JABI
JORNADAS ANDALUZAS DE BIOINFORMÁTICA
SEVILLA, 3-5 DE JULIO 2023

CENTRO PFIZER-UNIVERSIDAD DE GRANADA-JUNTA DE ANDALUCÍA DE GENÓMICA E INVESTIGACIÓN ONCOLÓGICA
GenYo

ibs.GRANADA
INSTITUTO DE INVESTIGACIÓN BIOSANITARIA

UNIVERSITAS GRANATENSIS PLUS ULTRA 1531

Discovery and validation of novel variants in unresolved Meniere's disease cases

Caridad Serrano Martínez[1,2,3,4], Alba Escalera-Balsera[1,2,3], Alberto M. Parra-Perez[1,2,4], Jose Antonio Lopez-Escamez[2,4,5], Alvaro Gallego-Martinez[1,2,3]

[1]Otology and Neurotology Group CTS495, Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica (GENYO), Granada, Spain; [2]Sensorineural Pathology Programme, CIBER - Center for Biomedical Research Network, Madrid, Spain; [3]Otology and Neurotology Group CTS495, Department of Otorhinolaryngology, ibs.GRANADA, Instituto de Investigación Biosanitaria, Granada, Spain; [4]Department of Surgery, Facultad de Medicina, Universidad de Granada. IBS.Granada, Granada, Spain; [5]Faculty of Medicine & Health, School of Medical Sciences, Kolling Institute, Meniere's Disease Neuroscience Research Program, St Leonards, Australia.

Background

An immense genetic heterogeneity is underlying more common markers, being **ultra-rare variants** that have gone unnoticed in prioritization and discovery protocols important players in complex disorders. Therefore, ultra-rare variants are **difficult** to find **recurrent** **between individuals sharing a similar phenotype in a complex disease background**. In this study, we selected **novel variants** in a cohort of **unresolved patients** with **Ménière's disease** (MD), in order to expand the genetic architecture associated with the disorder.

Methods

1. **Cohort**: 407 whole-exome sequenced MD patients.
2. **Calling quality filtering**: 1) Prefiltering by AB, GQ, DP, and VQSR; 2) No prefiltering VQSR (*Figure 1*).
3. **Annotation**: VEP for population frequencies, pathogenicity scores and ACMG criteria.
4. **Novelty filtering** against CSVS, gnomAD and TOPMED.
5. **Priorization of damaging variants:** CADD, constraint from gnomAD.
6. **Validation**: IGV and Sanger sequencing (*Figure 2*).

Conclusions

1. We have detected and validated different **multiplex novel variants** for unresolved MD patients from a WES cohort.

2. Potential **false negative novel variants may be missed during quality filtering,** so allele balance and genotype quality pre-filtering should be included for future analysis of candidate variants for MD to improve the yield after VQSR quality filtering.

Results

We proposed a specific workflow to consider **potential missing novel variants** in our MD WES cohort according to GATK and gnomAD standards. After prefiltering, we increased the yield of potential novel variants when compare with only VQSR filtering. From a selection of 21 high impact novel variants and 86 moderate impact novel variants, we kept 8 potential new candidate novel variants for MD *(Table 1)* after functional annotation priorization.

Figure 1. Proposed workflow for novel variant discovery. Two parallel VQSR filtering, *with* and *without* allele balance hard-prefiltering (right) were performed. Overlapping of resultant potential true positive variants can be observed in the attached Venn diagram (down) for Loss-of-Function (LoF) and Missense variants.

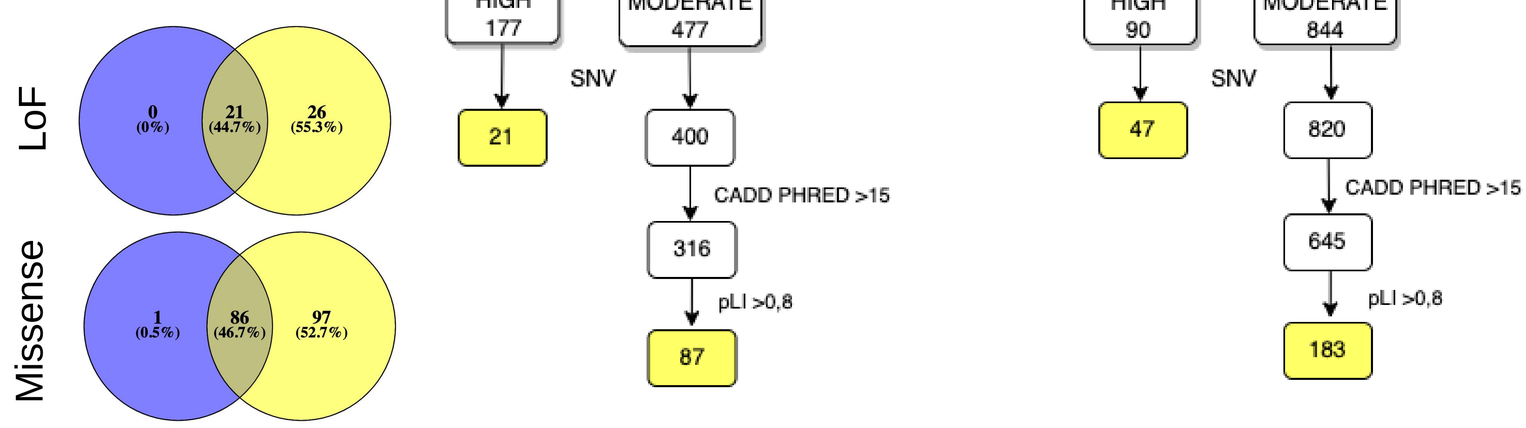

Table 1. Multiplex novel variants found in MD WES cohort for missense and LoF variant categories. These variants were evaluated as potential pathogenic through CADD and LOEUF scores. All these genes were found to be differentially expressed in inner ear.

	Gene name	Position	REF	ALT	CADD PHRED	LOEUF	Alelle Count
LoF	***NIN***	chr14:50758457	C	T	40	0.43	2
	NALCN	chr13:101057938	C	T	33	0.52	2
	COG4	chr16:70481763	C	T	34	0.71	2
Missense	***ACAN***	chr15:88868225	A	C	21.9	1.03	4
	FAM193A	chr4:2596130	C	T	23.8	0.96	2
	FAM193A	chr4:2646782	G	A	19.73	0.96	2
	DOCK4	chr7:111769578	C	T	28.9	0.83	3
	DOCK4	chr7:111915875	C	T	27.1	0.83	2

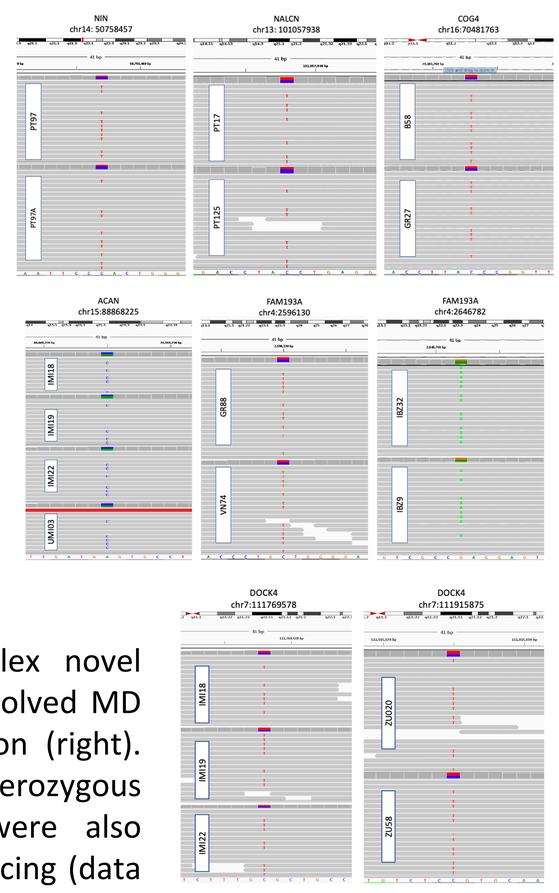

Figure 2. Validation of multiplex novel variants found in different unresolved MD patients through IGV visualization (right). All the variants found were heterozygous for each carrier. Variants were also validated through Sanger sequencing (data not shown).

Conflicts of interest: none declared.

References:
1. Lopez-Escamez JA, Carey J, Chung WH, et al. Diagnostic criteria for Menière's disease. J Vestib Res Equilib Orientat. 2015;25(1):1-7. doi:10.3233/VES-150549
2. Gallego-Martinez A, Requena T, Roman-Naranjo P, Lopez-Escamez JA. Excess of Rare Missense Variants in Hearing Loss Genes in Sporadic Meniere Disease. Front Genet. 2019;10:76. doi:10.3389/fgene.2019.00076
3. Zhao S, Agafonov O, Azab A, Stokowy T, Hovig E. Accuracy and efficiency of germline variant calling pipelines for human genome data. Sci Rep 10, Article number: 20222 (2020). Doi:10.1038/s41598-020-77218-4

Junta de Andalucía
Consejería de Salud y Familias

UNIÓN EUROPEA
Fondo Europeo de Desarrollo Regional (FEDER)
Una manera de hacer Europa

Instituto de Salud Carlos III

CuresWithinReach

Funding: ISCIII-PI20/1126; Andalusian Government EPIMEN-P20-00303 and GEN4PHEN-PI-0266-2021 and the support of CuresWithinReach and the Knight Family.