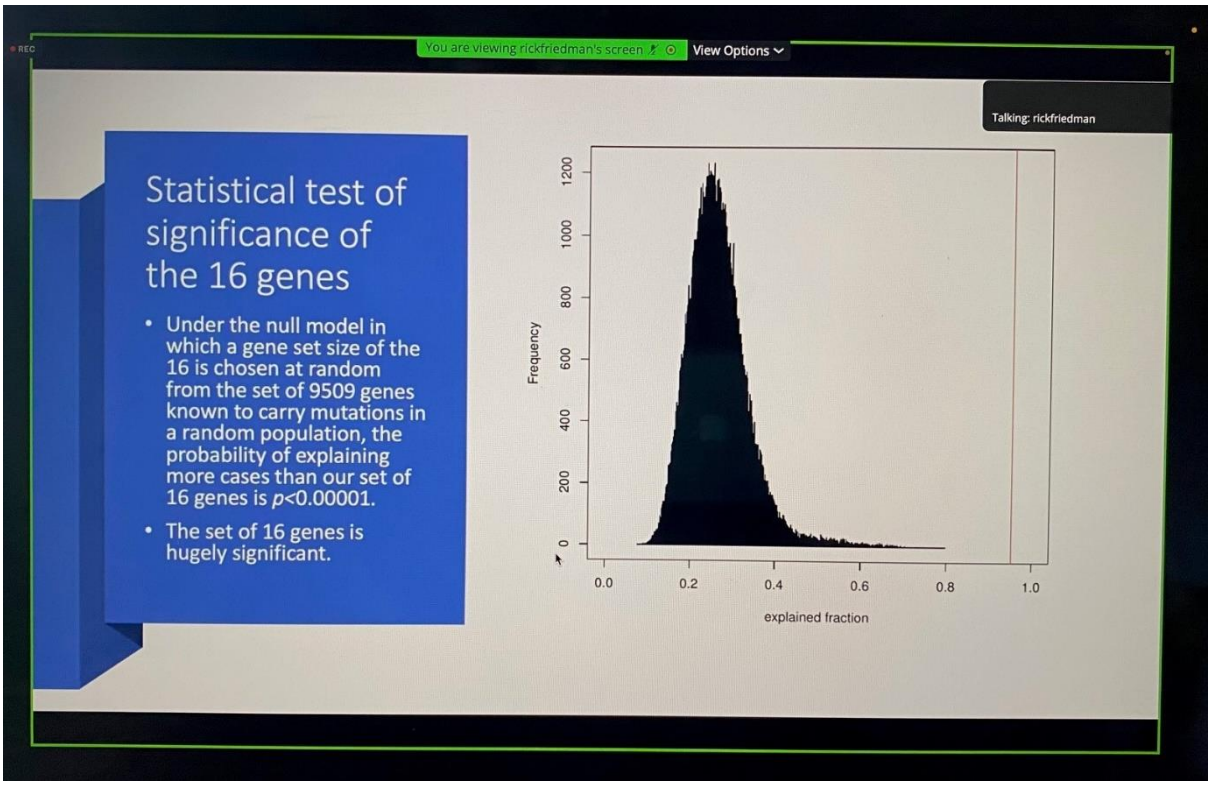

You are viewing rickfriedman's screen
View Options
Talking:
Variant Analysis: Network propagation
• STRING network specific to human cochlea (10,763 expressed genes, filtered by in-house RNA-seq of human cochlea)
• Network propagation from mutated genes (9509 unique genes - almost every expressed gene, Flag* genes removed)
• z-scores under null model (mutations are random).
• Look for functional clues using Gene Set Over-representation Analysis of 273 top genes with lfdr<0.2
*https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4267152/
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave

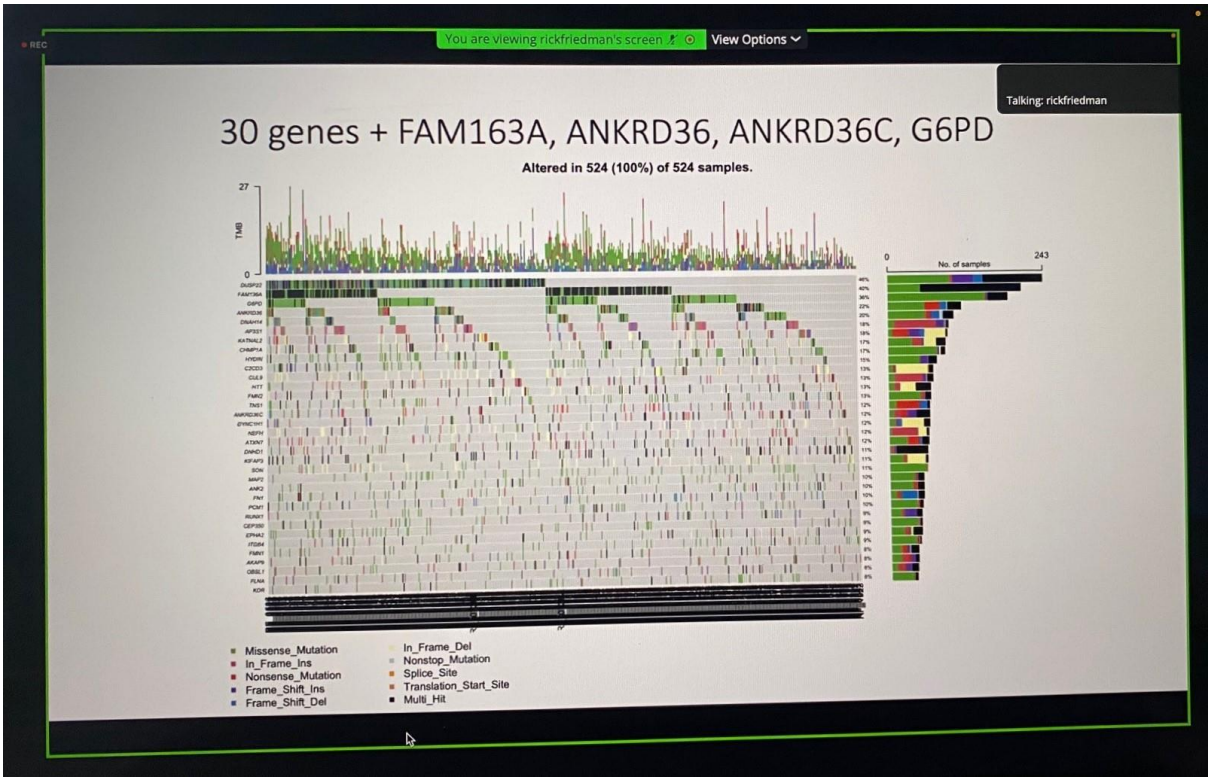

You are viewing rickfriedman's screen
View Options
Talking:
Over-representation Analysis
GO:0030705:cytoskeleton-dependent intracellular...
GO:0045785:positive regulation of cell adhesion
GO:1901214:regulation of neuron death
GO:0030902:hindbrain development
GO:0034329:cell junction assembly
GO:0007018:microtubule-based movement
GO:0048666:neuron development
GO:0030155:regulation of cell adhesion
GO:0051640:organelle localization
GO:0034330:cell junction organization
GO:0000902:cell morphogenesis
GO:0000904:cell morphogenesis involved in...
GO:0007010:cytoskeleton organization
GO:0030900:forebrain development
GO:0007155:cell adhesion
GO:0000226:microtubule cytoskeleton organization
GO:0007017:microtubule-based process
GO:0060322:head development
GO:0007420:brain development
GO:0007417:central nervous system development
0 1 2 3 4 5 6 7 8
-log10 (FDR)
Over-representation (or enrichment) analysis is a statistical method that determines whether genes from pre-defined sets are present more than would be expected (over-represented) in a subset of data
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave

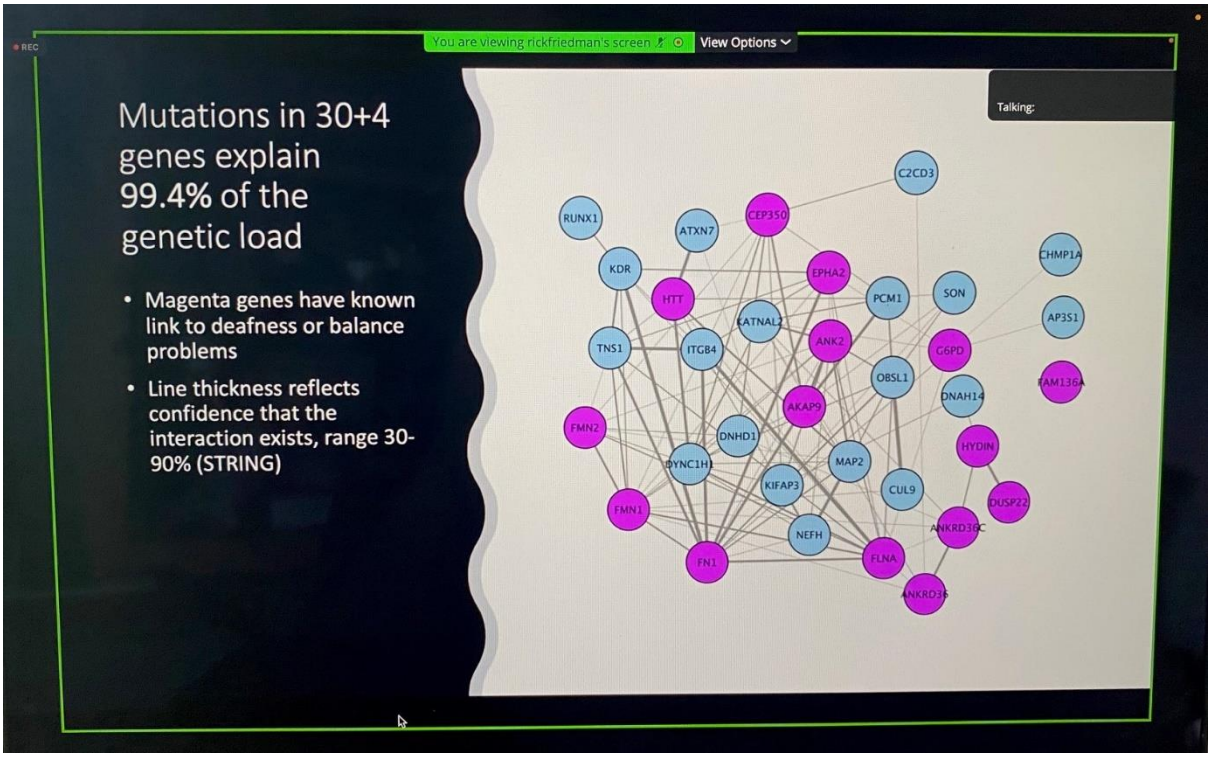

You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
Over-representation analysis: microtubule-based processes
• These 21 genes "explain" 95.3% of cases
Gene set: GO:0007017 microtubule-based process
FDR 0.0014179
P Value 1.3127e-7
Gene Set Size 480
Expected Value 5.6281
Overlap 21
Enrichment Ratio 3.7313
User ID | Gene Symbol | Gene Name
AKAP9 | AKAP9 | A-kinase anchoring protein 9
AP3S1 | AP3S1 | adaptor related protein complex 3 subunit sigma 1
ATXN7 | ATXN7 | ataxin 7
C2CD3 | C2CD3 | C2 calcium dependent domain containing 3
CEP350 | CEP350 | centrosomal protein 350
CHMP1A | CHMP1A | charged multivesicular body protein 1A
CUL9 | CUL9 | cullin 9
DNAH14 | DNAH14 | dynein axonemal heavy chain 14
DNHD1 | DNHD1 | dynein heavy chain domain 1
DYNC1H1 | DYNC1H1 | dynein cytoplasmic 1 heavy chain 1
FLNA | FLNA | filamin A
FMN2 | FMN2 | formin 2
HTT | HTT | huntingtin
HYDIN | HYDIN | HYDIN, axonemal central pair apparatus protein
KATNAL2 | KATNAL2 | katanin catalytic subunit A1 like 2
KIFAP3 | KIFAP3 | kinesin associated protein 3
MAP2 | MAP2 | microtubule associated protein 2
NEFH | NEFH | neurofilament heavy
OBSL1 | OBSL1 | obscurin like 1
PCM1 | PCM1 | pericentriolar material 1
SON | SON | SON DNA binding protein
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave

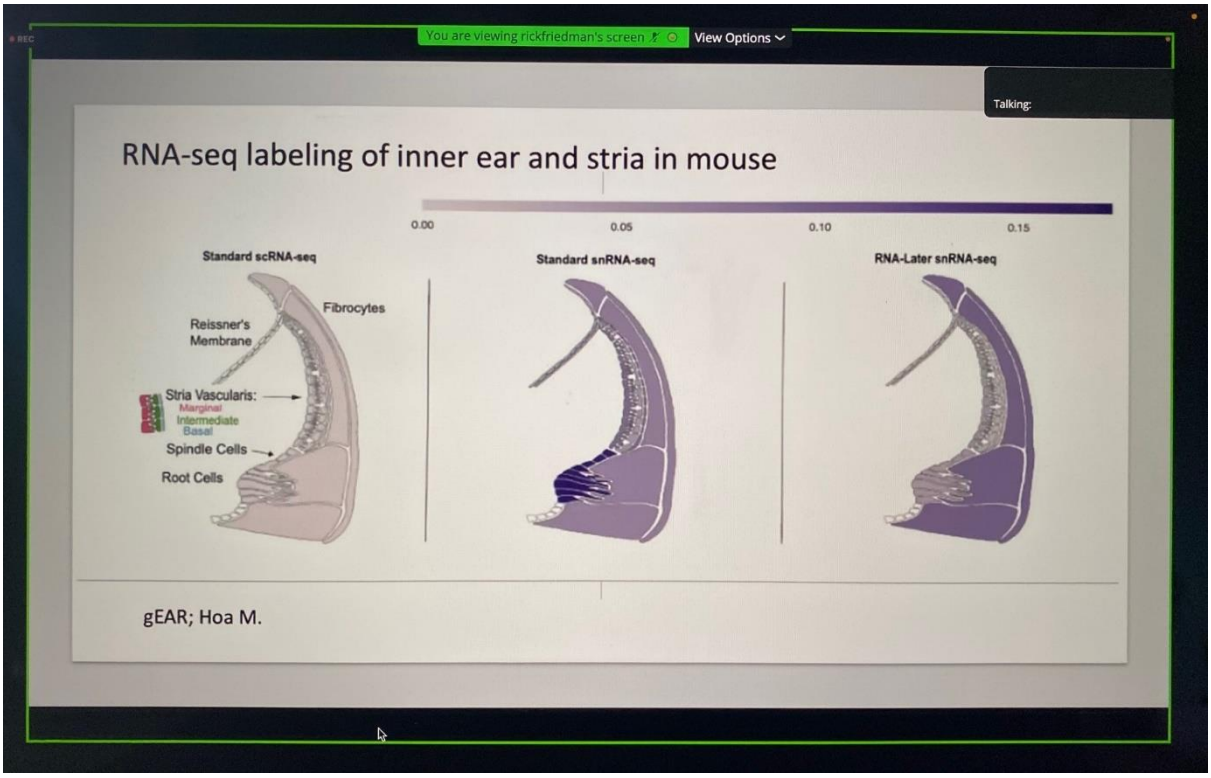

You are viewing rickfriedman's screen
View Options
Talking:
Over-representation analysis: cell junction assembly
• These 10 genes "explain" 77.2% of cases
Gene set: GO:0034329 cell junction assembly
FDR 0.044281
P Value 0.000024598
Gene Set Size 167
Expected Value 1.9581
Overlap 10
Enrichment Ratio 5.1070
User ID | Gene Symbol | Gene Name
ANK2 | ANK2 | ankyrin 2
DUSP22 | DUSP22 | dual specificity phosphatase 22
EPHA2 | EPHA2 | EPH receptor A2
FLNA | FLNA | filamin A
FMN1 | FMN1 | formin 1
FN1 | FN1 | fibronectin 1
ITGB4 | ITGB4 | integrin subunit beta 4
KDR | KDR | kinase insert domain receptor
RUNX1 | RUNX1 | runt related transcription factor 1
TNS1 | TNS1 | tensin 1
Unmute
Start Video
Participants
Chat
Show Captions
Reactions
Settings
More
Leave

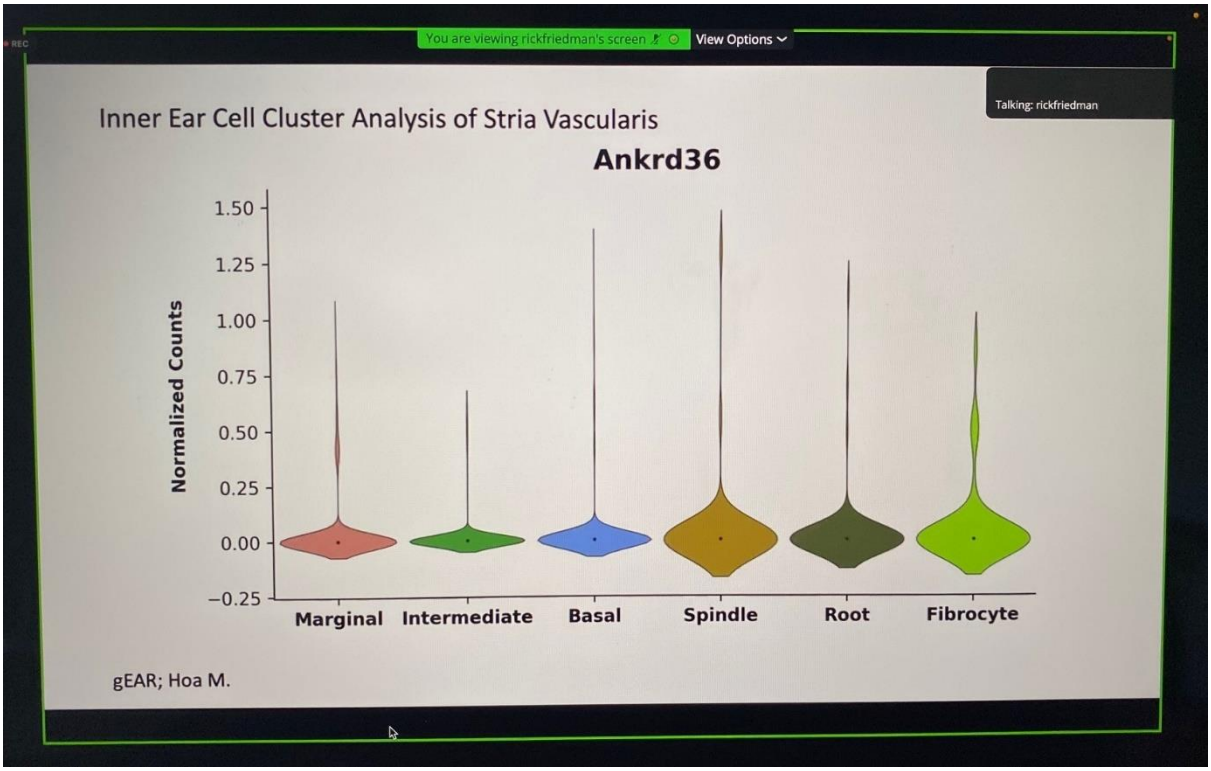

You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
These 16 functionally related genes explain 95.1% of cases
• Solitary genes are not known to interact with the rest of the genes (STRING)
• Line thickness reflects confidence that the interaction exists, range 30-90% (STRING)
• Magenta genes are linked to hearing disorders
ANKRD36C
C2CD3
ANKRD36
HYDIN
CHMP1A
KATNAL2
HTT
TNS1
ANK2
DUSP22
AP3S1
FMN2
KIFAP3
MAP2
DYNC1H1
FAM136A
FMN1
NEFH
FN1
STRING Analysis: software package assessing connectivity of proteins in cellular physiological pathways.

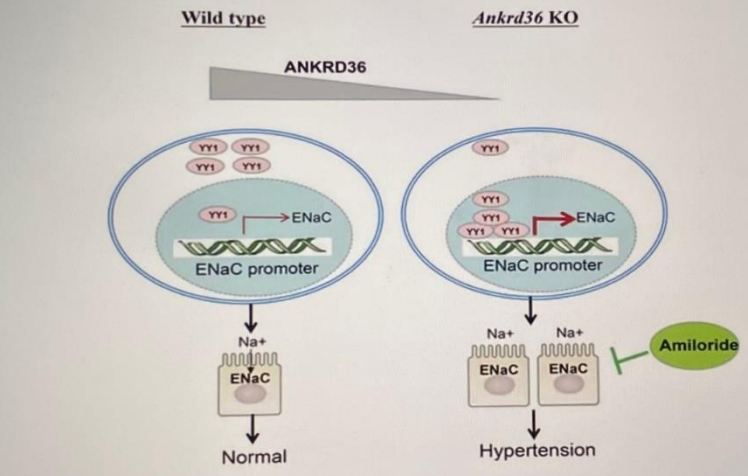

You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
Statistical test of significance of the 16 genes
• Under the null model in which a gene set size of the 16 is chosen at random from the set of 9509 genes known to carry mutations in a random population, the probability of explaining more cases than our set of 16 genes is $p<0.00001$.
• The set of 16 genes is hugely significant.
Frequency
0
200
400
600
800
1000
1200
0.0
0.2
0.4
0.6
0.8
1.0
explained fraction


You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
30 genes + FAM163A, ANKRD36, ANKRD36C, G6PD
Altered in 524 (100%) of 524 samples.
TMB
27
0
No. of samples
0
243
Missense_Mutation
In_Frame_Ins
Nonsense_Mutation
Frame_Shift_Ins
Frame_Shift_Del
In_Frame_Del
Nonstop_Mutation
Splice_Site
Translation_Start_Site
Multi_Hit


You are viewing rickfriedman's screen
View Options
Talking:
Mutations in 30+4 genes explain 99.4% of the genetic load
• Magenta genes have known link to deafness or balance problems
• Line thickness reflects confidence that the interaction exists, range 30-90% (STRING)
C2CD3
RUNX1
ATXN7
CEP350
CHMP1A
KDR
EPHA2
HTT
PCM1
SON
AP3S1
KATNAL2
TNS1
ITGB4
ANK2
G6PD
OBSL1
FAM136A
DNAH14
AKAP9
FMN2
DNHD1
HYDIN
DYNC1H1
MAP2
KIFAP3
CUL9
DUSP22
FMN1
ANKRD36C
NEFH
FN1
FLNA
ANKRD36


You are viewing rickfriedman's screen
View Options
Talking:
RNA-seq labeling of inner ear and stria in mouse
0.00
0.05
0.10
0.15
Standard scRNA-seq
Standard snRNA-seq
RNA-Later snRNA-seq
Fibrocytes
Reissner's Membrane
Stria Vascularis:
Marginal
Intermediate
Basal
Spindle Cells
Root Cells
gEAR; Hoa M.

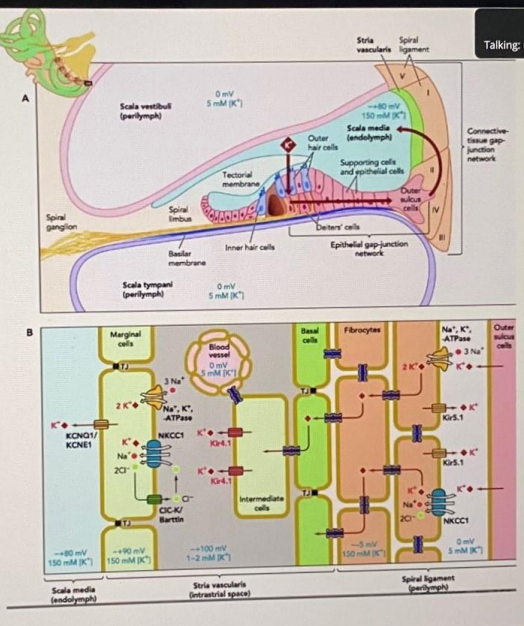

You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
Inner Ear Cell Cluster Analysis of Stria Vascularis
Ankrd36
Normalized Counts
1.50
1.25
1.00
0.75
0.50
0.25
0.00
−0.25
Marginal
Intermediate
Basal
Spindle
Root
Fibrocyte
gEAR; Hoa M.

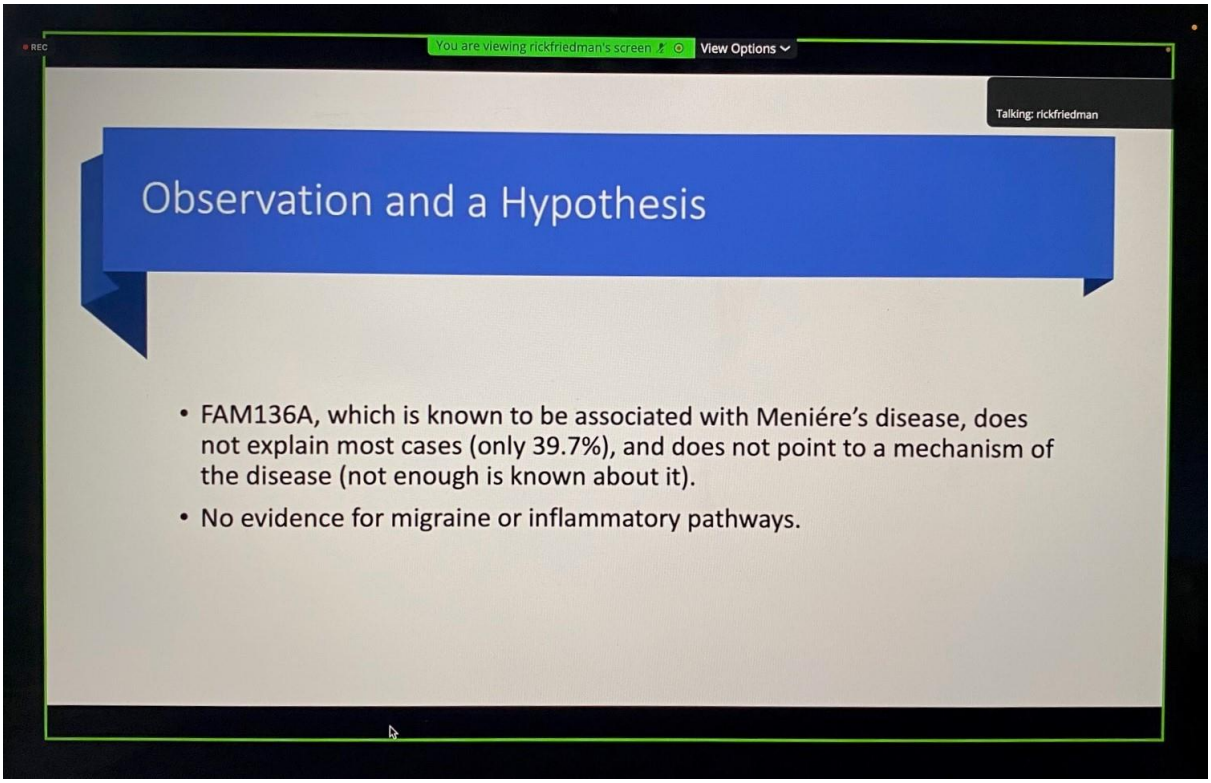


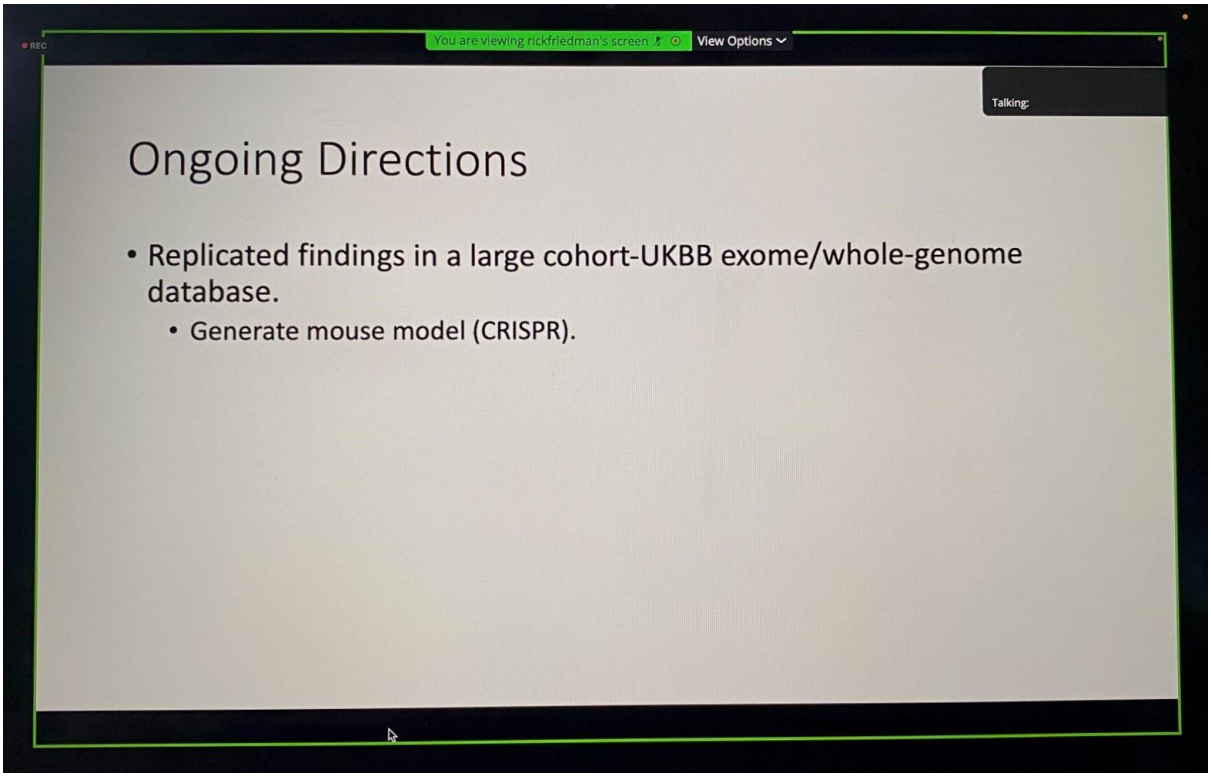
Yupeng Yan. Circulation Research. ANKRD36 Is Involved in Hypertension by Altering Expression of ENaC Genes, Volume: 129, Issue: 11, Pages: 1067-1081, DOI: (10.1161/CIRCRESAHA.121.319883)

Conclusions Based Upon Data: Cell-Cell/ECM interface

- Popular thought: increased endolymph production or reduced reabsorption.
- Aberrations in Cell-Cell/ECM interface leading to swelling within the endolymphatic space?




REC
You are viewing rickfriedman's screen
View Options
Talking: rickfriedman
Observation and a Hypothesis
• FAM136A, which is known to be associated with Meniére's disease, does not explain most cases (only 39.7%), and does not point to a mechanism of the disease (not enough is known about it).
• No evidence for migraine or inflammatory pathways.


REC
You are viewing rickfriedman's screen
View Options
Talking:
Ongoing Directions
• Replicated findings in a large cohort-UKBB exome/whole-genome database.
• Generate mouse model (CRISPR).

Next speaker:

Robert from Cochlear Australia:

- Could one therapy cure all – tinnitus, vertigo, hearing loss
- Are cochlear hydrops, vestibular hydrops and tinnitus 3 separate issues?
- Any damage to the inner ear can cause tinnitus

Prof Jeffrey Harris:

- Ruptured membranes in the inner ear can scar up
- Inheritance of MD is very low