Familial clustering and genetic heterogeneity in Meniere’s disease

The aims of this study were to estimate the prevalence of familial cases in patients with Meniere’s disease (MD) and to identify clinical differences between sporadic and familial MD. We recruited 1375 patients with definite MD according to the American Academy of Otolaryngology-Head and Neck Surgery criteria, obtaining the familial history of hearing loss or episodic vertigo by direct interview or a postal survey in 1245 cases in a multicenter study. Familial clustering was estimated by the recurrence risk ratio in siblings ($\lambda_s$) and offspring ($\lambda_o$) using intermediate and high prevalence values for MD in European population. A total of 431 patients (34%) reported a familial history of hearing loss or recurrent vertigo and 133 patients had a relative with possible MD. After clinical reevaluation, 93 relatives in 76 families were diagnosed of definite MD (8.4%), including three pairs of monozygotic twins. $\lambda_s$ and $\lambda_o$ were 16–48 and 4–12, respectively. We observed genetic heterogeneity, but most families had an autosomal dominant inheritance with anticipation. No clinical differences were found between sporadic and familial MD, except for an early onset in familial cases. We may conclude that MD has a strong familial aggregation and that sporadic and familial MDs are clinically identical.

Conflict of interest

The authors declare that they have no conflict of interest.

Key words: anticipation – autosomal dominant – endolymphatic hydrops – familial Meniere’s disease – sensorineural hearing loss – twins – vertigo
Meniere’s disease (MD) is an inner ear disorder characterized by episodic vertigo associated with sensorineural hearing loss (SNHL), tinnitus and/or aural fullness. Although its etiology is unknown, endolymphatic hydrops has been shown in histopathological examinations of human temporal bones (1, 2). However, current data support the hypothesis that endolymphatic hydrops is an epiphenomenon associated with a variety of inner ear disorders (3), and genetics and environmental factors contribute to its development. The prevalence of MD is about 0.5–1/1000 and it is more common in industrialized countries and in European descendent populations (4, 5). There is usually no gender preponderance for MD and the typical age of onset is 30–50 years (6). In 25–40% of cases, both the ears are affected (bilateral disease), leading to severe hearing impairment and chronic imbalance, resulting in a huge burden for the patient and reduced ability to work (7, 8). The bilateral MD is common (9, 10), but both sides are seldom affected simultaneously (11, 12).

Although the majority of cases with MD are considered sporadic, genetic factors probably confer susceptibility to MD (13). Of note, some patients report relatives with a history of vertigo or SNHL, and the frequency of familial cases is around 5–15% in European population (13, 14). Linkage studies in familial Meniere’s disease (FMD) have found candidate loci at 12p12.3 in Swedish (15) and 5q14–15 in German families (16), but families with recessive inheritance have been also described (17); however, no gene has been identified yet, and these findings point to a genetic heterogeneity (18). An autosomal dominant pattern of inheritance with incomplete penetrance has been described in several families (14, 16), but rare (minor allele frequency, MAF<5%) and heterozygotic de novo allelic variants may contribute to sporadic MD, and they are difficult to be identified with classical linkage studies. Anticipation including both earlier onset and tendency to more severe symptoms in successive generations has also been observed in MD in Swedish (15, 19), British (20) and German families with three affected generations (16). Our preliminary data in a large Spanish cohort also suggest familial aggregation, but a comprehensive study to determine the prevalence of FMD and the type of heredity in these families is missed.

MD has a clinical heterogeneity and the time course of the episodes of vertigo and hearing loss are variable (21, 22). Moreover, incomplete clinical variants could be missed according to the current American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) guidelines for diagnosis of MD (23), and this may underestimate the familial clustering in MD.

The aims of this study were to determine whether there is familial aggregation in MD and/or SNHL in the relatives of MD patients and to identify clinical differences between patients with and those without familial clustering.

### Material and methods

**The MD Spanish cohort**

We evaluated 1220 Spanish patients with MD. The clinical features of a subset of 690 patients with MD in the Spanish cohort have been described in detail previously (6, 21). Briefly, patients with a clinical diagnosis of definite MD according to the AAO-HNS criteria were included from October 2007 to June 2012 by establishing a common structured clinical database protocol to retrieve outcome measures. The 14 centers contributing to this cohort were distributed in 10 regions in Spain (Galicia, Asturias, Castilla-Leon, Navarra, Aragon, Madrid, Valencia, Extremadura, Andalucia, and Canary Islands) and were invited to participate on the basis of their academic profile and their staff expertise in MD.

**The MD North Italian cohort**

Patients from the Italian cohort were recruited at the outpatient clinic of the vestibular disorders Unit in San Raffaele Hospital, Milan (n = 143), and from Le Molinette Hospital in Turin (12 cases) from September 2007 to June 2012. All of them presented definite MD according to the AAO-HNS criteria. Patients with delayed hydrops were excluded and all phenotype
features were collected according to a structured clinical protocol in a database.

**Clinical evaluation of patients with MD**

All patients underwent complete otolaryngologic examination and audiologic evaluation with pure-tone audiometry. A basic neurotologic examination (spontaneous and gaze-evoked nystagmus, head-impulse test, head-shaking nystagmus and caloric test) was also performed. Hearing staging for each patient with definite MD was defined according to the AAO-HNS criteria (23). Patients with bilateral SNHL were considered to have metachronic SNHL, if a period of time >1 month to develop SNHL between the first- and the second-affected ear was observed. Episodes of vertigo were characterized by their frequency and duration as previously described (21), delayed hydrops was considered as a clinical variant of MD in the Spanish cohort and it was defined if the patient presented SNHL at least 1 month before the vertigo attacks.

The protocol of diagnosis included an examination by magnetic resonance imaging of the brain to exclude other possible causes of SNHL and vertigo.

The following clinical variables were assessed: sex, age of onset, unilateral or bilateral SNHL and ear affected, hearing loss at diagnosis, AAO-HNS hearing stage, frequency of vertigo during the last 6 months, Tumarkin crisis, delayed hydrops, AAO-HNS functional scale, duration of attacks, time-course of the disease, time since last attack and associated factors (recurrent headache, type of headache, history of autoimmune disease, and smoking habit).

**Estimation of familial aggregation**

Familial MD (FMD) was defined if a patient with definite MD had a relative in the first, second or third degree with diagnosis of definite MD, according to the AAO-HNS criteria. We considered parents, siblings, and offspring as first-degree relatives; aunts, uncles, nephews, nieces, half-siblings, grandparents, and grandchildren as second-degree relatives; and first cousins as third-degree relatives. More distant relatives were not included.

Familial aggregation was estimated by the recurrence-risk ratio for siblings (λs) and offspring (λo), after calculating the prevalence of MD between siblings and using intermediate (24) and high prevalence values (25) for MD in European descendent population. So,

\[ \lambda_s = \frac{N_s}{P_o} \]

where \( N_s \) = number of cases with a sibling affected and \( P_o \) = prevalence observed in the population (26).

Incomplete phenotypes were considered in the relatives of FMD cases presenting SNHL or episodic vertigo. We also could obtain information from 529 patients with MD to identify monozygotic and dizygotic twins among siblings.

**Exclusion criteria**

We excluded patients with vestibular migraine, benign paroxysmal positional vertigo, vestibular neuritis, ear surgery, recurrent infection of the middle ear, vestibular schwannoma, and any known cause mimicking MD, according to the diagnostic scale of the AAO-HNS (23).

**Statistical analyses**

Data were analyzed using SPSS Software 15.0 (SPSS Inc., Chicago, IL). Quantitative data are shown as means with SD. Qualitative variables were compared between patients with familial and sporadic MDs by using Pearson \( \chi^2 \) test with Yates’ continuity correction. Quantitative variables (age, time since onset of symptoms, hearing loss at diagnosis, and number of attacks in the last 6 months) were compared by unpaired two-tailed Student’s \( t \)-test.

**Results**

Estimation of familial clustering of MD

We retrieved clinical information from 1375 patients with MD. The familial history of ear disorders (hearing loss or episodic vertigo) was obtained in 1245 cases (missing rate 9%). From these 1245 cases, there were 142 cases without reliable information and we did not consider them for the calculations. The reasons to exclude these cases were deceased patients, incomplete clinical information in the clinical records and incomplete information at the clinical interview. A total of 431 patients (34%) reported a positive familiar history.
of hearing loss or recurrent vertigo. One hundred and thirty-three patients had a relative with hearing loss and recurrent vertigo consistent with possible MD. Figure 1 shows the stepwise method to identify the index cases and the 76 families with a proven family history of definite MD from the symptoms initially reported by their relatives with MD that were confirmed after clinical evaluation.

Forty of 1103 patients with MD had a sibling with MD and was 16–48. Ten patients had offspring with MD and was 4–12. Overall, FMD has a prevalence of 8.4% in our cohort. There were 93 cases (37 men and 56 women) from 76 multicase families (Table S1, supporting information). The mean age of onset in FMD was 42.9 ± 14.1 years (range 11–64), while sporadic cases started at 46 ± 13 years (p = 0.028.

Table 1 compares the clinical features between FMD and sporadic MD. Of note, we did not find any clinical difference between familial and sporadic MD, but the age of onset of the disease. MD has a light female preponderance, and bilateral SNHL occurred in 24% of sporadic and 32% of familial MD (p = 0.10). We also observed that metachronic SNHL was more common in familial than sporadic cases among patients with bilateral SNHL (p = 0.015).

Recurrent headache was a common complaint affecting 35% of sporadic and 41% of family cases (p = 0.05), but migraine was only found in 12% and 18% cases, respectively. Otolithic crisis of Tumarkin is observed in 20% of either familial or sporadic cases.

We tried to analyze the pattern of inheritance in each family to cluster the families according to their inheritance. Although genetic heterogeneity was found, most family cases were suspected to be autosomal dominant. Some recessive families with two affected cousins were identified, and mitochondrial inheritance cannot be excluded in a few families. Figure 2 shows the pedigrees of 10 selected families.

Thirty-five families have the affected patients in the same generation and anticipation could not be evaluated in these families. In addition, there were no reliable data to determine the age of onset of symptoms in another 23 families, because one of the affected subjects was deceased (7 families) or did not remember the exact age of onset. Finally, anticipation was found in 18 of the 19 evaluated families with cases in successive generations. We revised the parental origin in these 18 families to evaluate if there was any trend of genomic imprinting, but the trait was of paternal or maternal origin in 6 and 12 cases, respectively.

Migraine was a comorbid condition in 17 families, but migraine did not segregate with MD in most of the families. We only found co-segregation in family no. 5.

Evaluation of monozygotic or dizygotic twins

We identified five pairs of twins with at least one twin affected with MD. Three of them were monozygotic twins (families 17, 37 and 49) and all cases were affected. They did not have migraine or autoimmune disease. No history of possible MD was reported in their relatives, although a deaf-cousin was found in family 49, suggesting autosomal recessive inheritance. Two dizygotic twins were reported in the Spanish cohort and both cases have a healthy member. One pair consisted of a sporadic case of FMD and her healthy sister and another pair was found in family 54 with suspected autosomal dominant inheritance. Although reliable concordance rates in co-twins cannot be estimated from only five pairs, they also appear to indicate a strong genetic background in MD.

Discussion

The MD is a complex trait and multiple genes and environmental factors contribute to its development. The aggregation of a disease in families is the first observable clue for an underlying genetic susceptibility. Our study includes the largest cohort of patients with sporadic MD in European descendent population and it shows that MD has a strong familial clustering. Our data from 1245 cases with definite MD estimates that FMD is found in 8% of cases and confirm that MD has a genetic pre-disposition. The frequencies reported for FMD range from 4 to 20% and this variation is probably explained by the differences in criteria to define FMD (14, 16, 17, 27), although the prevalence of FMD may depend upon genetic variability or heterozygosity among different populations.

Some of the previous studies are based on questionnaires or did not use the AAO-HSN criteria to define MD. These could inflate the estimation of FMD because other vestibular disorders can present hearing loss and tinnitus such as vestibular migraine (28). To avoid an overestimation of FMD, we have required that two cases in first, second or third degree with criteria for definite MD were confirmed by clinical evaluation by an expert otoneurologist to accept the diagnosis of FMD. The second case was also considered as definite MD, despite of the patient was deceased, if there was
Familial clustering and genetic heterogeneity in Meniere’s disease

Table 1. Clinical characteristics of patients with sporadic and familial MD

<table>
<thead>
<tr>
<th>Variables</th>
<th>Familial MD (n = 93)</th>
<th>Sporadic MD (n = 1152)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>52.2 (13.9)</td>
<td>55.2 (12.8)</td>
<td>0.030</td>
</tr>
<tr>
<td>Female gender, n (%)</td>
<td>58 (62.4)</td>
<td>657 (56.1)</td>
<td>0.277</td>
</tr>
<tr>
<td>Age of onset, mean (SD)</td>
<td>42.9 (14.1)</td>
<td>46.0 (13.0)</td>
<td>0.028</td>
</tr>
<tr>
<td>Time since onset of symptoms (years), mean (SD)</td>
<td>9.1 (7.0)</td>
<td>9.0 (7.7)</td>
<td>0.741</td>
</tr>
<tr>
<td>Right ear affected, n (%)</td>
<td>26 (30.2)</td>
<td>421 (37.0)</td>
<td>0.165</td>
</tr>
<tr>
<td>Hearing loss (dB) at diagnosis, mean (SD)</td>
<td>49.6 (14.7)</td>
<td>51.4 (17.5)</td>
<td>0.519</td>
</tr>
<tr>
<td>Bilateral SNHL, n (%)</td>
<td>29 (31.2)</td>
<td>285 (24.4)</td>
<td>0.170</td>
</tr>
<tr>
<td>Synchronous SNHL, n = 114 (%)</td>
<td>5 (5.4)</td>
<td>109 (9.3)</td>
<td>0.015</td>
</tr>
<tr>
<td>Metachronic SNHL, n = 182 (%)</td>
<td>24 (25.8)</td>
<td>158 (13.5)</td>
<td></td>
</tr>
<tr>
<td>Time interval in months, mean (SD)</td>
<td>72 (70)</td>
<td>50 (71.6)</td>
<td>0.168</td>
</tr>
<tr>
<td>Delayed hydropsa, n (%)</td>
<td>6 (10.5)</td>
<td>61 (10.3)</td>
<td>0.951</td>
</tr>
<tr>
<td>Recurrent headache, n (%)</td>
<td>41 (46.6)</td>
<td>371 (35.8)</td>
<td>0.045</td>
</tr>
<tr>
<td>Type of headache, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Migraine</td>
<td>17 (18.3)</td>
<td>147 (12.5)</td>
<td>0.792</td>
</tr>
<tr>
<td>Tension type headache</td>
<td>24 (25.8)</td>
<td>190 (16.2)</td>
<td></td>
</tr>
<tr>
<td>History of autoimmune disease, n (%)</td>
<td>13 (15.7)</td>
<td>213 (19.6)</td>
<td>0.378</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>22 (25.9)</td>
<td>290 (26.8)</td>
<td>0.857</td>
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<tr>
<td>Number of attacks in last 6 months, mean (SD)</td>
<td>3.8 (11.4)</td>
<td>2.3 (4.5)</td>
<td>0.323</td>
</tr>
<tr>
<td>Drop-attacks (Tumarkin) crises, n (%)</td>
<td>15 (19.0)</td>
<td>198 (20.3)</td>
<td>0.782</td>
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<tr>
<td>Hearing stage, n (%)</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>6 (6.7)</td>
<td>106 (9.3)</td>
<td>0.810</td>
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<tr>
<td>2</td>
<td>21 (23.3)</td>
<td>247 (21.7)</td>
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</tr>
<tr>
<td>3</td>
<td>44 (48.9)</td>
<td>570 (50.0)</td>
<td></td>
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<tr>
<td>4</td>
<td>19 (21.1)</td>
<td>216 (19.0)</td>
<td></td>
</tr>
<tr>
<td>Functional scale, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15 (17.2)</td>
<td>195 (17.4)</td>
<td>0.534</td>
</tr>
<tr>
<td>2</td>
<td>21 (24.1)</td>
<td>335 (29.9)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21 (24.1)</td>
<td>307 (27.4)</td>
<td></td>
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<tr>
<td>4</td>
<td>17 (19.5)</td>
<td>171 (15.3)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11 (12.6)</td>
<td>93 (8.3)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2 (2.3)</td>
<td>19 (1.7)</td>
<td></td>
</tr>
</tbody>
</table>

*aDelayed hydrops: sensorineural hearing loss (SNHL) preceded at least 1 month ahead the episodes of vertigo. This condition was excluded in the Italian cohort.

A standard measure of familial aggregation is the recurrence-risk ratio of disease in siblings ($\lambda_s$) of an index case. So, $\lambda_s$ could be used to infer a genetic model of inheritance and under a multilocus multiplicative model, where the total $\lambda_s$ can be factored into locus-specific risk ratios (i.e. $\lambda_s = \lambda_1 \cdot \lambda_2 \cdots \lambda_i$), where $i$ = the number of disease loci (29). According to the low prevalence of sporadic MD in European population, we expect that several loci share the mutational target in MD (<100) and genetic heterogeneity is expected among different families (29, 30).

We have estimated familial aggregation in MD by using $\lambda_s$ and $\lambda_o$: MD is 16–48 times more common between siblings, and 4–12 times in offsprings than in the general population. As a measure of genetic risk, the limitations of $\lambda_s$ are well known: both shared genes and shared environment likely contribute to familial aggregation in complex diseases (31). The lower values of $\lambda_o$ compared to $\lambda_s$ were expected, since the onset of MD in the third or fourth decade of life would make possible that the offspring could develop MD several years later. However, parents and their offspring only shared the environment during the first two or three decades, but the $\lambda_o$ values indicate a moderate to high risk to first-degree relatives suggesting a genetic susceptibility in MD.

Our set of families presents different patterns of inheritance, although most of the families share an autosomal dominant pattern. This is the inheritance pattern most commonly described in FMD (14), and co-segregation with migraine has been reported in several families (32, 33), but some families do not segregate migraine (34). Five pedigrees (no. 10, 14, 42, 63 and 70) appear to have an autosomal recessive inheritance and another two families (17 and 44) with monozygotic twins could be caused by de novo mutations.
Our data confirm previous observation in FMD that some family members only had a partial syndrome or incomplete phenotype, with vestibular symptoms predominating (20, 34). We also found relatives with SNHL and episodic vertigo who could be classified as possible MD. The incomplete phenotypes in FMD could be explained by different mechanisms: (i) somatic mutations in the inner ear, (ii) multiple mutations at different loci, and (iii) mutations affecting mitochondrial DNA. First, although a number of studies has shown an apparent germline origin of low frequency mutations, \textit{de novo} somatic mutations have been found in exome sequencing studies comparing affected and unaffected tissues (29); second, multiple mutations at different loci can affect genes and regulatory elements (i.e. enhancers and transcription binding sites) and the additive effect of multiple loci will determine the incomplete or the full phenotype of MD; third, mutations affecting mitochondrial DNA are known to cause SNHL (35) and theoretically some families could harbor mutant heteroplasmic DNA and normal DNA. Mitochondrial mosaicism could also explain the incomplete phenotype observed in several families with FMD.

Anticipation in successive generation was found in 41 British families reported by Morrison (36), but this phenomenon was not observed in 15 of 16 Finnish families with MD (34). We observed anticipation in most of the families in our cohort, and maternal origin was observed in 12/18 families, suggesting maternal imprinting. Moreover, in eight pedigrees with anticipation, we cannot rule out mitochondrial inheritance. So, further studies are needed to investigate mitochondrial inheritance in FMD. Finally, it is possible that the anticipation phenomenon observed in FMD is the result of the improvement of diagnosis and the accessibility to healthcare systems in successive generations (14).

We identified two pedigrees with FMD and otosclerosis (families 5 and 47), both with autosomal dominant inheritance and anticipation. Moreover, we identified another four families with one case of MD and a relative with otosclerosis. It is possible that a common mechanism can explain the link between otosclerosis and MD. In fact, a histopathological study has found otosclerotic endolymphatic duct occlusion causing endolymphatic hydrops and MD (37). On
Familial clustering and genetic heterogeneity in Meniere’s disease

the other hand, cochlear otosclerosis with SNHL and vestibular symptoms can resemble MD (38). Finally, the same gene could pre-dispose individuals to both MD and otosclerosis, leading to different phenotypes.

Monozygotic or ‘identical’ twins have been widely studied to dissect the relative contributions of genetics and environment in human diseases. Interestingly, we identified three pairs of monozygotic twins with all members affected, suggesting that genetic factors determine the occurrence of MD. Brown described the first identical twins with Meniere-like phenotype (39). They were two men with deafness, but only one with dizzy spells for about 2 years; they had a conductive hearing loss on clinical and audiometric testing, and the diagnosis was probably otosclerosis. A previous study identified two pairs of monozygotic twins in pedigrees of families with migraine, episodic vertigo and MD (33), although one of the twins fulfilled criteria for definite MD, the other twin only had migraine and episodic vertigo without hearing loss. Despite the discordance for hearing loss observed in these twins, the same genetic background could lead to different syndromes, suggesting that the epigenetic and environmental factors are relevant in the final phenotype. Although the number of twins with MD described is very low, they also suggest a strong genetic component in MD.

We compare the clinical features between sporadic and familial cases of MD, but no clinical differences were found. FMD and sporadic MD are clinically indistinguishable, since the age of onset overlaps between familial and sporadic cases. All together, we do not have any epidemiological or clinical evidence to differentiate sporadic from familial MD, but familial clustering in siblings and offspring suggest a genetic background. Sporadic MD could be caused by rare variants or de novo mutations with moderate penetrance in successive generations as it occurs in neurodevelopmental disorders (40).

Conclusions

The MD has a strong familial aggregation. Although genetic heterogeneity is observed, most families have an autosomal dominant inheritance pattern. FMD is associated with anticipation in successive generations. Because there are no clinical differences between sporadic and familial MDs, sporadic MD could be caused by rare or de novo mutations with moderate penetrance.

Supporting Information

The following Supporting information is available for this article:
Table S1. Familial Meniere’s disease: pedigrees with at least two cases of definite MD. AD/AR, autosomal dominant/recessive inheritance; MT, mitochondrial inheritance; NA, not applicable (affected siblings).

Additional Supporting information may be found in the online version of this article.

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Ethics approval

The subject’s written informed consent to participate in the study was obtained according to the Helsinki guidelines and the Institutional Review Boards from each hospital approved the study.

References


