

1 **Rare Variants in the *OTOG* Gene Are a Frequent Cause of Familial**
2 **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.

55

INTRODUCTION

56 Meniere's disease [MD (OMIM 18600)] is a rare inner ear disorder with three major
57 symptoms: sensorineural hearing loss (SNHL), episodic vertigo and tinnitus (Lopez-
58 Escamez et al. 2015; Espinosa-Sanchez & Lopez-Escamez 2016). Hearing loss always
59 involves low and medium frequencies in one or both ears (unilateral or bilateral MD) at
60 the onset of the disease. However, MD also affects high frequencies in early or
61 advanced stages of the disease (Belinchon et al. 2011). Epidemiological studies indicate
62 that MD is most common in European population, suggesting a genetic predisposition
63 (Ohmen et al. 2013). Although the majority of MD patients are considered sporadic
64 (Frejo et al. 2016; Frejo et al. 2017), familial clustering has been reported in 8-9% of
65 sporadic cases in the European descendent (Requena et al. 2014), and in 6% of Korean
66 population (Lee et al. 2015), which also supports a genetic contribution to the disease
67 (Roman-Naranjo et al. 2017). MD shows a wide range of phenotypic variations among
68 patients, even within the same families (Lee et al. 2015b), and it is commonly
69 associated with migraine and systemic autoimmune disorders (Tyrrell et al. 2014; Cha
70 et al. 2008). Familial MD (FMD) shows an autosomal dominant (AD) pattern of
71 inheritance with incomplete penetrance and anticipation, showing an earlier onset
72 compared to sporadic cases (Morrison et al. 2009; Birgerson et al. 1987; Klar et al.
73 2006). Different whole exome sequencing (WES) based studies have identified several
74 genes related with FMD. Single nucleotide variants (SNV) in *DTNA*, *FAM136A*,
75 *PRKCB*, *DPT* and *SEMA3D* were identified in 4 different families AD inheritance with
76 incomplete penetrance (Requena et al. 2015; Martín-Sierra et al. 2016; Martín-Sierra et
77 al. 2017). However, these findings have not been replicated neither in other MD
78 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.

102

MATERIALS AND METHODS

103 **Patient assessment and selection**

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

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

117 **DNA extraction and whole exome sequencing**

118 Blood and saliva samples were taken from patients with MD to perform WES. DNA
119 samples were extracted with prepIT-L2P (DNA Genotek, Ottawa, Canada) and QIAamp
120 DNA Blood Mini Kit (Qiagen, Venlo, The Netherlands) using manufacturer's protocols
121 and quality controls previously described (Szczepek et al. 2019). DNA libraries were
122 prepared by using the SureSelect Human All Exon V6 kit (Agilent Technologies, Santa
123 Clara, CA, USA) and were paired-end sequenced on the Illumina HiSeq 4000 platform
124 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.

174

RESULTS

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

197 discarded for further analysis. The most significant finding was an enrichment of rare
198 missense variants in *OTOG* gene in our FMD cases against either NFE population from
199 ExAC (OR= 4.3(2.6-7.0), $p= 4.1 \times 10^{-8}$) or Spanish population (OR= 3.6(2.1-5.9), $p=$
200 7.1×10^{-6}). Nine different rare missense variants were found in *OTOG* in 14/46 non-
201 related families, existing 6 families with 2 or more shared variants (Table 1 & see
202 Figure 1, Supplemental Digital Content 2). The variants rs61978648 and rs61736002
203 were shared by individuals from 4 unrelated families (F2, F3, F4 & F5). Likewise, the
204 variants rs552304627 and rs117315845 were found in patients from other 2 unrelated
205 families (F1 & F14).

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

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

211 The hearing profile for the 14 patients (3 males, 11 females) with rare variants in *OTOG*
212 gene was studied (see Figure 2, Supplemental Digital Content 2, which shows the pure
213 tone audiograms for these patients). Ten of them showed bilateral hearing loss, 3 had
214 left-sided hearing loss and only 1 patient shown right-sided SNHL (see Table 1,
215 Supplemental Digital Content 1, which summarizes the clinical information of the
216 familial MD cases carrying variants in *OTOG* gene). From these 14 patients, 16 ears
217 from 12 patients showed a flat shaped audiogram (57.1%), 5 ears from 5 patients
218 showed a ski-slope shaped audiogram (17.8%), 3 ears from 3 patients showed a reverse-
219 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 ± 8.66 and the estimated hearing loss at
225 onset was 62.14 ± 12.83 for low frequencies (125-250-500 Hz) and 58.75 ± 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

252

253 Familial MD has an AD inheritance with incomplete penetrance (Morrison et al. 2009;
254 Requena et al. 2014), and few genes have been involved in singular families (Requena
255 et al. 2015; Martín-Sierra et al. 2016; Martín-Sierra et al. 2017). In this study, we have
256 found an enrichment of rare missense variants in several unrelated patients with FMD in
257 the *OTOG* gene. These variants were observed in 15 of 46 non-related families (33%
258 familial cases). Seven of the 15 families with rare variants in *OTOG* showed incomplete
259 penetrance (47%) and partial syndromes (episodic vertigo or hearing loss) were found in
260 relatives from 5 of 15 families (Morrison et al. 2009; Requena et al. 2015; Martín-Sierra
261 et al. 2016; Martín-Sierra et al. 2017). Most of these rare variants were found in 2, 3 or
262 4 unrelated individuals from different families with MD and they were considered
263 multiplex variants. However, the majority of the variants in *OTOG* found in non-
264 familial patients with early onset were not observed in other sporadic cases (singletons
265 variants).

266 *OTOG*, which encodes otogelin, was described for first time by Cohen-Salmon *et al*
267 (Cohen-Salmon et al. 1997). Otogelin is a 2925 amino acid protein
268 (ENST00000399391) constituted by several vWD and C8 domains, and a cysteine knot-
269 like domain in its C-terminal. It is mainly expressed in acellular structures which cover
270 the sensory inner ear epithelia: the tectorial membrane, the otoconial membranes and
271 the cupula over the cristae ampullaris of the semicircular canals. Because of its
272 localization in the extracellular structures overlying the stereocilia of the hair cells
273 involved in the mechanotransduction of sound and acceleration, this structural protein
274 plays an important role in both auditory and vestibular functions (Schrauwen et al.
275 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*^{tm1Prs} model, authors inactivated
279 *Otog* by deleting the first three exons. Vestibular dysfunction was detected at P4 in
280 *Otog*^{-/-}, observing anomalies in the saccule and utricle. The auditory function was
281 evaluated by Pleyer reflex, showing profound hearing impairment. The *Otog*^{+/-} 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*^{tm1Prs}, in *Otog*^{tw} 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*^{vbd/vbd}, cause a
288 frame-shift and a premature codon. *Otog*^{vbd/vbd} 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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471

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

GBA analysis

Nonsynonymous/splice variants with MAF < 0.001

Nonsynonymous/splice variants with MAF < 0.05

115 SNV

263 SNV

CADD value > 15

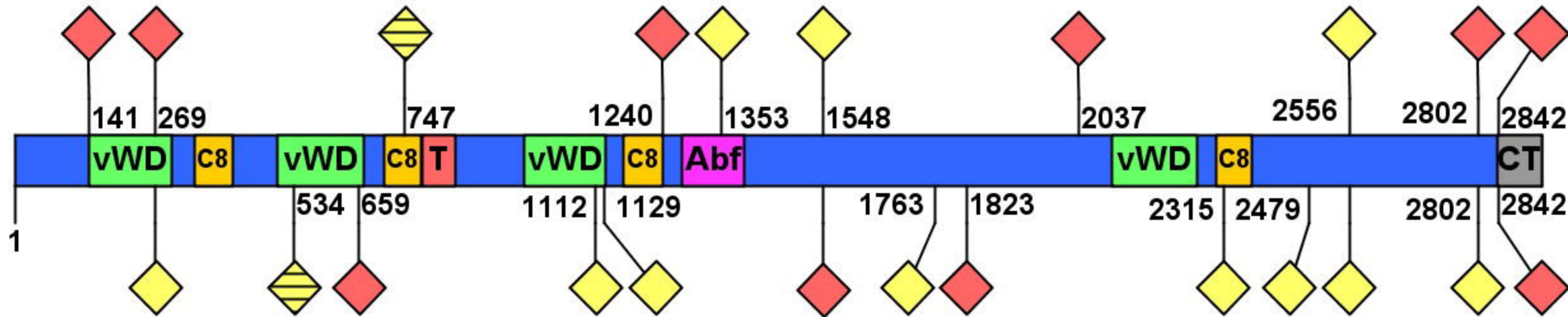
Described in public databases

75 SNV

214 SNV

Described in public databases

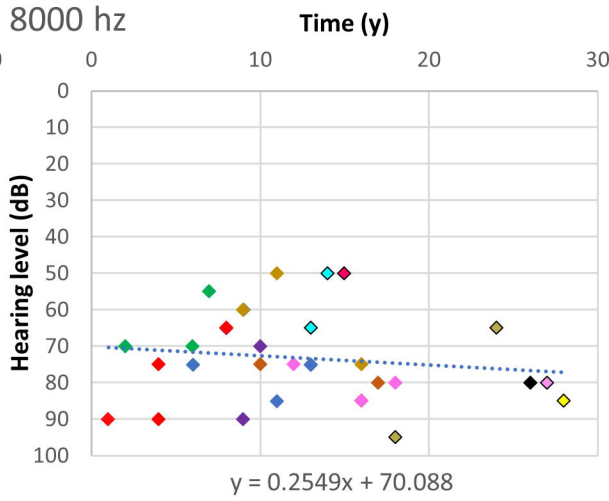
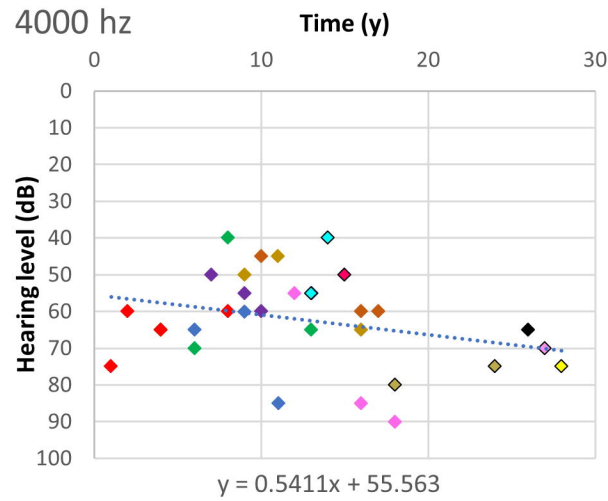
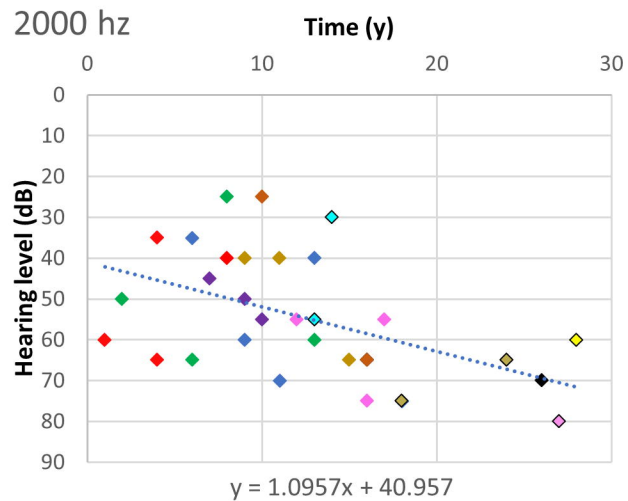
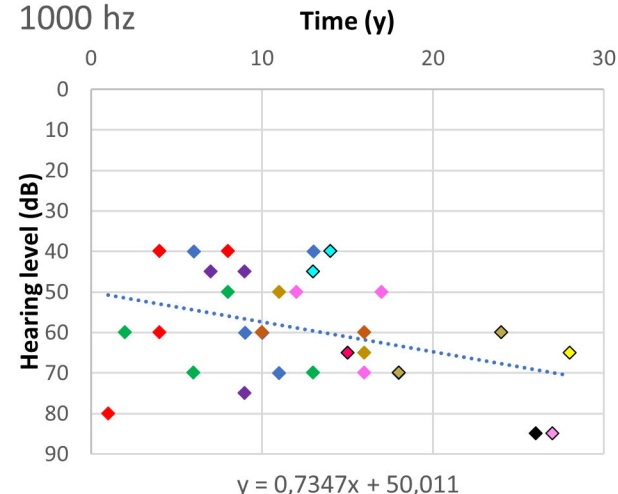
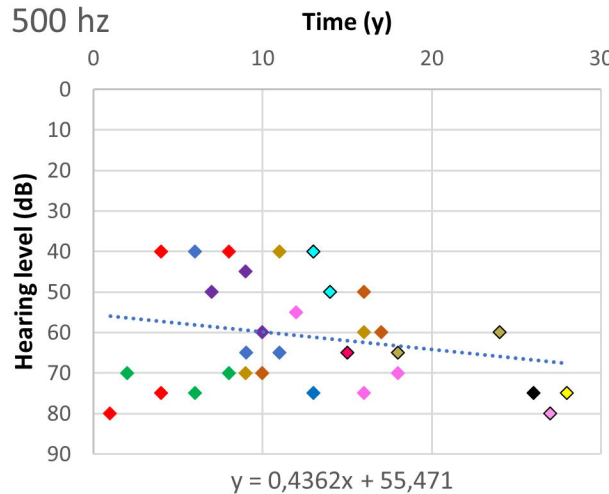
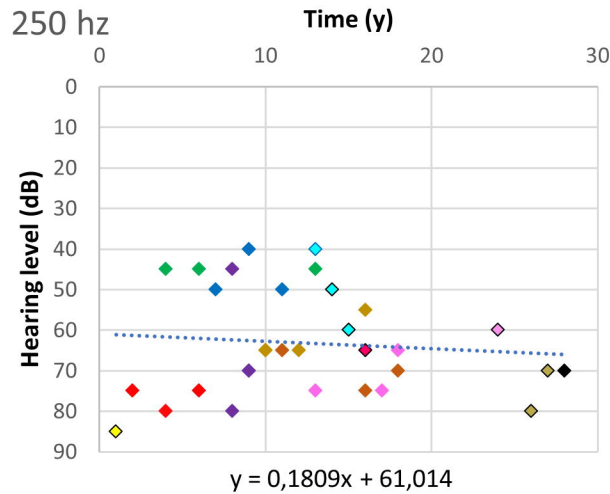
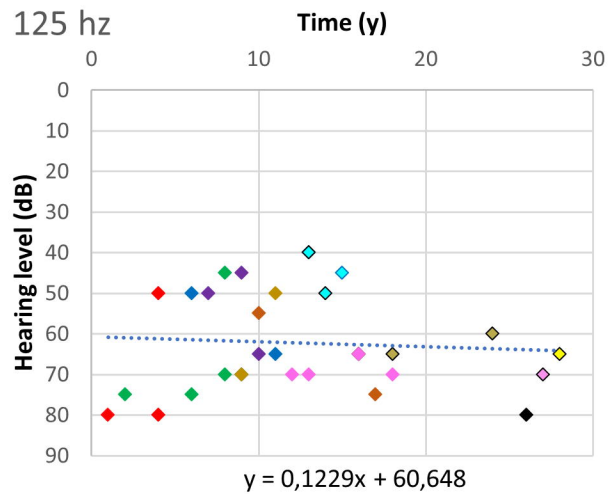
40 SNV

FMD**SMD**

Singleton

Multiplex

Novel



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