1. Lecture opened with statement that in 161 years no significant discoveries uncovering the cause of Ménière's or a viable treatment have happened.
2. Clinical Presentation-
    a. Vertigo,aural pressure,roaring tinnitus and fluctuating hearing loss
    b. Hearing loss begins with low frequencies, then proceeds to a tent like audiogram and then a progression downwards.
3. Mean age of onset is 38 years. Mean age of diagnosis is 50-54 years.
4. Impact on Quality of life is severe as shown in slide below based on a survey of patients.

Impact of Meniere's disease on Quality of Life

Study mean	Study population description
.900	Probability sample of children in San Diego County
.810	Pre-injury (baseline) trauma recovery project adult patients
.788	Probability sample of adults in San Diego County
.720	Adult patients with non–insulin-dependent diabetes
.700	Patients treated at UCSD Regional Burn Treatment Center, at point of maximum recovery
.650	Trauma recovery project patients six months after release from hospital
.649	Baseline score for AIDS patients treated with AZT
.634	Baseline score for AIDS patients treated with placebo
.616	Baseline score for Health Care Utilization Project, No. 1 (HCUP1) patients: ill adults with life-threatening diseases (AIDS, cancer, renal, pulmonary, etc.); live patient scores stayed at about this level throughout the study
.600	Mean QWB study end score for AIDS patients treated with AZT
.561	**Baseline, Ménière's disease patients**
.550	Elderly adults with severe chronic obstructive pulmonary disease
.506	Noninstitutionalized Alzheimer's patients
.505	**Ménière's disease patients, days with active episode of disease**
.457	Patients with AIDS or cancer, six days before death

Representative scores to aid interpretation of Ménière's disease study Quality of Well-being (QWB) results

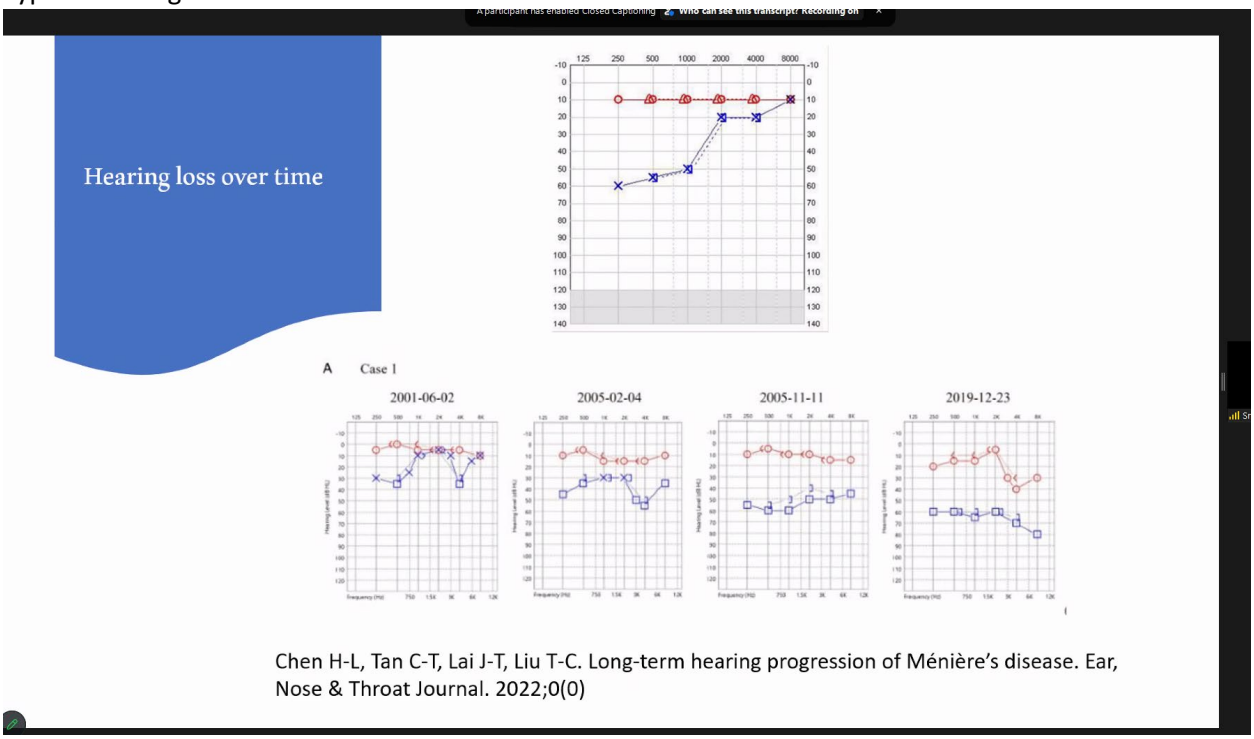
Source

Impact of Ménière's Disease on Quality of Life

Otology & Neurotology22(6):888-894, November 2001.

Anderson, J. P., & Harris, J. P. (2001). Impact of Meniere's disease on quality of life. Otology & Neurotology, 22(6), 888-894.

Typical hearing loss



Chen H-L, Tan C-T, Lai J-T, Liu T-C. Long-term hearing progression of Ménière's disease. Ear, Nose & Throat Journal. 2022;0(0)

Need to be careful with TX in case Bilateral develops

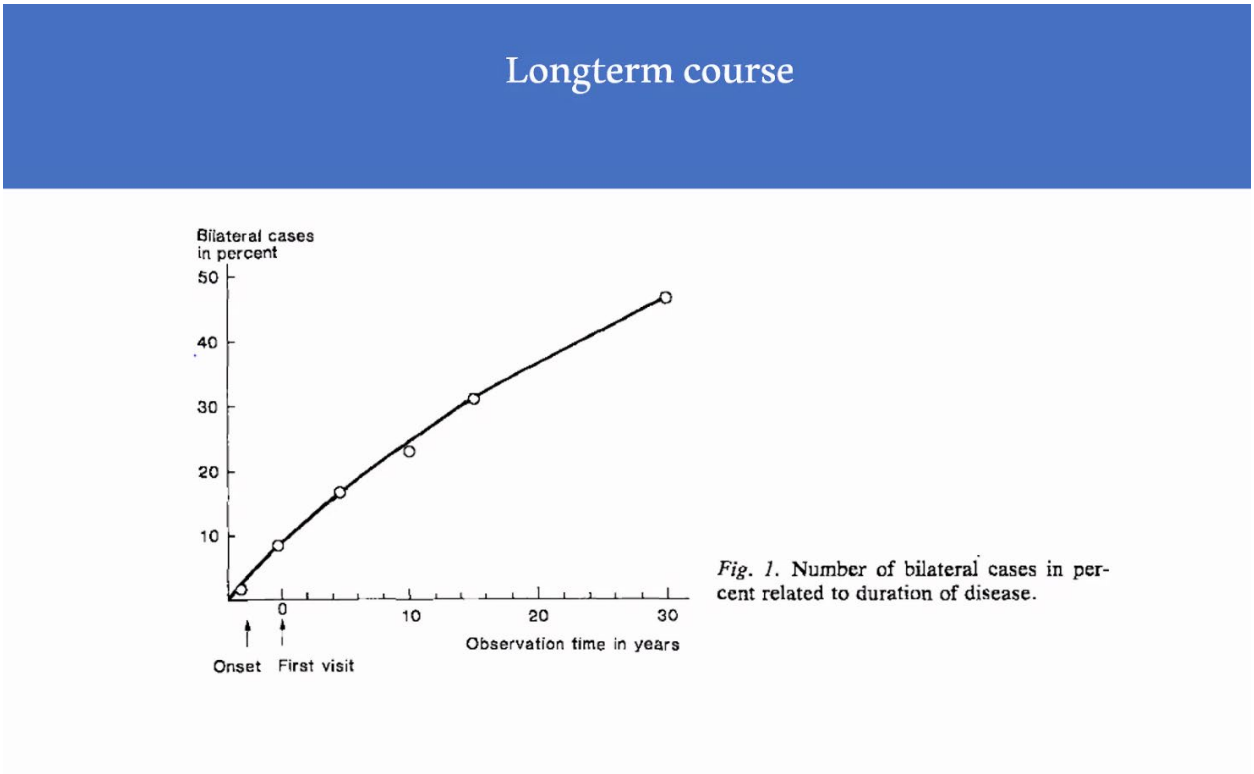
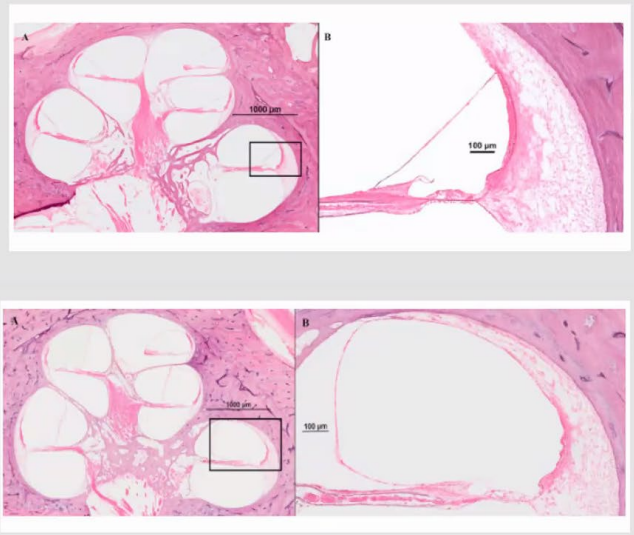


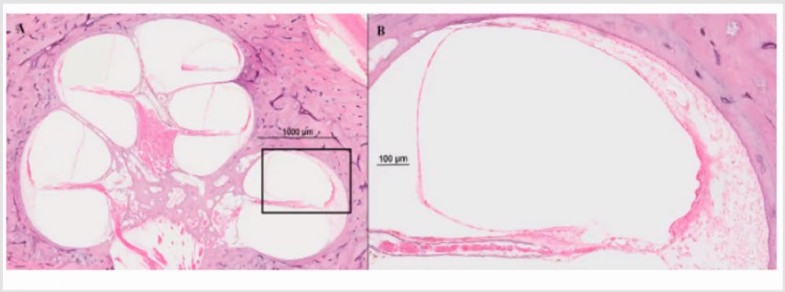
Fig. 1. Number of bilateral cases in percent related to duration of disease.

Histopathology of Meniere's disease



Courtesy of M. Paparella

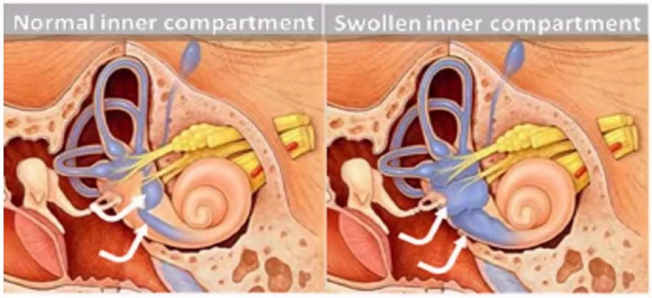
Meniere's disease is characterized by endolymphatic hydrops



Courtesy of M. Paparella

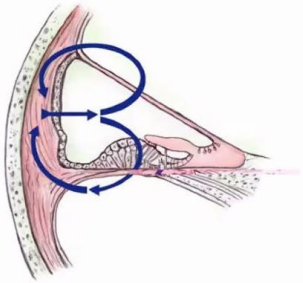
All Meniere's cases have endolymphatic hydrops but not all ears with endolymphatic hydrops have Meniere's

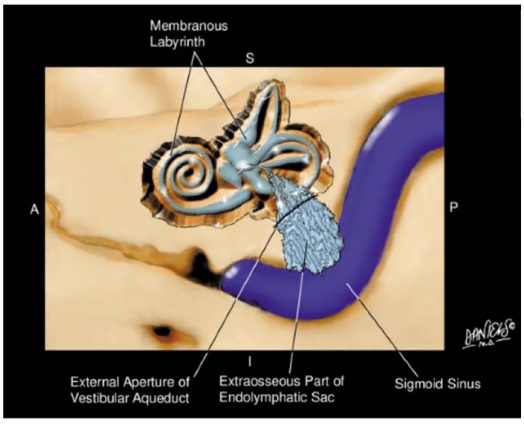
- Rauch SD, Merchant SN, Thedinger BA. Meniere's Syndrome and Endolymphatic Hydrops: Double-Blind Temporal Bone Study. *Annals of Otology, Rhinology & Laryngology*. 1989;98(11):873-883



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

- Radial flow
- Longitudinal flow





Why do MD ears have too much endolymph?

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

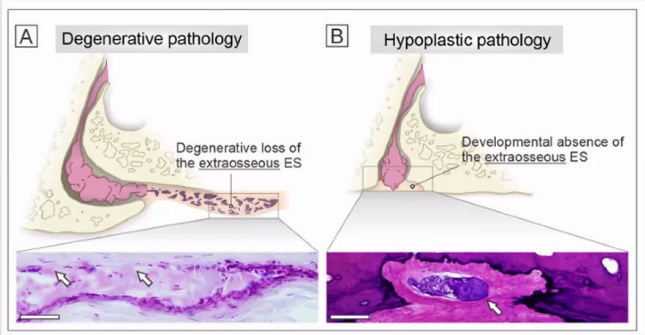
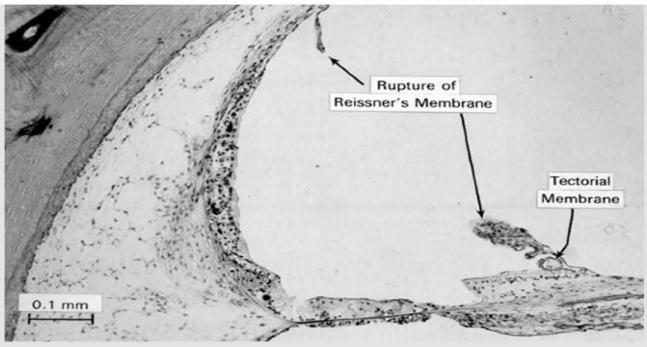


Figure 1. Schematic representation and histological examples (hematoxylin/eosin staining) of degenerative **(A)** and hypoplastic **(B)** ES pathologies, as present in cases with idiopathic endolymphatic hydrops. *Adapted from (37), according to the terms of the Creative Commons Attribution 4.0 International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

Another hypothesis

Histopathology of Meniere's disease "rupture theory"



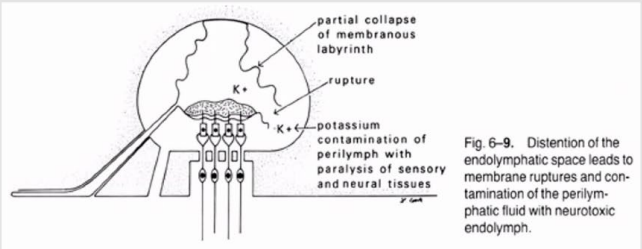
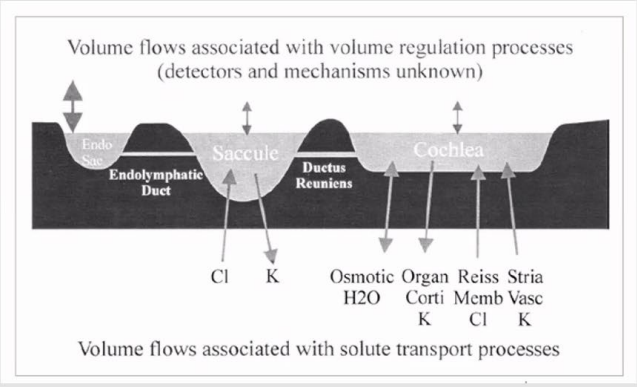


Fig. 6–9. Distention of the endolymphatic space leads to membrane ruptures and contamination of the perilymphatic fluid with neurotoxic endolymph.

After: H.F. Schuknecht

At steady state each compartment can manage quite well, but more absorption of fluid means the endolymphatic sac takes on more importance.

Inner ear fluid regulation and flow varies according to volume

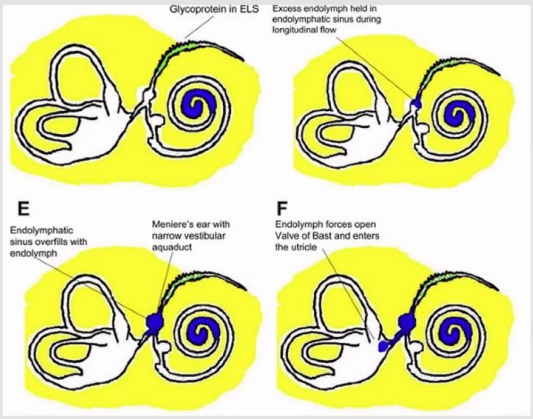


SALT, A.N. (2001), Regulation of Endolymphatic Fluid Volume. Annals of the New York Academy of Sciences, 942: 306-312

Bill Gibson's Hypothesis

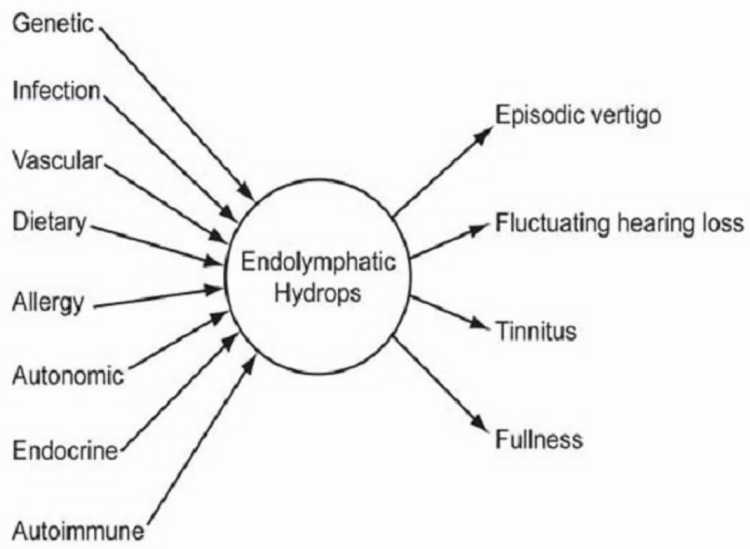
Why does e. hydrops lead to attacks of vertigo?

- Another hypothesis is that the endolymphatic sac malfunctions and there is reverse flow into utricle that initiates attacks of vertigo (Gibson)



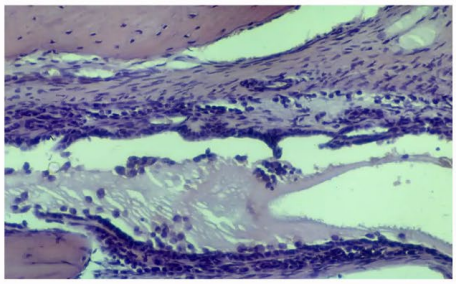
There are a number of known causes:

Polygenic traits often have an environmental trigger.



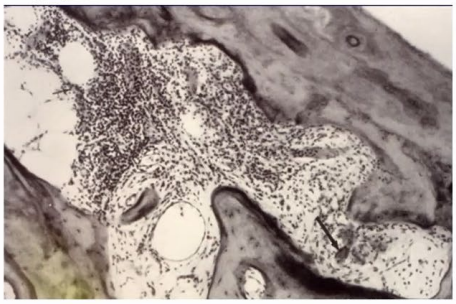
Examples of immunity and infection: Patients with Syphilis are known to have Meniere's like attacks:

Environmental triggers causing Meniere's-like syndromes



Tomiyama & Harris, Acta Otolaryngol. 1989 Mar-Apr;107 (3-4):202

Immune reactivity- sterile antigen or viruses "lymph node of inner ear"



Syphilitic inflammation

Courtesy of F. Linthicum, M.D.

Around 8% of families have genes known to cause Ménière's:

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).

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)		
			X^2	p
Total	376	377	23.2	<0.001

Genetic Evidence for an Ethnic Diversity in the Susceptibility to Ménière's Disease

Otology & Neurotology34(7):1336-1341, September 2013.

https://pubmed.ncbi.nlm.nih.gov/23598705/

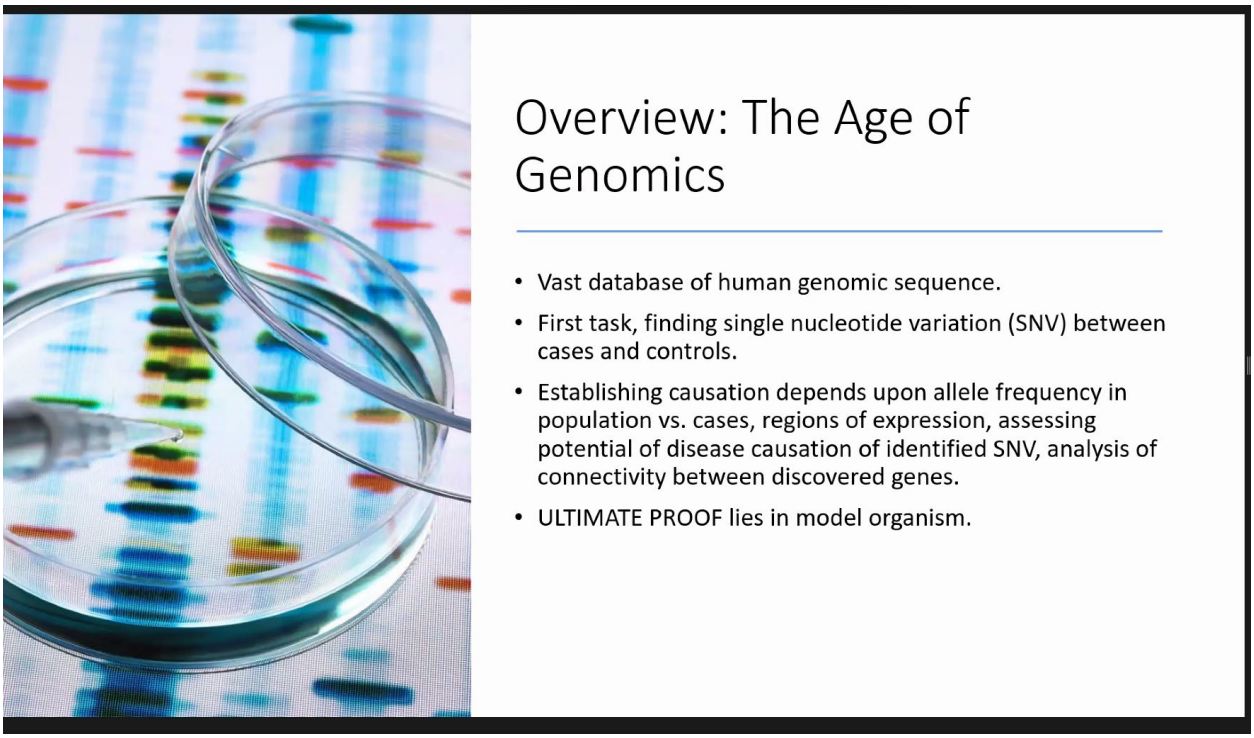
What do we think we know:

- 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

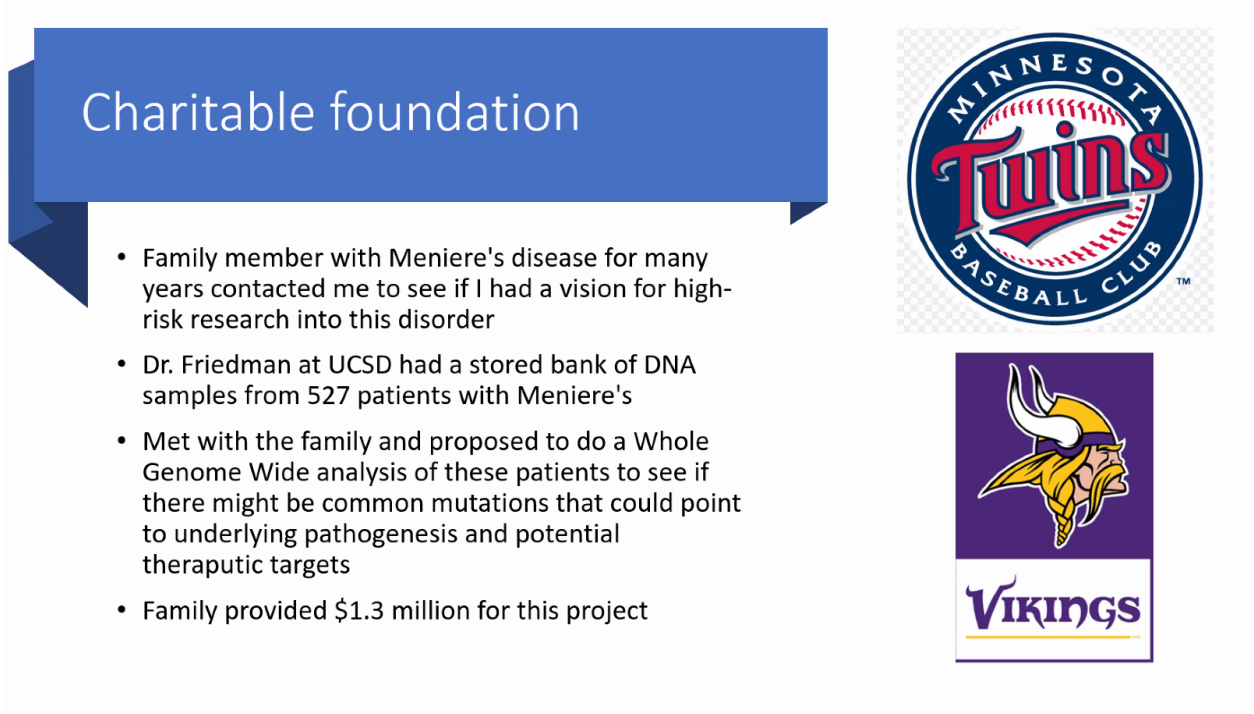
But we do not have any idea of what creates the trigger for endolymphatic hydrops

T

This portion was presented by Rick Friedman, a clinician scientist with many years' experience with Ménière's:



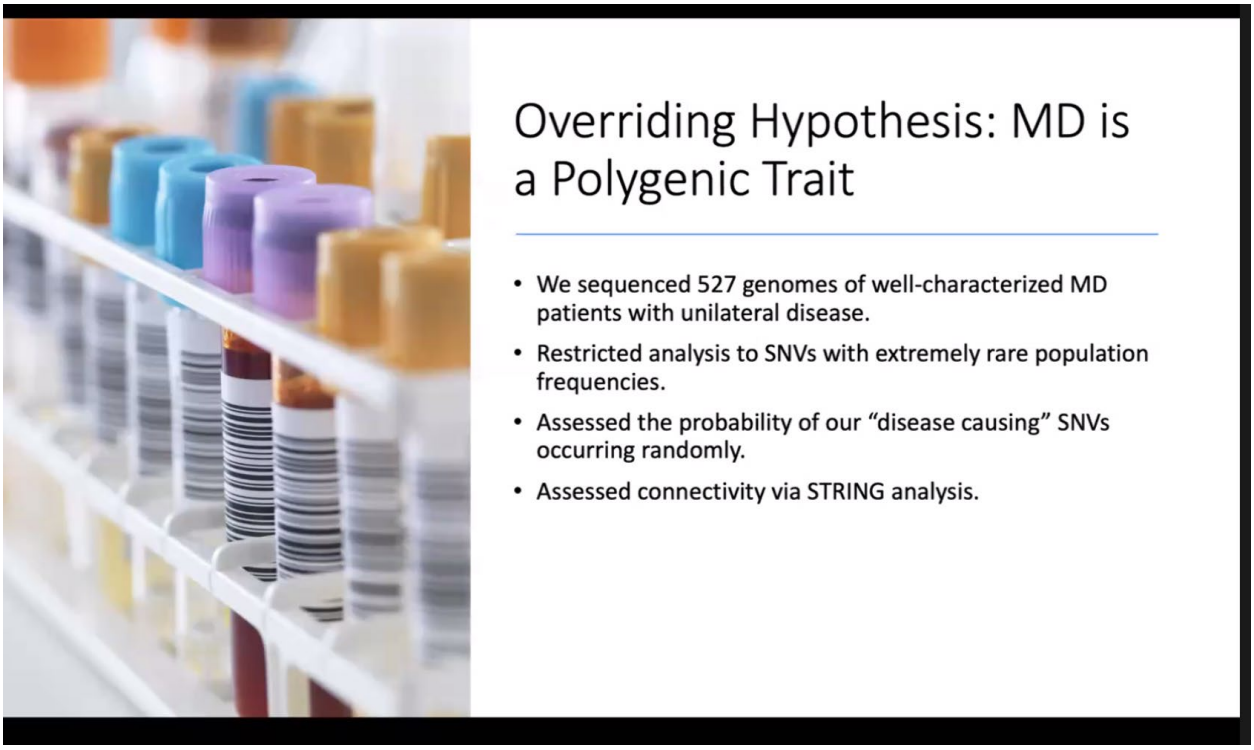
A family member of the group who owned the Minnesota Twins has Ménière's and they provided the money for this research project:



Full Disclosure

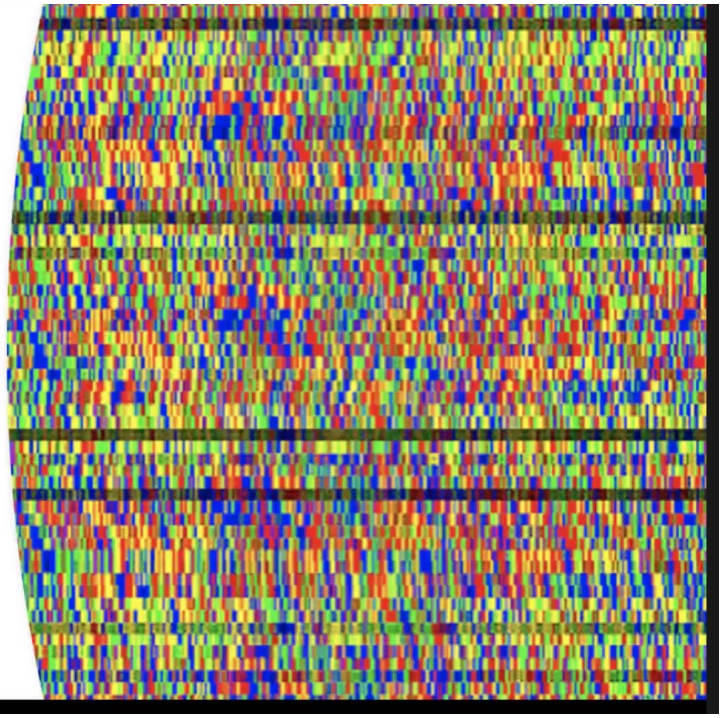
- I am not a geneticist
- From here on in I am reporting the results of the team from Computational Biology and the Genome Institute

Stopped at 527 as data was repeating and on the interest of cost:



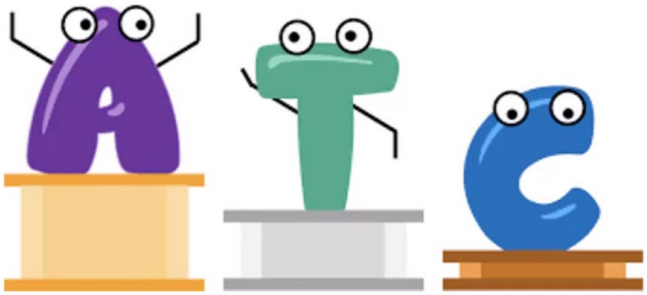
How to sort out large sequence data sets.

- 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.

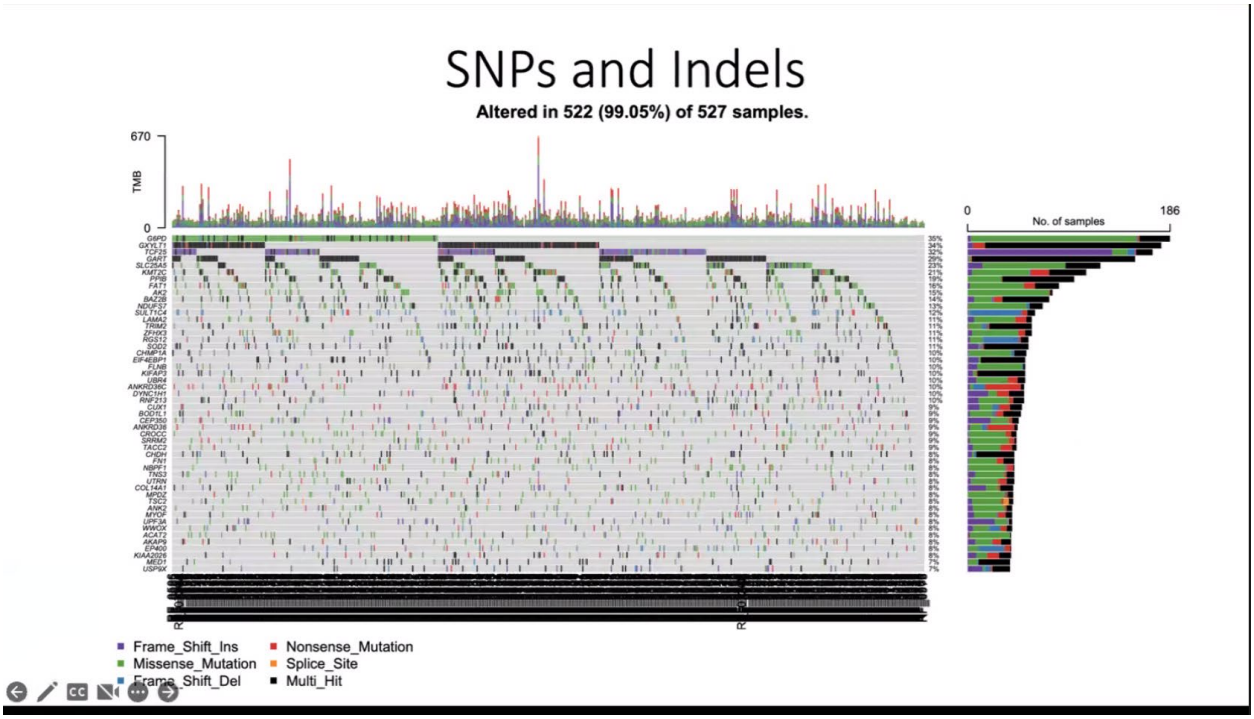


Variant Prioritization

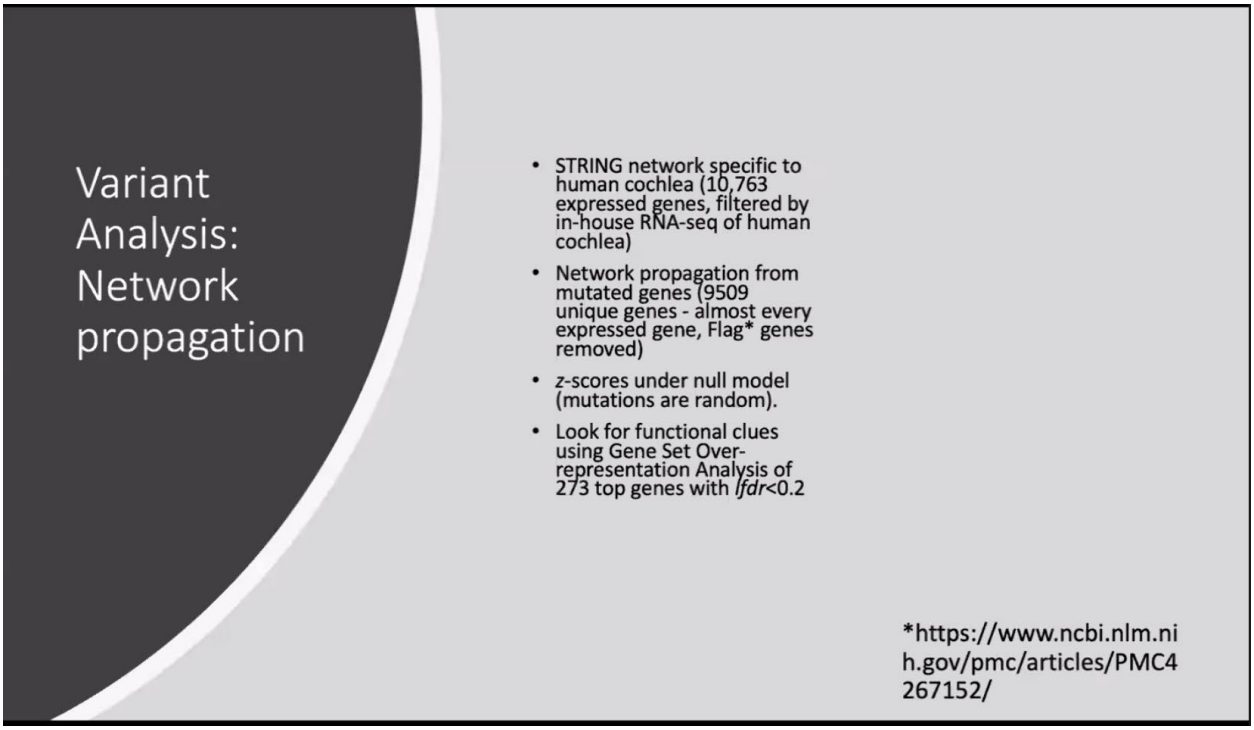
- 527 samples (definite MD):
  - ~4 million variants per sample
  - Filtered to 16606 unique variants
- Filtering criteria
  - Protein altering events
  - Gnomad v3 max population allele frequency < 0.01 (rare variants)
  - Deleterious according to SIFT and PolyPhen software
  - Expressed in adult mouse cochlea or human developing cochlea – single cell RNAseq in public data bases



Genes on the left panel were altered in 99% of Ménière's patients:



Wanted to know if the discoveries were linked.

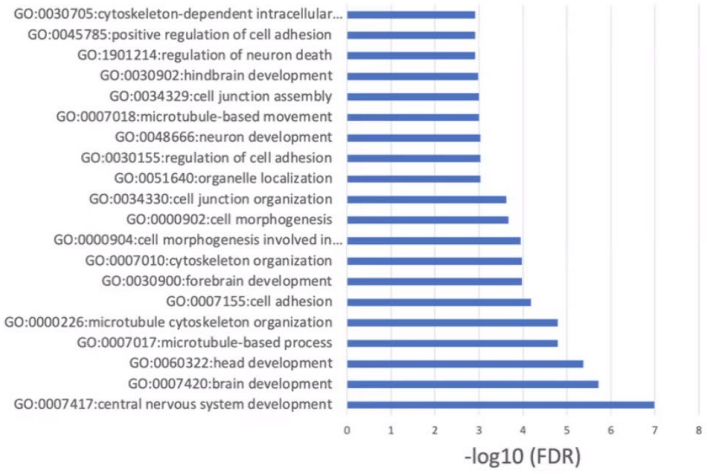


Highly significant discovery- IE the P Values very significant, indication the genes are responsible:

Frequency/*z*-score (top 30 genes) compared to population controls

	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	ENSG00000044	204	AK2	adenylate kinase 2	10.589534817	0.0063989197	0.15370019
28	ENSG00000123	29994	BAZ2B	bromodomain adjacent to zinc finger domain 2	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

Over-representation Analysis



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

Over-representation analysis: microtubule-based processes

- These 21 genes "explain" 95.3% of cases

Gene set: GO:0007017 microtubule-based process

FDR	P Value		
0.0014179	1.3127e-7		
Gene Set Size	**Expected Value**	**Overlap**	**Enrichment Ratio**
480	5.6281	21	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

Over-representation analysis: cell junction assembly

- These 10 genes "explain" 77.2% of cases

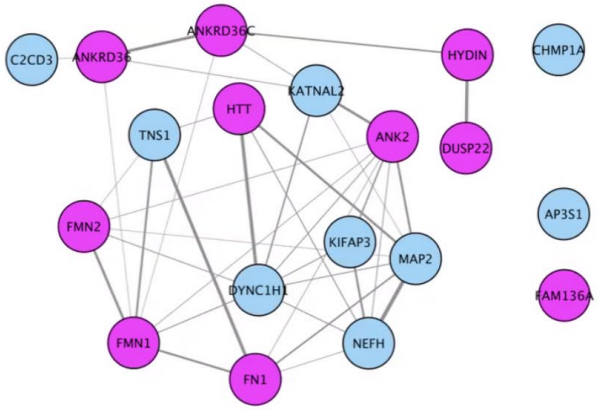
Gene set: GO:0034329 cell junction assembly

FDR	P Value		
0.044281	0.000024598		
Gene Set Size	**Expected Value**	**Overlap**	**Enrichment Ratio**
167	1.9581	10	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

These 16 functionally related genes explain 95.1% of cases

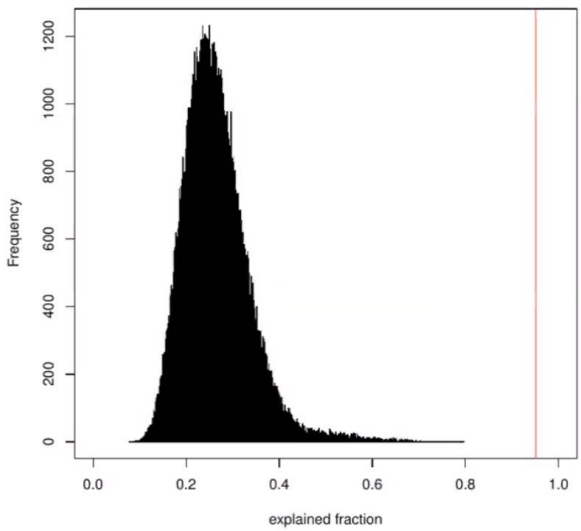
- Solitary genes are not known to interact with the rest of the genes (STRING)
- Line thickness reflects confidence that the interaction exists, range 30-90% (STRING)
- Magenta genes are linked to hearing disorders

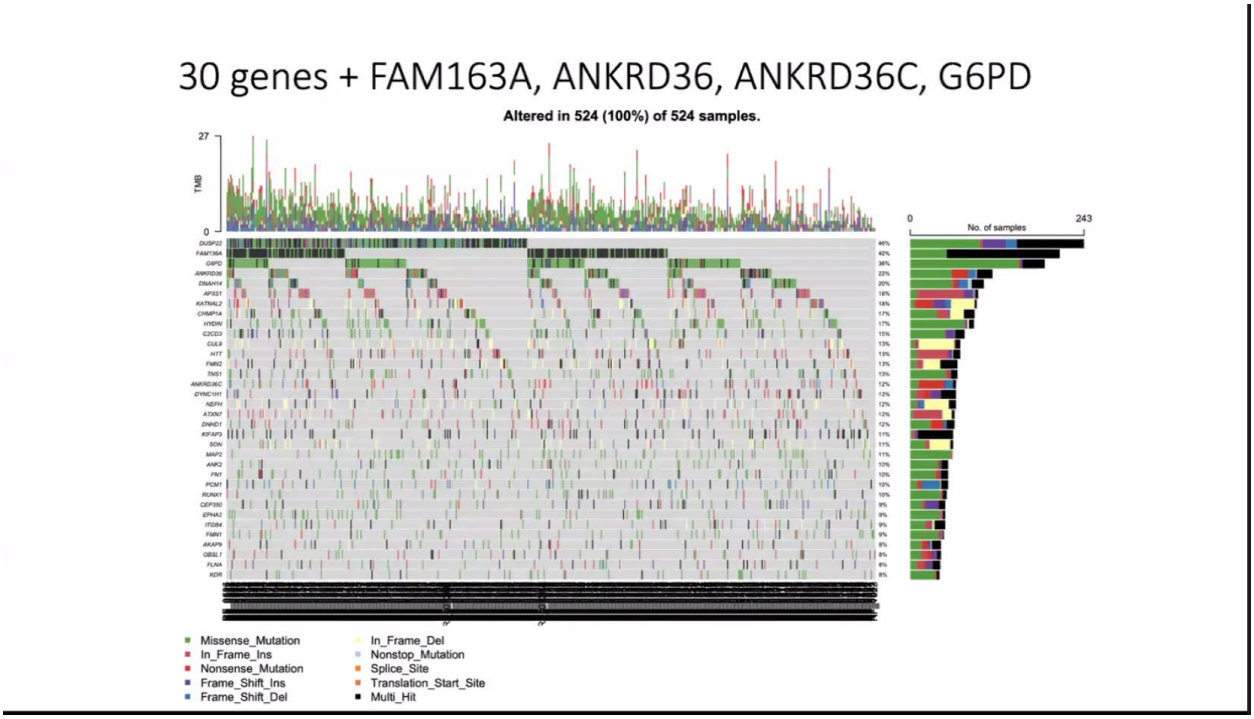


STRING Analysis: software package assessing connectivity of proteins in cellular physiological pathways.

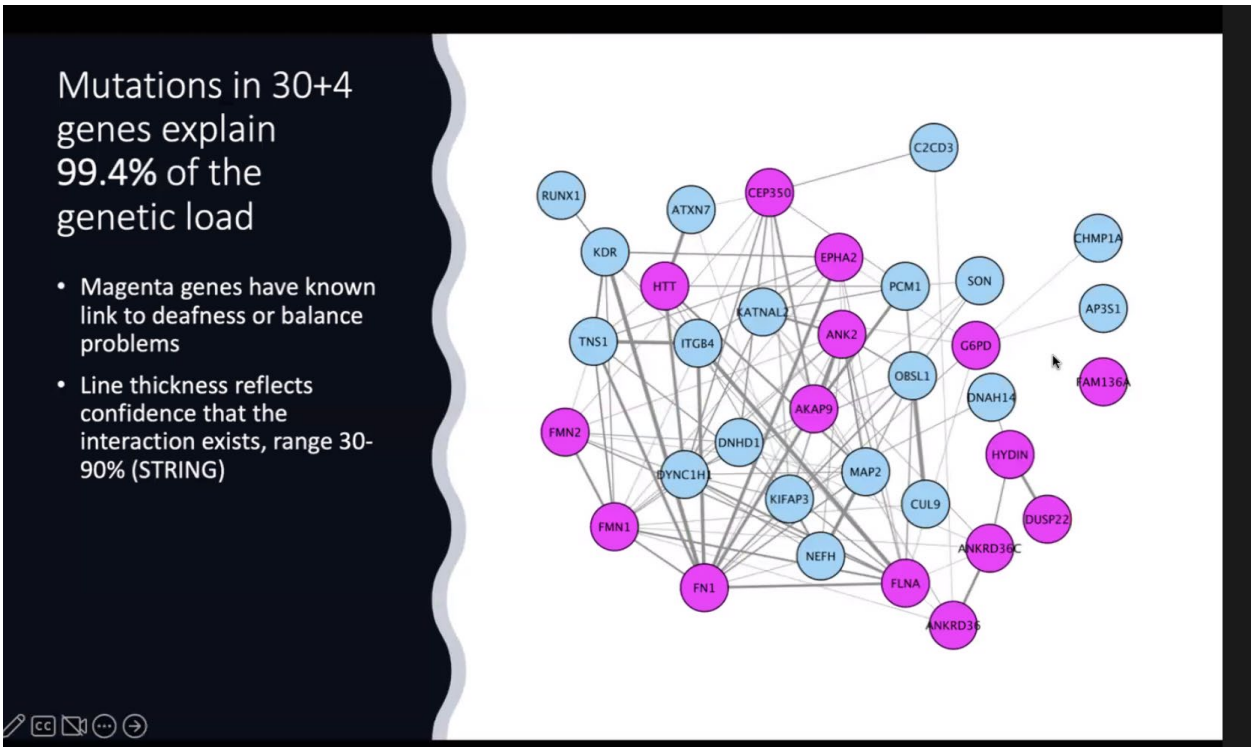
Statistical test of significance of the 16 genes

- 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.

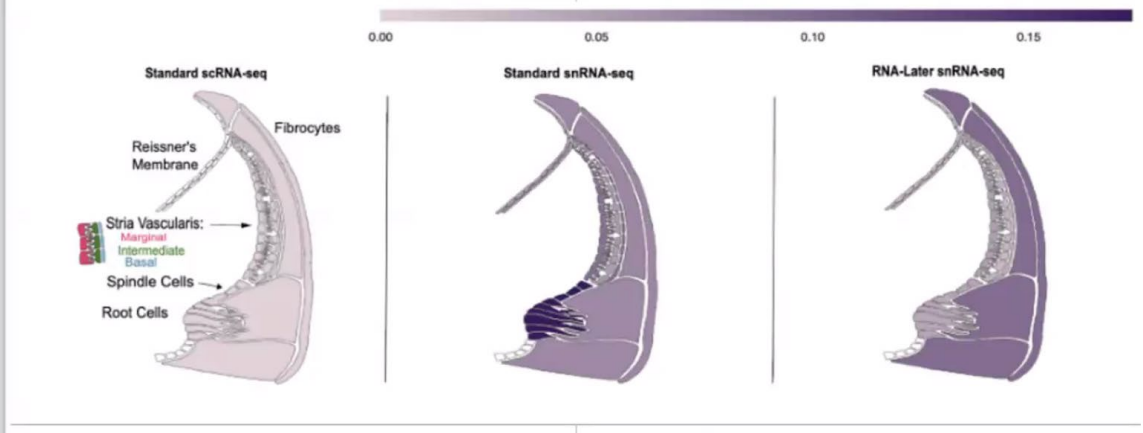




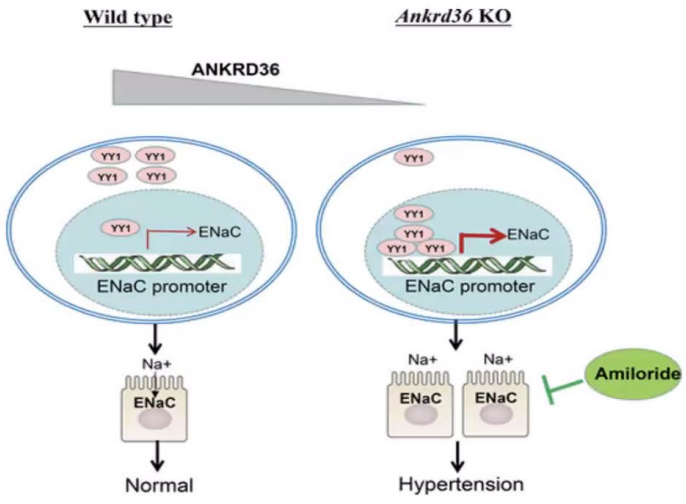
ANKRD 36 gene- only some have it:



RNA-seq labeling of inner ear and stria in mouse



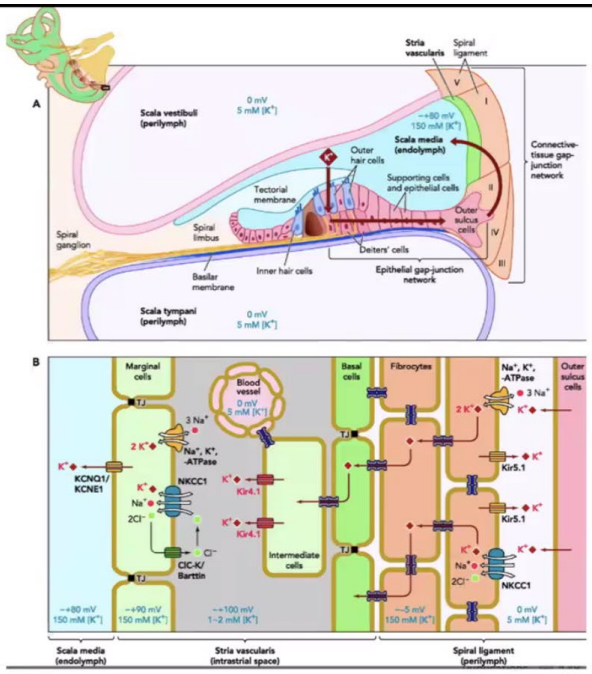
gEAR; Hoa M.



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?



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.

Ongoing Directions

- Replicated findings in a large cohort-UKBB exome/whole-genome database.
  - Generate mouse model (CRISPR).