

# **Rare Variants in the *OTOG* Gene Are a Frequent Cause of Familial Meniere's Disease**

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## 31 Abstract

32 **Objectives:** Meniere's disease (MD) is a rare inner ear disorder characterized by  
33 sensorineural hearing loss, episodic vertigo and tinnitus. Familial MD has been reported  
34 in 6-9% of sporadic cases, and few genes including *FAM136A*, *DTNA*, *PRKCB*,  
35 *SEMA3D* and *DPT* have been involved in single families, suggesting genetic  
36 heterogeneity. In this study, the authors recruited 46 families with MD to search for  
37 relevant candidate genes for hearing loss in familial MD.

38 **Design:** Exome sequencing data from MD patients were analyzed to search for rare  
39 variants in hearing loss genes in a case-control study. A total of 109 patients with MD  
40 (73 familial cases and 36 early-onset sporadic patients) diagnosed according to the  
41 diagnostic criteria defined by the Barany Society were recruited in 11 hospitals. The  
42 allelic frequencies of rare variants in hearing loss genes were calculated in individuals  
43 with familial MD. A single rare variant analysis (SRVA) and a gene burden analysis  
44 (GBA) were conducted in the dataset selecting one patient from each family. Allelic  
45 frequencies from European and Spanish reference datasets were used as controls.

46 **Results:** A total of 5136 single nucleotide variants in hearing loss genes were  
47 considered for SRVA in familial MD cases, but only one heterozygous variant in the  
48 *OTOG* gene (rs552304627) was found in two unrelated families. The GBA found an  
49 enrichment of rare missense variants in the *OTOG* gene in familial MD. So, 15/46  
50 families (33%) showed at least one rare missense variant in the *OTOG* gene, suggesting  
51 a key role in familial MD.

52 **Conclusions:** The authors found an enrichment of multiplex rare missense variants in  
53 the *OTOG* gene in familial MD. This finding supports *OTOG* as a relevant gene in  
54 familial MD and set the groundwork for genetic testing in MD.

# INTRODUCTION

Meniere's disease [MD (OMIM 18600)] is a rare inner ear disorder with three major symptoms: sensorineural hearing loss (SNHL), episodic vertigo and tinnitus (Lopez-Escamez et al. 2015; Espinosa-Sanchez & Lopez-Escamez 2016). Hearing loss always involves low and medium frequencies in one or both ears (unilateral or bilateral MD) at the onset of the disease. However, MD also affects high frequencies in early or advanced stages of the disease (Belinchon et al. 2011). Epidemiological studies indicate that MD is most common in European population, suggesting a genetic predisposition (Ohmen et al. 2013). Although the majority of MD patients are considered sporadic (Frejo et al. 2016; Frejo et al. 2017), familial clustering has been reported in 8-9% of sporadic cases in the European descendent (Requena et al. 2014), and in 6% of Korean population (Lee et al. 2015), which also supports a genetic contribution to the disease (Roman-Naranjo et al. 2017). MD shows a wide range of phenotypic variations among patients, even within the same families (Lee et al. 2015b), and it is commonly associated with migraine and systemic autoimmune disorders (Tyrrell et al. 2014; Cha et al. 2008). Familial MD (FMD) shows an autosomal dominant (AD) pattern of inheritance with incomplete penetrance and anticipation, showing an earlier onset compared to sporadic cases (Morrison et al. 2009; Birgerson et al. 1987; Klar et al. 2006). Different whole exome sequencing (WES) based studies have identified several genes related with FMD. Single nucleotide variants (SNV) in *DTNA*, *FAM136A*, *PRKCB*, *DPT* and *SEMA3D* were identified in 4 different families AD inheritance with incomplete penetrance (Requena et al. 2015; Martín-Sierra et al. 2016; Martín-Sierra et al. 2017). However, these findings have not been replicated neither in other MD families nor sporadic MD (SMD) cases.

79 WES continues to be an efficient tool to determine disease-causing variants (Williams et  
80 al. 2016; Adams & Eng 2018; Suwinski et al. 2019), although the monogenic  
81 hypothesis in FMD should be reconsidered to achieve results beyond private rare  
82 variants for singular families. Thus, the “one variant-one disease” hypothesis, described  
83 for classic Mendelian inheritance cannot explain the incomplete penetrance or variable  
84 expressivity observed in MD (Martín-Sierra et al. 2017) and more complex inheritance  
85 models are needed (Cooper et al. 2013; Kousi & Katsanis 2015). Oligogenic and  
86 multiallelic models have been already applied in different diseases, such as Parkinson  
87 (Lubbe et al. 2016) and Huntington’s disease (Lee et al. 2015a), explaining changes in  
88 disease progression and phenotypic variability. Furthermore, a digenic inheritance of  
89 deafness was reported by variants in *CDH23* and *PCDH15* (Zheng et al. 2005), and  
90 recently, an enrichment of rare missense variants in certain SNHL genes, such as *GJB2*,  
91 *SLC26A4* or *USH1G*, was found in a large cohort of SMD cases (Gallego-Martinez et  
92 al. 2019), supporting the hypothesis of multiallelic inheritance in MD.

93 More than 150 genes have been associated to deafness (Azaiez et al. 2018), and 116 of  
94 them are related with non-syndromic SNHL (Van Camp G. 2018).

95 In this study, we have investigated the genetic background of FMD, focusing on SNHL  
96 genes by analyzing 46 families with MD by WES. We have found an enrichment of rare  
97 missense variants in the *OTOG* gene compared with non-Finnish European (NFE) and  
98 Spanish populations. The *OTOG* gene, which encodes otogelin, has been previously  
99 associated with deafness and imbalance and causes autosomal recessive deafness 18B  
100 (Simmler et al. 2000a; Schraders et al. 2012). A total of 15 families out of 46 showed, at  
101 least, one rare missense variant in this gene, suggesting a key role of otogelin in MD.

# MATERIALS AND METHODS

## Patient assessment and selection

A total of 73 MD patients from 46 different families with one or more affected first-degree relatives, and 36 sporadic MD cases with an age of onset younger than 35 were recruited. Patients were diagnosed following the diagnostic criteria described by the International Classification Committee for Vestibular Disorders of the Barany Society (Lopez-Escamez et al. 2015). A complete hearing and vestibular assessment was carried out in all cases, including a brain magnetic resonance imaging to exclude other causes of neurological symptoms. Serial pure tone audiograms were retrieved from clinical records to assess hearing loss since the initial diagnosis. A summary of the clinical information of these patients is presented in the Supplemental Digital Content 1 (see Table 1 to Table 3, Supplemental Digital Content 1).

This study protocol was approved by the Institutional Review Board for Clinical Research (MS/2014/02), and a written informed consent to donate biological samples was obtained from all subjects.

## DNA extraction and whole exome sequencing

Blood and saliva samples were taken from patients with MD to perform WES. DNA samples were extracted with prepIT-L2P (DNA Genotek, Ottawa, Canada) and QIAamp DNA Blood Mini Kit (Qiagen, Venlo, The Netherlands) using manufacturer's protocols and quality controls previously described (Szczepek et al. 2019). DNA libraries were prepared by using the SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA) and were paired-end sequenced on the Illumina HiSeq 4000 platform at 100X coverage. Raw reads were stored in two FASTQ files for each individual.

## 125 **Bioinformatic analysis**

### 126 **Dataset generation and processing**

127 Analysis-ready BAM files and VCF files were generated from raw unmapped reads  
 128 using the GATK Best Practices pipeline. Reads were aligned to the GRCh37/hg19  
 129 human reference genome using the BWA-MEM algorithm. For obtaining the final  
 130 dataset, SNV and small structural variants were filtered according to its Variant Quality  
 131 Score Recalibration (VQSR) and depth of coverage (DP) values. Thus, variants were  
 132 excluded if their VQSR value were under the VSQR threshold or their average DP < 10.  
 133 Variants were functionally annotated using ANNOVAR version 2018Apr16. RefSeq  
 134 was used for gene-based annotation and the Exome Aggregation Consortium (ExAC)  
 135 database, the Combined Annotation Dependent Depletion (CADD) scores and the  
 136 dbNSFP database (v3.0) were used for filter-based annotation.

### 137 **Sensorineural hearing loss gene set**

138 The SNHL gene set was generated by using three different databases: the Hereditary  
 139 Hearing Loss Homepage (Van Camp G. 2018), the Deafness Variation Database  
 140 (Azaiez et al. 2018) and Harmonizome (Rouillard et al. 2016), containing a total of 116  
 141 genes related with SNHL (see Table 4, Supplemental Digital Content 1).

### 142 **Data analysis and prioritization strategy**

143 Two pipelines and filtering/prioritization strategies were conducted to search for rare  
 144 variants as we have previously described (Gallego-Martinez et al. 2019). The first was a  
 145 single rare variant analysis (SRVA) for studying individual families; the second  
 146 approach was a gene burden analysis (GBA) to obtain a gene-level mutational profile  
 147 (Figure 1). For these analyses only one patient from each family was selected.

148 Whenever possible, the patient selected was in the last generation. Sporadic cases with  
149 an early onset were also investigated to search for singleton variants in candidate genes  
150 in both analyses.

151 All variants were assessed according to the standards and guidelines described by the  
152 American College of Medical Genetics and Genomics (ACMG) and the Association for  
153 Molecular Pathology (AMP) (Richards et al. 2015). Variants not described in the NFE  
154 population from ExAC and the Spanish population from CSVS were discarded to  
155 minimize false calls and population-specific variants (Shearer et al. 2014). Selected  
156 variants were checked in patients BAM files with IGV and/or sequenced by Sanger  
157 sequencing to minimize false calls.

# **158 Statistics and databases**

159 Two independent datasets were used as reference to compare the observed MAF in  
160 FMD and to calculate odds ratios (OR): NFE population from ExAC and the  
161 Collaborative Spanish Variant Server (CSVS) database (Lek et al. 2016; Dopazo et al.  
162 2016).

163 For each selected variant in the SRVA, OR with 95% confidence interval (CI) were  
164 calculated using the MAF values from the CSVS database (N=1,579) and the NFE  
165 population (N=33,365) from ExAC.

166 For GBA, we counted the total exonic alternate alleles per gene in our cohort against the  
167 two reference datasets. After calculating OR with 95% CI, we obtained one-sided p-  
168 values that were corrected for multiple testing by the total number of variants found in  
169 each gene following the Bonferroni approach.

170 Standard audiometric evaluations for air and bone conduction elicited by pure tones  
171 from 125 to 8000 Hz were retrieved from the clinical records to analyse the time course

172 of the hearing profile in FMD cases with candidate variants. Regression analysis was  
173 performed to estimate the outcome of hearing loss for each frequency.

# RESULTS

174

## 175 Main genetic findings in familial MD

### 176 Single rare variant analysis

177 A total of 5136 variants located in SNHL genes were considered in FMD cases. After  
 178 applying quality controls (QC), 4247 SNV remained. Only 75 nonsynonymous or splice  
 179 site SNV fulfilled the MAF ( $<0.001$ ) and CADD ( $>15$ ) filtering criteria (Figure 1).  
 180 From them, 40 SNV were already described in the NFE population or Spanish  
 181 population, but only one SNV was found in more than one family (see Table 5,  
 182 Supplemental Digital Content 1, which shows the rare variants found in the SRVA for  
 183 FMD cases). This heterozygous variant located in *OTOG* gene was observed in cases  
 184 from two unrelated families (F1 & F14). The variant chr11:17574758G>A  
 185 (rs552304627; p.V141M), which is in the last nucleotide of the fourth exon in the  
 186 *OTOG* canonical transcript (ENST00000399391), is likely pathogenic according to the  
 187 ACMG and AMP guidelines. This multiplex variant is located in a Von Willebrand  
 188 Factor D-type domain (vWD) with a MAF=.0008 in NFE population, and multiple *in*  
 189 *silico* tools supported a likely pathogenic effect of this variant (SIFT score=.001; M-  
 190 CAP=.153; CADD=28.2; GERP++ =5.36).

191 The rest of the rare SNV were considered private familial variants because none of them  
 192 were found in other FMD cases. None small structural variant (insertion or deletion)  
 193 was found in any SNHL genes.

### 194 Gene burden analysis

195 Seventy-three genes with 214 SNV with a MAF $<0.05$  were retained after QC and  
 196 filtering steps. Most of the genes (74%) carried less than 3 variants, thus they were

discarded for further analysis. The most significant finding was an enrichment of rare missense variants in *OTOG* gene in our FMD cases against either NFE population from ExAC (OR= 4.3(2.6-7.0),  $p= 4.1 \times 10^{-8}$ ) or Spanish population (OR= 3.6(2.1-5.9),  $p= 7.1 \times 10^{-6}$ ). Nine different rare missense variants were found in *OTOG* in 14/46 non-related families, existing 6 families with 2 or more shared variants (Table 1 & see Figure 1, Supplemental Digital Content 2). The variants rs61978648 and rs61736002 were shared by individuals from 4 unrelated families (F2, F3, F4 & F5). Likewise, the variants rs552304627 and rs117315845 were found in patients from other 2 unrelated families (F1 & F14).

In addition, a novel variant in *OTOG* not included in the GBA was found in two cases from a 15th family (F34). This variant, located in exon 18 (chr11:17594747C>A), was found in heterozygous state affecting the sequence of the C8 domain. The distribution of the variants found in *OTOG* is scattered across the gene sequence (Figure 2).

## **Hearing profile in familial patients with rare variants in *OTOG***

The hearing profile for the 14 patients (3 males, 11 females) with rare variants in *OTOG* gene was studied (see Figure 2, Supplemental Digital Content 2, which shows the pure tone audiograms for these patients). Ten of them showed bilateral hearing loss, 3 had left-sided hearing loss and only 1 patient shown right-sided SNHL (see Table 1, Supplemental Digital Content 1, which summarizes the clinical information of the familial MD cases carrying variants in *OTOG* gene). From these 14 patients, 16 ears from 12 patients showed a flat shaped audiogram (57.1%), 5 ears from 5 patients showed a ski-slope shaped audiogram (17.8%), 3 ears from 3 patients showed a reverse-slope shaped (10.7%) and 4 ears had a normal pure-tone audiogram (14.2%).

220 A regression analysis was done to estimate the hearing loss at onset and the outcome for  
221 each frequency. We found a negative correlation at 1000 Hz ( $R^2=.143$ ;  $p=.033$ ) and  
222 2000 Hz ( $R^2=.246$ ;  $p=.004$ ). There was no statistical correlation at 125 Hz, 250 Hz, 500  
223 Hz, 4000 Hz nor 8000 Hz, suggesting no progression at these frequencies (Figure 3).  
224 The age of onset of the symptoms was  $41.93 \pm 8.66$  and the estimated hearing loss at  
225 onset was  $62.14 \pm 12.83$  for low frequencies (125-250-500 Hz) and  $58.75 \pm 14.1$  for high  
226 frequencies (1000-2000-4000 Hz).

## 227 **Early onset sporadic MD**

228 The same analytical pipeline was used in a series of patients with sporadic MD with an  
229 age of onset younger than 35 (see Figure 3, Supplemental Digital Content 2). For the  
230 SRVA, we found 60 nonsynonymous or splice site SNV with  $MAF < 0.001$  and CADD  
231  $> 15$  in SNHL genes. Among them, one variant was found in two sporadic cases and  
232 another variant was also found in a familial case. The rest of the SNV were considered  
233 simplex variants found in singletons and none of them were homozygous (see Table 6,  
234 Supplemental Digital Content 1, which shows the rare variants found in the SRVA for  
235 SMD cases).

236 A heterozygous nonsynonymous SNV in *OTOG* gene was found in two unrelated  
237 sporadic MD cases (S1 and S23). The variant chr11:17632279C>T (rs779658224;  
238 p.A1823V) is located in exon 35 of the canonical transcript of *OTOG* gene and it is a  
239 variant of uncertain significance (VUS) according to the ACMG and AMP guidelines.  
240 This variant has a  $MAF=.0005$  in the NFE population from ExAC and it is not  
241 described in the Spanish population from the CSVS. In addition, a heterozygous  
242 nonsynonymous SNV in *OTOGL* gene was found in one sporadic case and in one  
243 familial case (S27 and F31). The variant chr12:80752642T>G (rs145929269;

244 p.C2068G) is located in exon 51 of the canonical transcript of *OTOGL* gene  
 245 (ENST00000458043). This region encodes a cysteine-rich region and this variant was  
 246 also classified as a VUS according to the ACMG and AMP guidelines.

247 For the GBA, we found 12 rare SNV in *OTOG* gene in patients with early onset MD  
 248 (see Table 7, Supplemental Digital Content 1). However, in contrast with the results  
 249 obtained in FMD cases, there was not an excess of rare variants in this gene against  
 250 neither the NFE population from ExAC (OR=2.1(1.2-3.7),  $p=.11$ ) nor Spanish  
 251 population (OR=2(1.1-3.5),  $p=.20$ ) (Figure 2).

# DISCUSSION

Familial MD has an AD inheritance with incomplete penetrance (Morrison et al. 2009; Requena et al. 2014), and few genes have been involved in singular families (Requena et al. 2015; Martín-Sierra et al. 2016; Martín-Sierra et al. 2017). In this study, we have found an enrichment of rare missense variants in several unrelated patients with FMD in the *OTOG* gene. These variants were observed in 15 of 46 non-related families (33% familial cases). Seven of the 15 families with rare variants in *OTOG* showed incomplete penetrance (47%) and partial syndromes (episodic vertigo or hearing loss) were found in relatives from 5 of 15 families (Morrison et al. 2009; Requena et al. 2015; Martín-Sierra et al. 2016; Martín-Sierra et al. 2017). Most of these rare variants were found in 2, 3 or 4 unrelated individuals from different families with MD and they were considered multiplex variants. However, the majority of the variants in *OTOG* found in non-familial patients with early onset were not observed in other sporadic cases (singletons variants).

*OTOG*, which encodes otogelin, was described for first time by Cohen-Salmon *et al* (Cohen-Salmon et al. 1997). Otogelin is a 2925 amino acid protein (ENST00000399391) constituted by several vWD and C8 domains, and a cysteine knot-like domain in its C-terminal. It is mainly expressed in acellular structures which cover the sensory inner ear epithelia: the tectorial membrane, the otoconial membranes and the cupula over the cristae ampullaris of the semicircular canals. Because of its localization in the extracellular structures overlying the stereocilia of the hair cells involved in the mechanotransduction of sound and acceleration, this structural protein plays an important role in both auditory and vestibular functions (Schrauwen et al. 2016).

276 The effects of variants in otogelin were first demonstrated in the orthologous gene in a  
277 mouse model. Three mouse models have been generated for evaluating the phenotypic  
278 changes resulting from *OTOG* variants. In the *Otog*<sup>tm1Prs</sup> model, authors inactivated  
279 *Otog* by deleting the first three exons. Vestibular dysfunction was detected at P4 in  
280 *Otog*<sup>-/-</sup>, observing anomalies in the saccule and utricle. The auditory function was  
281 evaluated by Pleyer reflex, showing profound hearing impairment. The *Otog*<sup>+/-</sup> mice did  
282 not present any anomalies (Simmler et al. 2000a). The second model is the twister (*tw*)  
283 mice, mice with a spontaneous recessive mutation entailing absence of *Otog* expression.  
284 Similarly to *Otog*<sup>tm1Prs</sup>, in *Otog*<sup>tw</sup> the vestibular dysfunction was detected at P4, and the  
285 hearing loss was progressive and moderate to severe/profound (Simmler et al. 2000b).  
286 The last mouse model published is the otogelin ENU-induced mouse model. In this  
287 model, a homozygous variant at the splice donor site of intron 29, *Otog*<sup>vbd/vbd</sup>, cause a  
288 frame-shift and a premature codon. *Otog*<sup>vbd/vbd</sup> mice shown abnormal hearing and  
289 vestibular functions (El Hakam Kamareddin et al. 2015).

290 Four variants have been described in *OTOG* gene causing DFNB18B. Schraders *et al.*  
291 were the first to describe causative variants in *OTOG*. A homozygous 1bp deletion,  
292 c.5508delC (p.Ala1838Profs\*31) in four related patients, and two compound-  
293 heterozygous variants, c.6347C>T (p.Pro2116Leu) and c.6559C>T (p.Arg2187\*) in  
294 other two related patients, were described to cause hearing loss and vestibular  
295 dysfunction (Schraders et al. 2012). More recently, a homozygous nonsense variant  
296 c.330C>G (p.Tyr110\*) in a Korean patient has been identified, showing early-onset  
297 mild hearing loss without vestibular dysfunction (Yu et al. 2019).

298 In contrast to studies mentioned above, *OTOG* variants found in this study were all in  
299 heterozygous state and, despite 6 FMD cases and 3 SMD cases studied had two or more  
300 variants, compound heterozygous variants could not be demonstrated because samples

301 from the parents were not available and *OTOG* variant segregation could not be fully  
302 assessed in each family. However, the variants chr11:17574758G>A and  
303 chr11:17663747G>A found in F14 were also identified in his mother, the variants  
304 chr11:17578774G>A and chr11:17632921C>T found in F5 were also identified in her  
305 sister (II-7), and a novel variant chr11:17594747C>A not considered for the GBA were  
306 found in F34 and her brother. Furthermore, variants located in untranslated regions  
307 (UTRs) and promoter regions, which modulate gene expression and different protein  
308 features (Chatterjee & Pal 2009; Buckland 2006), could not be evaluated because of the  
309 study design. Altogether, the results obtained by GBA suggested a different genetic  
310 architecture in FMD cases and SMD cases, since the enrichment of rare variants in  
311 *OTOG* gene was only found in FMD cases and most of the variants found in sporadic  
312 cases with early onset were singletons (not observed in multiple individuals).

313 Each region of the cochlea is specifically stimulated by a specific frequency. Thus, the  
314 base of the cochlea mainly responds to high-frequency sounds, whereas the apex  
315 responds to low-frequency sounds, frequencies mostly affected in MD (Robles &  
316 Ruggero 2017; Nakashima et al. 2016). Of note, otogelin shows a tonotopic gene  
317 expression in mice (Yoshimura et al. 2014). *OTOG* gene showed a 2.43-fold change in  
318 expression for apex vs base, making this gene a possible candidate for SNHL in MD. In  
319 addition, an RNA-seq study of the inner ear from patients with normal hearing showed a  
320 high expression of *OTOG* gene in the vestibule (Schrauwen et al. 2016), which could  
321 explain the vestibular dysfunction in patients with pathogenic variants in this gene.

322 The audiograms of FMD patients who carried rare variants in *OTOG* gene showed a  
323 moderate-to-severe flat hearing loss ~60 dB since the first years of onset involving all  
324 frequencies. Low-frequency hearing had slight variations throughout the years, while a  
325 negative correlation was found at mid (1000Hz) and high-frequency (2000Hz) hearing.

326 Data from F14 were considered as an outlier and discarded because his hearing profile  
327 was not comparable to the rest of FMD patients (see Figure 2, Supplemental Digital  
328 Content 2). Since all frequencies are involved since the onset of the disease, we can  
329 speculate that the damage of the tectorial membrane mediated by mutations in otogelin  
330 will involve the entire cochlea from base to apex.

331 According to our results, the clinical picture of patients with mutations in *OTOG* would  
332 be a female of 43 years old with sudden or rapidly progressive flat SNHL around 60 dB  
333 and vertigo attacks with a family history of MD, vertigo or early onset SNHL.

334 In conclusion, we have found an enrichment of rare missense variants in the *OTOG*  
335 gene in FMD cases. These findings support a multiallelic contribution in MD, where  
336 *OTOG* gene seems to be playing a relevant role in the pathophysiology of hearing and  
337 vestibular functions in MD.

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## Figure legends

Figure 1: Flowchart summarizing the bioinformatic analysis on familial MD cases. On the left, single rare variant analysis (SRVA) and prioritization pipeline. On the right, the gene burden analysis (GBA) pipeline. SNV, single nucleotide variants; CADD, Combined Annotation Dependent Depletion Score.

Figure 2: Variants distribution across OTOG gene domains. On the upper part, variants which were found in familial Meniere disease (FMD) cases. On the bottom part, variants which were found in sporadic Meniere disease (SMD) cases. Yellow-colored variants indicate variants found in only one case, whereas red-colored variants represent variants found in 2 or more cases in a cohort. vWD, von Willebrand factor type D domain; T, Trypsin inhibitor-like domain; Abf, Alpha-L-arabinofuranosidase B domain; CT, Cysteine knot domain.

Figure 3: Scattered plot showing air conduction hearing thresholds obtained and the duration of the disease for each frequency in familial MD cases. Regression equations and estimated hearing loss at the onset are displayed below the charts.

## Supplemental Digital Content

Supplemental Digital Content 1.docx

Supplemental Digital Content 2.docx

**Table 1. Rare Variants Found in the Gene Burden Analysis in *OTOG* Gene for Familial MD Cases.**

| Variant position | Exon | Families       | Sporadic cases | MAF FMD      | MAF ALL MD    | MAF NFE | MAF CSVS | CADD  | Domain |
|------------------|------|----------------|----------------|--------------|---------------|---------|----------|-------|--------|
| 11:17574758G>A   | 4    | F1; F14        | –              | 0.041 (3/73) | 0.028 (3/109) | 0.00080 | 0.0033   | 24.8  | vWD    |
| 11:17578774G>A   | 7    | F2; F3; F4; F5 | S24            | 0.068 (5/73) | 0.055 (6/109) | 0.0090  | 0.017    | 15.95 | vWD    |
| 11:17594747C>A*  | 18   | F34            | –              | 0.027 (2/73) | 0.018 (2/109) | –       | –        | 22.2  | C8     |
| 11:17621218C>T   | 30   | F6; F7         | –              | 0.027 (2/73) | 0.018 (2/109) | 0.0026  | 0.0033   | 34    | C8     |
| 11:17627548G>A   | 32   | F14            | –              | 0.012 (1/73) | 0.009 (1/109) | 0.0056  | 0.0054   | 23.6  | Abf    |
| 11:17631453C>T   | 35   | F8             | S11; S24       | 0.012 (1/73) | 0.028 (3/109) | 0.017   | 0.014    | 12.89 | –      |
| 11:17632921C>T   | 35   | F2; F3; F4; F5 | –              | 0.068 (5/73) | 0.046 (5/109) | 0.0015  | 0.0054   | 7.71  | –      |
| 11:17656672G>A   | 45   | F10            | S9             | 0.013 (1/73) | 0.018 (2/109) | 0.0034  | 0.0039   | 31    | –      |
| 11:17663747G>A   | 52   | F1; F13; F14   | S7             | 0.055 (4/73) | 0.046 (5/109) | 0.0058  | 0.0054   | 19.41 | –      |
| 11:17667139G>C   | 54   | F9; F11; F12   | S12; S20       | 0.082 (6/73) | 0.073 (8/109) | 0.019   | 0.017    | 27.2  | CT     |

\* This novel variant was not included in the gene burden analysis. Abbreviations: MAF FMD, minor allele frequency in familial MD; MAF ALL MD, minor allele frequency in all familial and non-familial MD cases ; NFE, Minor allele frequency in non-Finnish European population; CSVS, Collaborative Spanish Variant Server; Abf, alpha-L-arabinofuranosidase B domain; CADD, Combined Annotation Dependent Depletion Score; CT: cysteine knot domain; vWD, von Willebrand factor type D domain.

**5136 SNV in SNHL genes**

**Quality control**

**4247 SNV**

**SRVA analysis**

Nonsynonymous/splice  
variants with  $MAF < 0.001$

**115 SNV**

CADD value  $> 15$

**75 SNV**

Described in public  
databases

**40 SNV**

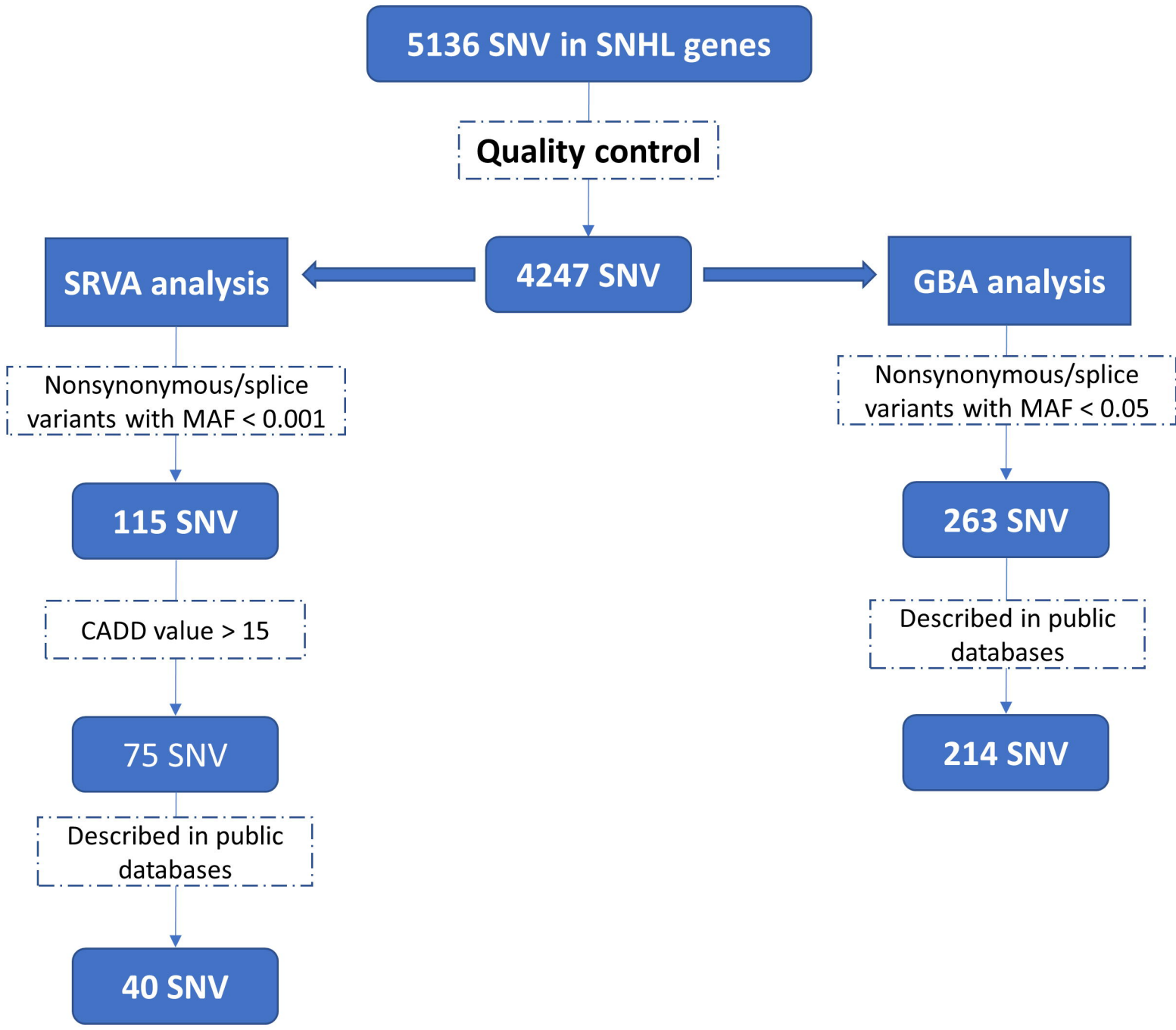
**GBA analysis**

Nonsynonymous/splice  
variants with  $MAF < 0.05$

**263 SNV**

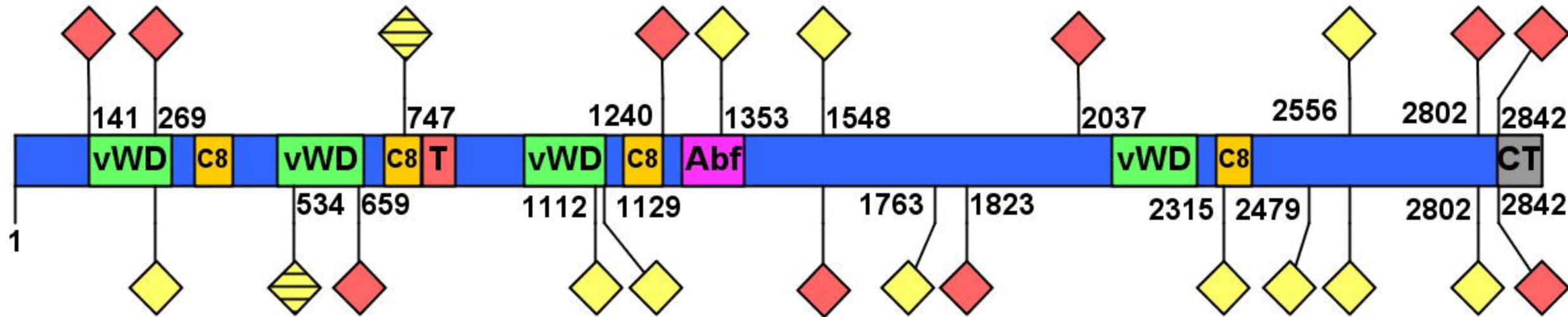
Described in public  
databases

**214 SNV**



**FMD**

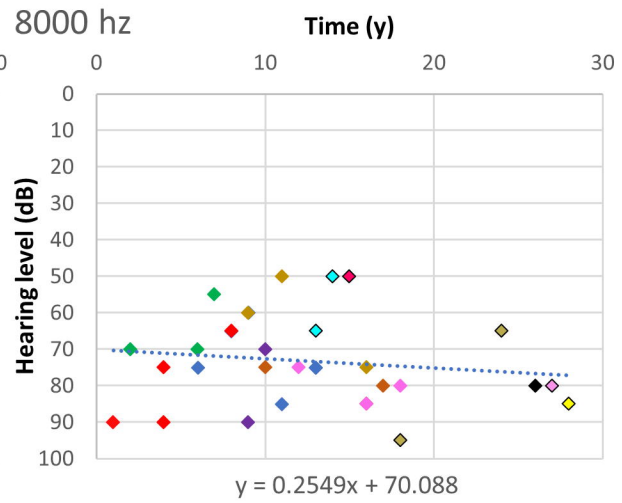
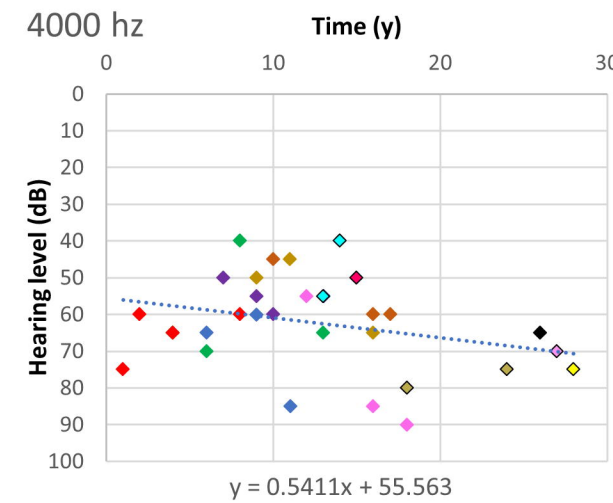
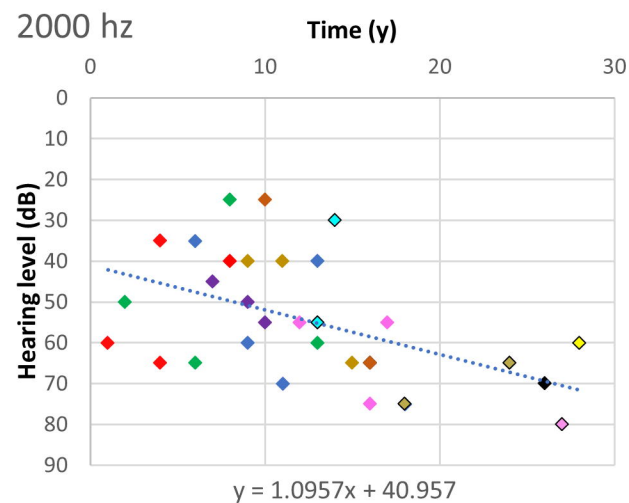
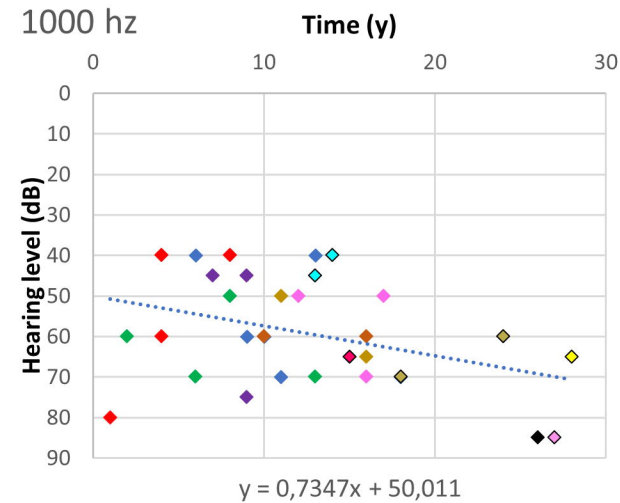
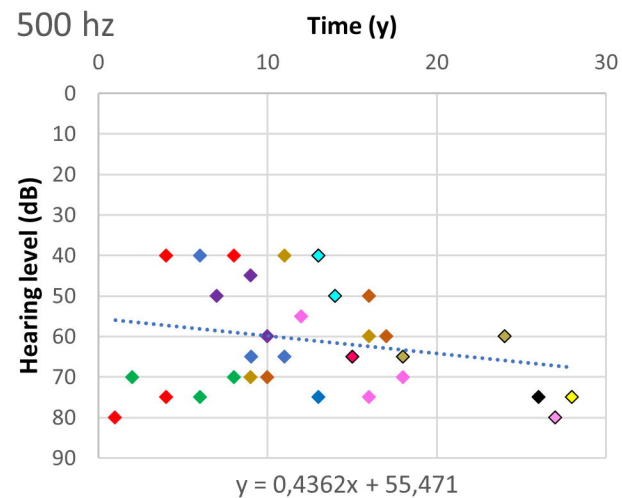
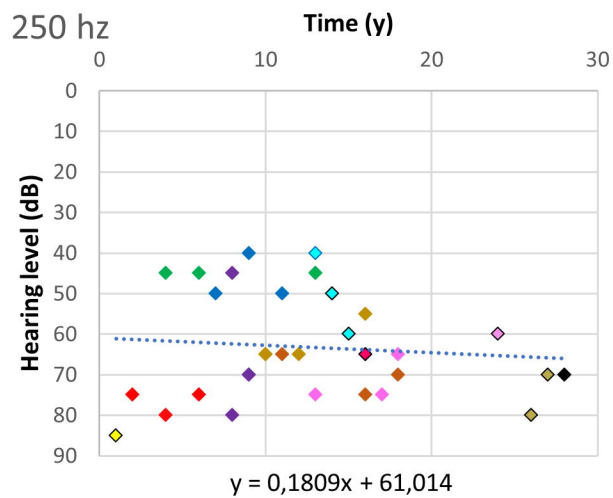
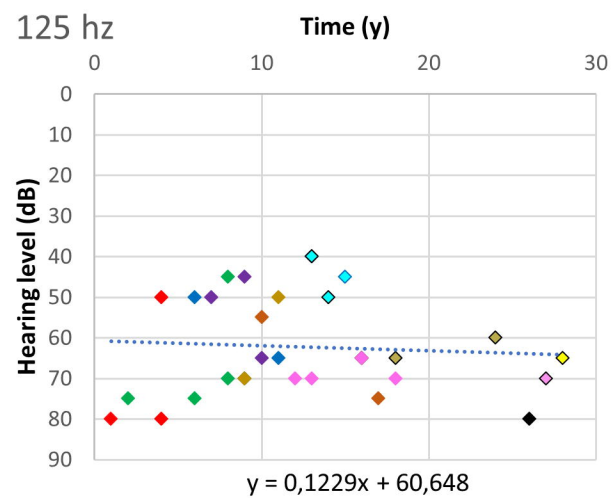
**SMD**



**Singleton**

**Multiplex**

**Novel**



- F1
- F2
- F3
- F4
- F5
- F6
- F7
- F8
- F9
- F10
- F11
- F12
- F13