EPIDEMIOLOGY AND GENETICS OF VESTIBULAR DISORDERS

EDITED BY: Jose Antonio Lopez-Escamez, Alan G. Cheng, Eva Grill and Tien-Chen Liu

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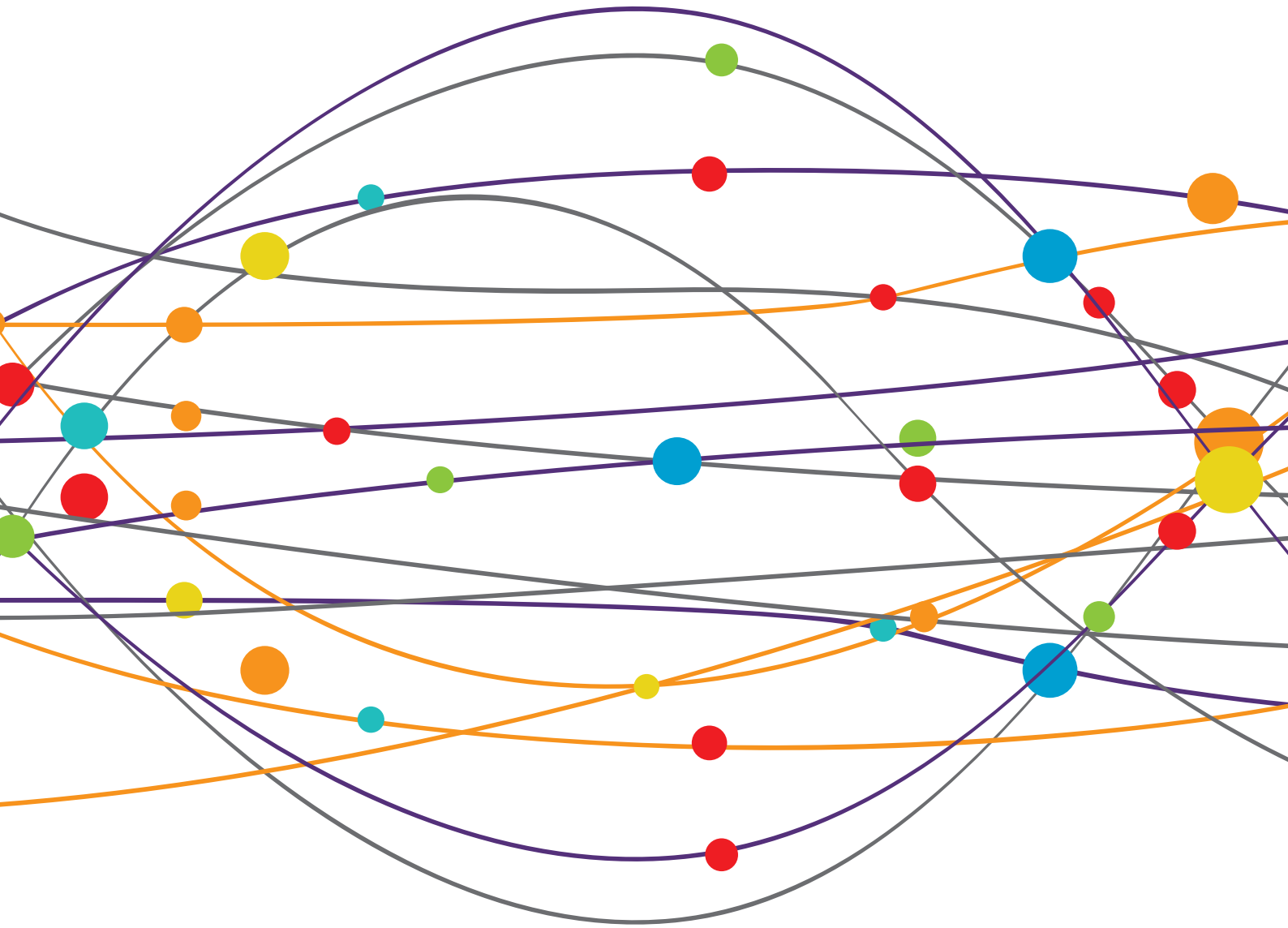

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EPIDEMIOLOGY AND GENETICS OF VESTIBULAR DISORDERS

Topic Editors:
Jose Antonio Lopez-Escamez, Universidad de Granada, Spain
Alan G. Cheng, Stanford University, United States
Eva Grill, Ludwig Maximilian University of Munich, Germany
Tien-Chen Liu, National Taiwan University, Taiwan

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Editorial: Epidemiology and Genetics of Vestibular Disorders

Jose A. Lopez-Escamez[1,2,3,4]*, *Alan G. Cheng*[5], *Eva Grill*[6,7,8] *and Tien-Chen Liu*[9]

[1] Otology and Neurotology Group CTS495, Department of Genomic Medicine, GENYO - Centre for Genomics and Oncological Research - Pfizer/University of Granada/Junta de Andalucía, Parque Tecnologico de la Salud (PTS), Granada, Spain, [2] Department of Otolaryngology, Instituto de Investigación Biosanitaria, ibs.GRANADA, Hospital Universitario Virgen de las Nieves, Granada, Spain, [3] Division of Otolaryngology, Department of Surgery, Universidad de Granada, Granada, Spain, [4] Sensorineural Pathology Programme, Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Madrid, Spain, [5] Department of Otolaryngology - Head and Neck Surgery, Stanford University School of Medicine, Stanford, CA, United States, [6] Institute for Medical Information Processing, Biometrics and Epidemiology, Ludwig-Maximilians-Universität München (LMU) Munich, Munich, Germany, [7] German Center for Vertigo and Balance Disorders, University Hospital Munich, Ludwig-Maximilians-Universität München (LMU) Munich, Munich, Germany, [8] Munich Centre of Health Sciences, Ludwig-Maximilians-Universität München (LMU) Munich, Munich, Germany, [9] Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan

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Michael Strupp, Ludwig Maximilian University of Munich, Germany

Reviewed by:
Jorge Kattah, University of Illinois at Chicago, United States
Nese Celebisoy, Ege University, Turkey
Maurizio Versino, ASST Settelaghi, Italy

***Correspondence:**
Jose A. Lopez-Escamez
antonio.lopezescamez@genyo.es

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Editorial on the Research Topic

Epidemiology and Genetics of Vestibular Disorders

Vestibular disorders (VD) include a heterogeneous set of neuro-otological conditions. Peripheral and central VD such as vestibular migraine (VM) or Menière's disease (MD) are among the more frequently encountered disease entities, but there is also a large group of rare cerebellar disorders (1–3).

This issue of Frontiers in Neurology is dedicated to recent developments and new methodological findings in the epidemiology and genetics of VD. Of note, heritability has been largely ignored in VD as epidemiological evidence based on familial aggregation and twin studies are scarce (4, 5). Familial clustering suggests a genetic contribution in some VD, including VM, MD, and spinocerebellar and episodic ataxias (6, 7). Paz-Tamayo et al. show epidemiological evidence to support heritability in VM including familial aggregation and ethnic-specific differences in the occurrence of this condition.

However, a better characterization is needed to define syndromes and symptoms that overlap between individuals with a vestibular episodic syndrome and those with peripheral and central bilateral vestibular loss.

Precise history taking is the first and essential step for the diagnosis of vestibular disorders, and the systematic gathering of clinical information is particularly relevant in the primary care setting (Strobl et al.). The distribution of diagnoses in patients with VD is different in primary care and specialized neuro-otology clinics, and therefore they have different needs. Primary care professionals would benefit from training on maneuvers for repositioning otoliths, the diagnosis and treatment of different types of headaches, the identification of cardiovascular risk factors including orthostatic hypotension, and the appreciation of unwanted effects of some of the most commonly used drugs (Domínguez-Durán et al.) In an opinion paper, Maarsingh and van Vugt propose 10 practical vestibular tools for primary care physicians. In addition, machine learning techniques applied on large datasets have a huge potential to provide a decision support system for diagnosis and treatment in neuro-otology (Vivar et al.), including the classification of central and peripheral VD (8).

The assessment of disability is also a major issue in the elderly population and presbyvestibulopathy shows an important subjective perception of disability, particularly in women (Soto-Varela et al.). For this reason, co-morbidities should be carefully considered in patients with vestibular dysfunction (Malmström et al.), this approach being used to define clinical subgroups of patients (9, 10). Bilateral vestibulopathy (BVP) is a heterogeneous clinical condition characterized by a hypofunction of the vestibular nerves or labyrinths on both sides and quantitative assessment of the vestibulo-ocular reflex is needed to differentiate it from presbyvestibulopathy (11). In a retrospective study, Mancino-Moreira et al. classify patients into four clinical subgroups according to the symptoms: recurrent vertigo with BVP, rapidly progressive BVP, slowly progressive BVP, and slowly progressive BVP with ataxia.

Despite the huge progress in the definition and classification of vestibular disorders performed by the International Classification Committee, Dlugaiczyk et al. illustrate that there are still patients whose recurrent vestibular symptoms cannot be attributed to any of the recognized episodic vestibular syndromes, including MD (12), VM (13), benign paroxysmal positional vertigo (14), vestibular paroxysmia (15), orthostatic vertigo (16), or transient ischemic attacks (17). This category has been defined as recurrent vestibular symptoms not otherwise specified and it is composed of individuals with an incomplete phenotype not fulfilling the diagnostic criteria for MD or VM.

Research about the genetics of vestibular disorders in an emerging topic, including MD (18–20), and this volume offers some outstanding pictures that contribute to a better understanding of neurotological disorders. Gu et al. combine RNAseq and data mining to define potential MD genes in the stria vascularis. Shew et al. report microRNA profiles in the perilymph and serum of patients with MD that may serve as potential biomarkers of the condition. The diagnosis and prognosis of MD is likely to be improved by the presence of endolymphatic sac (ES) hypoplasia, under the hypothesis that ES hypoplasia critically predisposes the inner ear to develop bilateral MD (Bächinger et al.).

Rujescu et al. report an allelic variant conferring susceptibility to vestibular neuritis, indirect evidence for an involvement of Herpes simplex virus in this condition. Mei et al. highlight the role of genetic sequencing to develop personalized medicine in VD. As an example, Oh et al. report the *TRPM7* gene in a Korean family with four affected individuals with vestibular migraine as the first candidate gene for familial vestibular migraine by exome sequencing. Choi et al. also report MD-like symptoms in the 22q11.2 deletion syndrome, targeting the *TBX1* gene. Moreover, epigenetic regulation by circular RNAs may explain susceptibility for intracranial aneurysms rupture (Huang et al.).

This is only the beginning. Genetic research in VD is still in its infancy. The development of cellular and animal models of vestibular disorders is needed to carry out functional validation of candidate genes obtained in human studies (21, 22). Gene replacement therapy can successfully repair auditory and vestibular hair cells and preserve organ function in genetic mouse models (23).

AUTHOR CONTRIBUTIONS

JL-E wrote the original draft, assembled and incorporated comments from the co-authors, and crafted the final draft. All co-authors contributed to manuscript review and revision.

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SYSTEMATIC REVIEW
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Systematic Review of Prevalence Studies and Familial Aggregation in Vestibular Migraine

Ana Paz-Tamayo[1], *Patricia Perez-Carpena*[2,3]* *and Jose A. Lopez-Escamez*[1,3,4]

[1] Division of Otolaryngology, Department of Surgery, Universidad de Granada, Granada, Spain, [2] Department of Otolaryngology, Instituto de Investigación Biosanitaria ibs.GRANADA, Hospital Universitario San Cecilio, Granada, Spain, [3] Otology & Neurotology Group CTS495, Department of Genomic Medicine, GENYO - Centre for Genomics and Oncological Research – Pfizer/University of Granada/Junta de Andalucía, PTS, Granada, Spain, [4] Department of Otolaryngology, Instituto de Investigación Biosanitaria ibs.GRANADA, Hospital Universitario Virgen de las Nieves, Granada, Spain

Background: Vestibular migraine (VM) is complex disorder consisting of episodes of migraine and vertigo with an estimated prevalence of 1–3%. As migraine, it is considered that VM has genetic predisposition; however, evidence to support a genetic contribution has not been critically appraised.

Objective: The aim of this systematic review is to assess available evidence in scientific publications to determine the role of inheritance in VM.

Methods: After performing the quality assessment of the retrieved records, 31 studies were included (24 epidemiological reports and 7 genetic association studies in families or case-control in candidate genes). We gathered data about prevalence of VM in different populations and in families, and also about the genetic findings reported. In addition, other variables were considered to assess the heritability of VM, such as the ancestry, the age of onset or the familial history of vertigo and migraine.

Results: The estimated prevalence of VM was different between black (3.13%), white (2.64%) and Asian (1.07%) ethnicities. The reported prevalence of VM in migraine patients is higher in European countries (21%) than in Asian countries (10%). Moreover, the prevalence of the migraine-vertigo association in families is 4–10 times higher than the prevalence reported in the general population (sibling recurrence risk ratio λ_S = 4.31–10.42). We also found that the age of onset is lower in patients with simultaneous onset of symptoms and in those who have familial history for migraine and/or vertigo, suggesting anticipation. Although some genetic studies have reported few allelic variants associated to MV, replication studies are needed to validate these results.

Conclusions: The available evidence to support heritability in VM is limited. Variability in prevalence depending on ethnicity and geographic location suggests a combined genetic and environmental contribution to VM. However, the familial aggregation observed in VM support genetic and shared familial environmental effects that remarks the necessity of twins and adoptees-based epidemiological studies to estimate its heritability.

Keywords: vestibular migraine, heritability, prevalence, genetics, vestibular disorders, epidemiology

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Reviewed by:
Chiara Di Resta, Vita-Salute San Raffaele University, Italy
Habib Georges Rizk, Medical University of South Carolina, United States

***Correspondence:**
Patricia Perez-Carpena
percarpena@gmail.com

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INTRODUCTION

Migraine is a complex multifactorial disorder characterized by headache attacks associated with a constellation of neurological symptoms. In approximately one-third of patients, headaches are preceded by transient focal sensorial symptoms, so-called auras, involving visual and hearing systems. Genetic factors contribute to the clinical spectrum of migraine and multiple common variants have been associated with migraine with and without aura (Sutherland and Griffiths, 2017; de Boer et al., 2019). The diagnostic criteria of migraine were standardized according to the International Headache Society (Headache Classification Committee of the International Headache Society, 2018). Epidemiological evidence supports that familial aggregation with an early disease onset, particularly for the aura subtype, indicating a higher genetic susceptibility in migraine with aura (Russell et al., 1996; Ulrich et al., 1999; Mulder et al., 2003; Stewart et al., 2006).

Vestibular migraine (VM) consist of a subgroup of patients with migraine where the sensorial symptoms involve the vestibular system. VM is characterized by recurrent episodes of vertigo, a current or past history of migraine and simultaneous occurrence of both symptoms during crisis (Li et al., 2019). VM is one of the most common causes of recurrent vertigo. Prevalence studies have described MV as a frequent disease (0.9–2.7%), with variations in the frequency according to the population of study (Neuhauser et al., 2006; von Brevern et al., 2007; Formeister et al., 2018). However, VM is underdiagnosed, due to variability and overlap of symptoms with other causes of vertigo and to normal neurological examination and neuroimaging (Li et al., 2019).

The diagnostic criteria for VM were jointly developed by the Barany Society and the International Headache Society in 2012 (Lempert et al., 2012), and they differentiate probable and definite VM. Criteria for definite VM were included in the appendix of the 3rd edition of the International Classification of Headache Disorders (ICHD-III), one step forward to consider VM as an independent entity (Headache Classification Committee of the International Headache Society, 2018).

Migraine is a complex and multifactorial disorder with a large genetic component (Sutherland and Griffiths, 2017). Therefore, VM could also present a genetic predisposition (Knezevic et al., 2018; Rainero et al., 2019).

Familial clustering in VM has been occasionally observed, supporting the hypothesis of a genetic contribution to VM (Espinosa-Sanchez and Lopez-Escamez, 2015). Some studies reported families with an autosomal dominant inheritance with a moderate to high penetrance; however, no causative mutations have been found (Kim et al., 1998; von Brevern et al., 2006). Moreover, some loci segregating VM have been identified by linkage analysis in some few families with affected individuals in 22q12 (Lee et al., 2006) and 5q35 (Bahmad et al., 2009). These results suggest polygenic inheritance for VM; however, evidence to support a genetic contribution has not been critically appraised, and more research in this area is needed.

The aim of this systematic review is to assess available evidence in scientific publications to determine the role of inheritance in VM. So, we gathered information about the prevalence of VM in different populations, including family studies and twin studies, and we analyzed the quality of reported findings in genetic studies.

MATERIALS AND METHODS

Study Design

This review has followed the Preferred Reported Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009) (**Supplementary Table 1**).

According to the methodology for systematic reviews, the issues related to the PICO question are listed below and the studies have been selected according to the following characteristics:

- Participants: Patients diagnosed with VM.
- Intervention: Measurement of prevalence of the disease, estimation of familial aggregation, estimation of concordance in monozygotic/dizygotic twins, measurement of the presence of certain genes or loci, measurement of other demographic characteristics (age of onset of symptoms, familial history of migraine, vertigo or the vertigo-headache association).
- Control: Controlled and uncontrolled studies.
- Main results: Variation in the prevalence of the disease among different populations, families and twins compared to the general population.
- Secondary results: Reported associations with certain genes or loci; variation in the prevalence of other demographic characteristics (age of onset or family history).
- Study design: Case-control studies, family studies, twin concordance studies, cross-sectional studies and case series.

Search Strategy

The article search was performed on May 23, 2020 in the PubMed and Scopus databases, with the following combination of keywords: ("vestibular migraine" OR "migrainous vertigo" OR "migraine associated vertigo" OR "migraine related vertigo") AND ("epidemiology" OR "prevalence" OR "inheritance" OR "heritage" OR "heritability" OR "genes" OR "genetics" OR "families" OR "familial" OR "twins"). The search was limited to articles published in the last 25 years. Additional records identified through the list of references or other sources were also included.

Exclusion Criteria

Records that met the following characteristics were excluded from the review:

- Animal studies.
- Studies in child population.
- Articles published in languages other than English or Spanish.

Data Collected

Two independent reviewers (APT, PPC) analyzed the scientific papers that met the selection criteria. Each article was reviewed to extract the relevant data for the purpose of this work. The main data that were extracted from the studies were those referring to indicators of heritability, since the main objective

of this review is to assess whether VM is a disease that has a genetic contribution. These heritability criteria in multifactorial disorders are estimated by comparing the variation of the prevalence of the disease among population with different ethnic background and by familial aggregation studies calculating the sibling recurrence risk ratio (λ_s) using the Falconer formula (Wickramaratne and Hodge, 2001).

The following information was also collected from descriptive and genetic studies: first author and year of publication, country, study design, main objective, sample size, MV diagnostic criteria, ancestry, sex of patients with VM, mean age of patients with VM and mean age of VM onset. From descriptive studies information on VM prevalence, target population, and family history of MV was also extracted.

Data Synthesis

Information on the prevalence of VM have been collected in epidemiological studies. Mean values and standard deviation have been calculated for the age of onset of migraine and vertigo. Prevalence of VM in families was estimated from different studies and pooled to calculate the recurrence risk among siblings (λ_s). Statistical analyses were performed using Microsoft Excel and SPPS software.

Quality Assessment

The quality of each study has been assessed through the Cochrane Collaboration Tool (Higgins et al., 2019) and the risk of bias was summarized in **Supplementary Table 2**. Furthermore, the quality of genetic studies has also been evaluated according to the criteria defined to assess genetic studies in quantitative traits with extreme phenotype (Amanat et al., 2020).

RESULTS

Thirty-one studies with a total sample size of 41,127 individuals were finally included in this revision, according to the eligibility criteria. A flowchart detailing the selection of studies is included in **Figure 1**.

Cross-Sectional Studies and Case Series

Twenty-four descriptive epidemiological studies were included (16 cross-sectional studies, 5 case series, and 3 familial studies) (**Supplementary Table 3**). These studies estimated the prevalence of VM in different populations, geographical areas or families and reported clinical and demographic features of this disease. Five studies were population-based, while the rest were hospital-based.

Only 5 studies mentioned the ancestry and 3 of them included data about the prevalence of VM. An earlier onset of the disease was found in families when they were compared to non-familiar VM patients.

A female preponderance was observed in VM, although four studies did not report this information. Nineteen studies reported the mean age of patients with VM, however, only 11 described the age at VM onset. Thus, familial history of VM patients was detailed in seven studies.

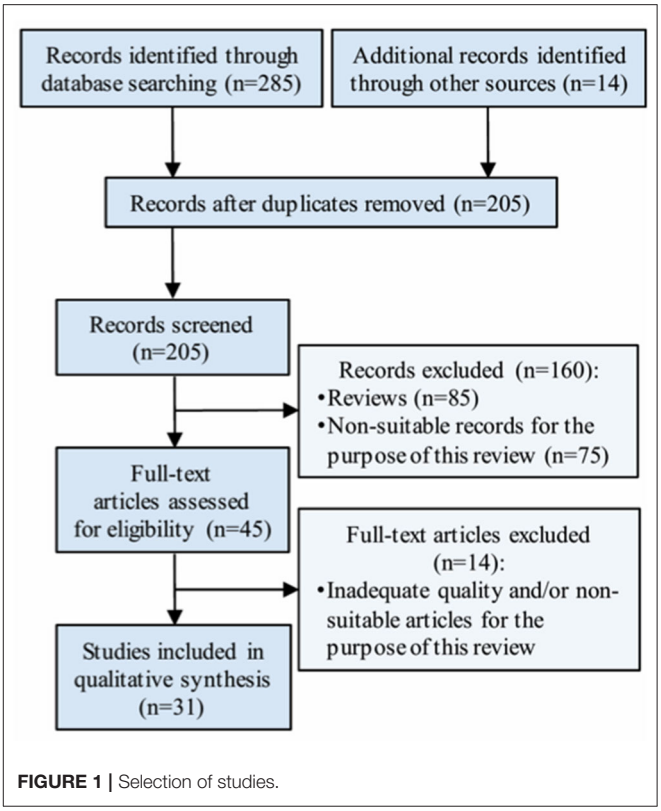

FIGURE 1 | Selection of studies.

Genetic Studies

Seven genetic studies were included (3 case-control studies and 4 genetic linkage analysis in families) (**Table 1**). The main purpose of these studies was to find mutations or loci in families associated with the disease. One of the case-control studies reported a significant association of the allelic variant rs770963777 in the *HTR6* gene with VM in a small cohort of Han Chinese descendants. Two of the studies reported significant associations of VM with certain genome regions: a locus in chromosome 5q35, and the PROGINS variant of the *PRG* gene, respectively. Another study found significant association between chromosome 22q12 and benign recurrent vertigo, which was a common diagnosis established for patients with migraine and episodic vertigo before the VM diagnostic criteria were established.

Three studies reported the ancestry, specifically Han Chinese in one of the studies, Caucasian in another one and German, Bosnian and Turkish in the last one. Female preponderance was also observed in all genetic studies.

Prevalence of VM According to the Geographic Area or Ancestry

Two population-based studies estimated the prevalence of VM in the general population, ranging from 0.89 to 2.70% in Germany and US, respectively. However, most studies were hospital-based and the authors estimated the prevalence of VM in migraine patients and in outpatient clinics, showing some differences. Five studies used the Barany Society diagnostic criteria for VM, and

TABLE 1 | Summary of the six genetic studies in patients with vestibular migraine.

References	Country	Study design	Main objective	Sample size	Main findings	Diagnostic criteria (VM)	Ancestry	Gender of VM patients (% women)	Mean age of VM patients (years)	Mean age of VM onset (years)
Wu et al. (2020)	China	Case-control	To investigate the association of rs770963777 in HTR6 gene with VM	- 92 VM - 100 controls	Significant association of VM with rs770963777(C/T)	Barany/IHS	Han Chinese	52.2%	44.2 ± 9.3	Not available
Peddareddygari et al. (2019)	USA	Genome-wide linkage analysis in one family using microsatellite markers	To test whether vertigo and motion sickness are inherited through different susceptibility genes than migraine	29	Non-significant results	- ICHD-I: migraine - *ad hoc* clinical criteria: vertigo and motion sickness	Not available	90%	Not available	10.5 ± 4
Bahmad et al. (2009)	USA/Brazil	Genome-wide linkage analysis in one family using microsatellite markers	To map the genetic locus for familial VM and to define the progression of the disease in one family	23	Significant association of VM with a region of chromosome 5q35	Neuhauser et al.	Not available	50%	60.7 ± 20.6	- 12± 4.7 (migraine) - 39.2 ± 6.2 (vertigo)
Lee et al. (2008)	USA	Genetic linkage analysis in one family	To analyze phenotypic and genetic features of a family with VM in order to assess its inheritance pattern.	46	- Non-significant results - Non-significant association with chromosome 11q in most affected women	- ICHD-I: migraine - *ad hoc* criteria: VM	Not available	87.5%	47.6 ± 15.4	- 14.7 ± 6 (migraine) - 36.2 ± 9.1 (vertigo) - 13.5 ± 9.2 (simultaneous onset of both symptoms)
Lee et al. (2007)	USA	Case-control	To test the association of female hormonal genes (PGR and ESR1) with VM	- 150 MV - 145 controls	Significant association of VM with PROGINS variant of progesterone receptor	- IHS: migraine - *ad hoc* clinical criteria: vertigo	Caucasian	83.4%	Not available	Not available
von Brevern et al. (2006)	USA/Germany	Case-control	To test whether mutations in CACNA1A, ATP1A2, SCN1A and CACNB4 confer susceptibility to VM	- 14 MV - 46 controls	Non-significant results	Neuhauser et al. (modified)	German, Turkish, Bosnian	64.3%	50 ± 11.1	- 21.3 ± 7.5 (migraine) - 37.9 ± 14.4 (vertigo) - 35.3 ± 13.4 (simultaneous onset of both symptoms)
Lee et al. (2006)	USA	Genome-wide linkage analysis in families	To genetically define BRV and its association with migraine.	257	- 31.2% prevalence of migraine and BRV association - Significant association of chromosome 22q12 with BRV	- IHS criteria for migraine - *ad hoc* clinical criteria for vertigo	Not available	84.4%	Not available	Not available

VM, Vestibular Migraine; ICHD, International Classification of Headache Disorders; IHS, International Headache Society; BRV, Benign Recurrent Vertigo.

TABLE 2 | Prevalence of vestibular migraine depending on geographic area and target population (outpatients clinics or hospital-based studies).

References	Continent	Country	Target population	Number of patients	Diagnostic criteria	Prevalence of VM
Power et al. (2018)	Oceania	Australia	Outpatients in a balance disorders clinic	90	Barany/IHS	41% definite and probable VM
Yollu et al. (2017)	Europe	Turkey	Patients with migraine	100	Barany/IHS	21% definite VM
Hazzaa and El Mowafy (2016)	Africa	Egypt	Outpatients in a dizziness clinic	446	Barany/IHS	22% definite VM
Akdal et al. (2015)	Europe	Turkey	Patients with migraine	871	- ICHD-II for migraine. - *ad hoc* clinical criteria for vestibular symptoms	- 62% vertigo - 76% vestibular symptoms (vertigo and motion sickness)
Akdal et al. (2013)	Europe	Turkey	Patients with migraine	1,880	- ICHD-II for migraine. - *ad hoc* clinical criteria for vestibular symptoms	20.3% vestibular symptoms (vertigo and/or dizziness and/or motion sickness)
Jay-du Preez and van Papendorp (2011)	Africa	South Africa	Patients visiting a general practitioner	717	Neuhauser et al.	1.67% (definite and probable VM)
Uneri (2004)	Europe	Turkey	Patients with BPPV	476	ICHD-I for migraine	54.8% migraine
Van Ombergen et al. (2015)	Europe	Belgium	Patients attending an ORL clinic	407	Barany/IHS	- 4.3% definite VM - 5.6% probable VM
Vuković et al. (2007)	Europe	Croatia	Patients with migraine	327	Neuhauser et al.	23.2% definite VM
Neuhauser et al. (2001)	Europe	Germany	- Patients with migraine - Patients attending a dizziness clinic	- 200 with migraine - 200 attending a dizziness clinic	Neuhauser et al.	- 9% definite VM in patients with migraine - 7% definite VM in patients attending a dizziness clinic
Cho et al. (2016)	Asia	South Korea	Patients with migraine	631	Barany/IHS	- 10.3% definite VM - 2,5% probable VM
Tungvachirakul et al. (2014)	Asia	Thailand	Patients attending a neurotology clinic	167	Neuhauser et al.	34.7% definite VM

VM, Vestibular Migraine; IHS, International Headache Society; ICHD, International Classification of Headache Disorders; BPPV, Benign Paroxysmal Positional Vertigo.

showed a prevalence of VM ranging from 4.3% in Belgium to 22% in Egypt (**Table 2**).

One population-based study performed in US described that 13.9% of VM patients were black, while 79.8% of VM patients were white, which results in a significant difference. After the analysis of the results from this population-based survey, we calculated the prevalence of VM for each ethnicity, resulting in a VM prevalence of 3.13% in African descendants, 2.64% in Europeans and 1.07% in Asian descendent population.

Familial Aggregation

Three studies reported that the migraine-vertigo association was more frequent in siblings of patients with VM than in the general population.

On the other hand, some cross-sectional and case series studies described a familial history of migraine, vertigo or both among VM patients. So, a familial history of migraine was reported in 70% of VM patients and 21.4% of VM patients had a familial history of VM.

The sibling recurrence risk ratio (λ_s) for the migraine-vertigo association was calculated by comparing the prevalence of this association in families with that prevalence in the general population, to assess the familial aggregation for VM (**Table 3**). Our results also showed a moderate familial aggregation ($\lambda_s = 4.31–10.42$).

Age of VM Onset

We compared the age of VM onset in different studies, particularly, the age of migraine onset, the age of vertigo onset and the simultaneous onset of vertigo and migraine, including a brief analysis on sex distribution (**Table 4**). So, we found that the mean age of onset in patients with simultaneous presentation of vertigo and migraine was 22.7 ± 10.4 years; however, for patients with a metachronic presentation of symptoms, the mean age of

TABLE 3 | Prevalence of migraine-vertigo association in families and sibling recurrence risk (λ_s).

References	Sample size	Number of siblings with migraine and vertigo	Total number of siblings	Prevalence of migraine and vertigo in siblings (%)	λ_s
Peddareddygari et al. (2019)*	29	4	15	26.67	8.33
Bahmad et al. (2009)	23	4	12	33.33	10.42
Cha et al. (2008)	69	4	29	13.79	4.31
Lee et al. (2008)	46	2	14	14.29	4.46
Lee et al. (2006)	257	13	57	22.81	7.13
Oh et al. (2001)	287	20	68	29.41	9.19
Oliveira et al. (1997)	19	5	18	27.78	8.68
Total	730	52	213	24.41	7.63

**Studies based on Barany Society diagnostic criteria for VM.*

TABLE 4 | Sex distribution and age of onset (mean ± standard deviation) of migraine, vertigo, and patients with simultaneous onset of both symptoms.

References	Number of patients with VM and non-simultaneous onset of migraine and vertigo (sex distribution, F/M)	Age of migraine onset (years)	Age of vertigo onset (years)	Number of patients with VM and simultaneous onset of migraine and vertigo	Simultaneous onset for migraine and vertigo (years)
*Beh et al. (2019)	129	–	44.3 ± 13.7	–	–
*Peddareddygari et al. (2019)	10 (9/1)	–	10.5 ± 4	–	–
Teggi et al. (2018)	260	21.8 ± 9	37.4 ± 13.1	19	19.8 ± 2.1
Martínez et al. (2017)	14 (14/0)	16.3 ± 8.2	31.7 ± 11.8	27	24 ± 12
*Cohen et al. (2011)	147	30.7	38.7	–	–
Bahmad et al. (2009)	6 (2/4)	12 ± 4.7	39.2 ± 6.2	–	–
Lee et al. (2008)	6 (5/1)	14.7 ± 6	36.2 ± 9.1	2	13.5 ± 9.2
*Vuković et al. (2007)	169 (migraine with vestibular symptoms)	–	25.3	–	–
von Brevern et al. (2006)	11 (6/5)	21.3 ± 7.5	37.9 ± 14.4	3	35.3 ± 13.4
*Neuhauser et al. (2001)	33	22 ± 11	35 ± 14	–	–
*Oh et al. (2001)	20	–	34.15 ± 16.9	–	–
Oliveira et al. (1997)	8 (5/3)	15.5 ± 10.1	–	–	–
Female total	41	15, 2+/-7, 4	33, 7+/- 15, 9	21	24, 8+/- 12, 8
Male total	14	19, 9+/-10, 1	37, 5+/-14, 3	11	23, 6+/-13, 3
Total	813	24 ± 8.9	35.6 ± 12.4	51	22.7 ± 10.4

*VM, Vestibular Migraine. *These studies do not report data on simultaneous age of onset; therefore, they were included in the "patients with non-simultaneous onset" category.*

onset for migraine was 24 ± 8.9 years, and 35.6 ± 12.4 year for vertigo.

To sum up, we observed that 6 studies recorded data about audiological symptoms in VM. The prevalence and distribution of these audiological symptoms in VM is shown in **Table 5**.

DISCUSSION

The association between migraine and vertigo has been known for a long time; however, it has not been considered as an independent entity until the last decade (56). The current diagnostic criteria were published in 2012 (Lempert et al., 2012), therefore, only 8 of the studies included in this revision applied these criteria for the diagnosis of VM. The criteria proposed by Neuhauser et al. (2001) were reported in 8 of the studies, and in the rest of the publications, the authors used ICHD criteria for migraine and *ad-hoc* clinical criteria for vertigo. One of the family studies did not report any diagnostic criteria, but later this family was reported as familial VM.

There are several evidence to support heritability in complex traits such as vestibular disorders: (a) differences in the prevalence of the condition according to the ethnic background;

TABLE 5 | Prevalence of audiological symptoms in patients with VM.

References	*N*	Audiological symptoms reported	Number of patients with otological symptoms	Prevalence
Teggi et al. (2018)	252	Tinnitus (during vertigo attacks)	27	0.11
		Ear fullness (during vertigo attacks)	22	0.09
		Hearing loss (during vertigo attacks)	10	0.04
Yollu et al. (2017)	21	Sensorineural hearing loss (SNHL) according to average hearing threshold	2	0.09
		SNHL acc. to low frequency	6	0.28
		SNHL acc. to high frequency	11	0.52
Van Ombergen et al. (2015)	65	Tinnitus	35	0.54
		Decreased hearing	3	0.05
		Hearing loss	5	0.25
Cha et al. (2008) (families study)	20	Migraine and MD symptoms (migraine/MD)	19	0.13
Lee et al. (2007)	150	Migraine and MD symptoms (migraine/MD)	19	0.13
Neuhauser et al. (2006)	33	Cochlear symptoms (during vertigo attacks)	12	0.36
		Tinnitus (during vertigo attacks)	5	0.15
		Aural fullness (during vertigo attacks)	5	0.15
		Hearing loss (during vertigo attacks)	3	0.09
Total	541	Hearing loss	46	0.085
	350	Tinnitus	67	0.191

(b) familial aggregation with early onset and anticipation; and (c) high concordance of the phenotype in monozygotic twins and adoptees with biological parents when they are compared with dizygotic twins or adoptive parents, respectively (Gallego-Martinez et al., 2018).

The population-based study from Formeister et al. (2018) showed a higher prevalence of VM in African American or Europeans than in Asian descendent population, suggesting a possible genetic contribution. It should be interesting to compare the prevalence of VM in East Asia in a large cohort with the Asian American descendent to assess the environmental effect on VM.

Moreover, the prevalence of VM in migraine patients seems to be higher in European countries (23% in Croatia, 21% in Turkey) (Vuković et al., 2007; Yollu et al., 2017) than in Asian countries (10% in South Korea) (Cho et al., 2016). However, the diagnosis of VM in neurotology clinics shows a great variation ranging from a 7% reported in Germany (Neuhauser et al., 2001), 22% in Egypt (Hazzaa and El Mowafy, 2016) to 41% in Australia (Power et al., 2018). These data suggest differences in the diagnostic criteria used rather than ethnic differences in VM and evidence that population-based studies are needed to estimate the prevalence of VM.

Familial aggregation is usually reported in rare diseases, and suggests a combined effect of genetic and environmental factors (Requena et al., 2014a). This type of studies compares the prevalence of a disease in individuals of the same generation within a family with the prevalence of that disease in general population. In order to do this, the sibling recurrence risk ratio (λ_s) is calculated. That proportion allows us to know how many times the disease is more frequent between the siblings of the affected individual, as compared with the general population. Our systematic review show that familial studies (Oliveira et al., 1997; Oh et al., 2001; Lee et al., 2006, 2008; Cha et al., 2008; Bahmad et al., 2009; Peddareddygari et al., 2019) showed a higher prevalence of the migraine-vertigo association than the observed prevalence of that association in the general population (3.2%) (Neuhauser et al., 2001), and also higher than the 2.7% prevalence of VM in general population (Formeister et al., 2018). We calculated λ_s for the migraine-vertigo association, which resulted in a risk ranging between 4–10 times higher than in general population.

Most of the studies included in this review reported information about age of onset of migraine and vertigo. Some of them described a significantly lower age of onset in patients with VM, as compared with controls affected either by migraine or vertigo (Vuković et al., 2007; Akdal et al., 2013). By comparing the age of VM onset in several studies, we found that there was an earlier onset of symptoms in those patients with simultaneous presentation of migraine and vertigo, as compared with those with metachronic onset of symptoms.

Early and simultaneous onset of symptoms observed in familial VM suggests genetic anticipation, a phenomenon of progression of severity of an inherited disorder in successive generations frequently found in neurological disorders and associated with expansion of nucleotide repeats (Carpenter, 1994). Teggi et al. (2018) reported a significantly lower age of migraine onset in patients with a familial history of migraine or VM, as compared with those without it. These results were also reported in some of the included familial studies (Lee et al., 2008; Bahmad et al., 2009; Peddareddygari et al., 2019),

in which age of migraine onset seems to be lower than in non-familial cases.

The genetic association studies retrieved have reported allelic variants in the *HTR6* and *PRG* genes associated with VM (Lee et al., 2007; Wu et al., 2020). Replication studies are needed to validate these results.

The genetic contribution to vestibular diseases, including VM is largely unknown (Requena et al., 2014b; Gallego-Martinez et al., 2018). The application of high throughput sequencing technologies in multiplex families with deep phenotyping will target candidate genes and clarify disease mechanisms and facilitate the genetic diagnosis of VM in clinical practice (Di Resta and Ferrari, 2018; Prodan Žitnik et al., 2018).

Limitations of This Study

Most of the genetic studies included in this revision are based on linkage analysis of multicase families. The majority of them were published more than a decade ago and were performed before the development of high-throughput DNA massively parallel sequencing technology. Only two of them found 2 loci associated with VM, but none of them found the causal mutations.

The current diagnostic criteria for VM were published in 2012, so previous studies applied different probably broader criteria. This variability in the diagnostic criteria is a selection bias that might influence the estimated prevalence and therefore, could limit the comparability between studies. Moreover, the number of studies analyzing VM heritability could be considered low, so few of them share the same variables, resulting in a limited comparability.

Future Perspectives

This systematic review provides some evidences to support a genetic contribution in VM, including familial aggregation. There is a need to perform twins and adoptees studies to estimate heritability in VM. Therefore, whole genome sequencing studies in multicase families are needed to find genetic variants conferring susceptibility to this disease.

CONCLUSIONS

1. Clinical studies seem to report differences in the prevalence of VM according to the ethnic origin and country.
2. Family aggregation studies show a higher prevalence of migraine and vertigo in families compared to general population, with a moderate risk of recurrence among siblings and possible anticipation.
3. Since there are no twins studies published, there is a need to perform such studies to estimate heritability in VM.

AUTHOR CONTRIBUTIONS

JL-E conceived the study design and develop the scientific arguments. AP-T and PP-C performed literature search, quality assessment of the studies, and interpretation of data. All authors drafted the manuscript and revised the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2020.00954/full#supplementary-material

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High-risk Allele for Herpes Labialis Severity at the IFNL3/4 Locus is Associated With Vestibular Neuritis

Dan Rujescu[1†], Marko Herrling[2†], Annette M. Hartmann[1], Stephan Maul[1], Ina Giegling[1], Bettina Konte[1] and Michael Strupp[2,3]*

[1] Department of Psychiatry, Psychotherapy and Psychosomatics, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany, [2] German Center for Vertigo and Balance Disorders, University Hospital Munich, Munich, Germany, [3] Department of Neurology, University Hospital Munich, Munich, Germany

Objective: Vestibular neuritis (VN) is a peripheral vestibular disorder leading to a sudden loss of unilateral vestibular function. Although the underlying etiological mechanisms for disease development are not yet known, there is evidence that a latent infection with herpes simplex virus type 1 (HSV-1) might be involved. The polymorphism rs12979860 has been associated with the severity of recurrent herpes labialis and hepatitis C virus (HCV) clearance and treatment outcome and is located within the first intron of the IFNL4 gene on chromosome 19.q13.2. This case control study was conducted to evaluate the association of rs12979860 with VN occurrence.

Methods: DNA was extracted from EDTA blood of 151 VN patients and 1,775 healthy controls. Genotyping of rs12979860 was performed using iPLEX and MassARRAY Matrix Assisted Laser Desorption Ionization—Time of Flight (MALDI-TOF) mass spectrometry. For association analyses, an additive, dominant and recessive logistic regression model was calculated, using age and sex as covariates.

Results: A significant association of rs12979860 with VN was obtained for the additive [OR = 1.51 (1.18–1.92); $p = 9.23 \times 10^{-4}$] and dominant models [OR = 2.15 (1.48–3.13); $p = 5.86 \times 10^{-5}$], with the T allele being more frequent in the VN group.

Conclusion: By detecting a significant association of the rs12979860-T risk allele for herpes labialis severity with susceptibility to VN, this study gives further indirect evidence for an involvement of HSV-1 in VN pathology, thereby strengthening the virus hypothesis.

Keywords: vestibular neuritis, HSV-1, IFNL3, IFNL4, vertigo

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****Correspondence:***
Annette M. Hartmann
annette.hartmann@uk-halle.de

† These authors have contributed equally to this work

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INTRODUCTION

Vestibular neuritis (VN) is characterized by an acute onset of sustained spinning vertigo, oscillopsia, postural imbalance, nausea and vomiting due to a sudden loss of unilateral vestibular function. The estimated incidence of VN ranges between 3.5 and 15.5 per 100,000 subjects (1, 2) with a recurrence rate of about 2% (3) to 10.7% (4).

The mechanisms contributing to the development of VN have not yet been identified. According to the leading virus hypothesis, VN is caused by reactivation of a latent herpes simplex virus type 1 (HSV-1) infection, which leads to an accumulation of HSV-1 DNA and latency associated transcript (LAT) in cells hosting the virus (5–8). The virus hypothesis is also supported by a

mouse model in which vestibular dysfunction and vestibular ganglion infection were induced after inoculation of HSV-1 and 2 (9), as well as by the observation of elevated acute phase proteins in the blood and an elevated percentage of CD40-positive peripheral blood mononuclear cells in VN patients (10, 11). The latter is associated with thrombotic events, so that proinflammatory processes and the associated increased expression of CD40 on monocytes and macrophages may lead to microvascular occlusion via aggregation of platelets with these cells (11, 12). In contrast to other complex diseases such as schizophrenia (13), little is known about the genetic factors underlying vestibular disorders. For VN, a genome-wide association study (GWAS) conducted in 151 VN patients and 2,609 healthy controls was recently published, with three genome-wide significantly associated loci with a link to viral replication (14). In addition to the virus hypothesis, immune-mediated processes were assumed to cause VN, since an unbalanced CD4/CD8 quotient was found in VN patients, which is similar to the findings in patients with multiple sclerosis [for review see (15)]. However, there has been no further progress in this area in recent years.

In a study by Griffiths et al. (16) an association of the single nucleotide polymorphism (SNP) rs12979860 with severity and recurrence rate of herpes labialis was observed, in which a dose-dependent effect of the T allele was determined. This is in line with studies conducted in patients with hepatitis C virus (HCV) infections. In a GWAS on HCV infected individuals, T allele carriers showed a significantly higher rate of chronic persistence of the virus, whereas C allele carriers showed a higher incidence of spontaneous virus clearance (17). In addition, the T allele was associated with non-response to antiviral therapy with pegylated interferon λ (IFN- λ) and ribavirin (18, 19) and also with decreased *IFNL3* mRNA levels (20). Interestingly, a reduced INF-λ response was also observed in patients with recurrent herpes labialis caused by HSV-1 compared to seropositive controls without recurrences (21).

The SNP rs12979860 is localized on chromosome 19q13.2 in a region containing the *IFNL* genes and was initially assigned to the promotor region of *IFNL3*. In fact, it is also located in the first intronic region of the *IFNL4* (pseudo)gene. Transcription of this gene depends on the dinucleotide variant rs368234815 located in the first exon of *IFNL4*. While TT renders IFNL4 to a pseudogene, the ΔG variant leads to a frameshift that generates an open reading frame and enables protein expression (22). The ΔG allele of rs368234815 and the T allele of rs12979860 are in strong linkage disequilibrium (LD) in European and Asian ancestries ($r^2 = 0.9$, European, $r^2 = 1.0$, Asian) (22), but considerably less in African populations ($r^2 \sim 0.7$) (23).

IFN- λs (or type III IFNs) mediate antiviral, antibacterial and antifungal effects, with the four previously known INF- λ ligands (IFN- λ1-4) having different affinities to their receptor (IFNLR), which is a heterodimer consisting of an a- (IL28RA) and a b-subunit (IL10RB) showing a highly variable cell type specific expression pattern [for review see (24)]. Similar to type I IFNs (IFN-α and β), IFN- λs are able to inhibit replication of several virus types, e.g., HSV-1 (25), HSV-2 (26), and HCV (27). However, IFN- λ4 differs from the other type III IFNs in its kinetics, its earlier release during viral infections and its ability to induce the expression of genes that negatively regulate IFN response (28). Interestingly, it has been shown that expression of *IFNL4* mRNA *per se* was associated with a reduced HCV clearance and a poorer response to antiviral therapy. Thus, a possible functional relationship between the described polymorphisms and the associated phenotypes was proposed (20, 22).

Since reactivation of a latent HSV-1 infection is discussed as a cause for VN and severity and recurrence rates of herpes labialis caused by HSV-1 infection are associated with rs12979860, a variation localized in the IFNL4 gene, an influence of this variation on VN pathology could be hypothesized. Therefore, a case-control study investigating the association of rs12979860 with VN was conducted.

MATERIALS AND METHODS

Study Population

Participants of European ancestry included in the study were recruited from the Greater Munich area (Germany) and clinical interviews were conducted at the German Center for Vertigo and Balance disorders, the Department of Neurology (patients) and the Institute for Psychiatry (controls), at the Ludwig-Maximilians-University Munich from 1997 to 2016. Detailed medical histories of the participants and their first-degree relatives were assessed using a semi-structured interview.

Patients

The cohort of VN patients has already been described elsewhere (14). In brief, 151 patients who met the diagnostic criteria for unilateral VN (29) were included in the study. The diagnosis was based on the patient's medical history, the clinical examination and, in ambiguous cases, further examinations such as MRI and caloric testing. The key symptoms, all of which had to last for at least 72 h, were a history of acute/subacute onset of sustained spinning vertigo, oscillopsia, gait imbalance and nausea or vomiting. Horizontal-torsional peripheral vestibular spontaneous nystagmus toward the unaffected ear, a pathological head impulse test (HIT) toward the affected side, suppressed by visual fixation, and postural imbalance with Romberg fall toward the affected ear were required in the neurological examination. In addition, caloric testing had to show a hypo- or unresponsiveness of the affected horizontal canal with an >25% asymmetry between both sides. A video HIT was performed in all patients with a gain of the vestibulo-ocular reflex < 0.7 required on the affected side. Patients with any evidence for other peripheral and central vestibular or ocular motor disorders (see below) were excluded. In addition, a history of acute hearing loss, brainstem or cerebellar symptoms as well as clinical evidence for a central ocular motor lesion, i.e., skew-deviation, saccadic smooth pursuit, gaze-evoked nystagmus, normal head-impulse test led to exclusion from the study (30). Patients with recurrent VN as well as with chronic hepatitis B, C or HIV infection were not included.

Healthy Controls

DNA samples from 1,775 unrelated healthy subjects of European ancestry were taken from the Phenomics and Genomics Sample (PAGES), which is comprised of ∼3,000 controls, 1,000 schizophrenia patients and 300 individuals with other diagnoses. The Structured Clinical Interview (SCID I and SCID II) and the Family Assessment Module were performed on all healthy volunteers (31–33). Individuals with a history of VN (self-reported) as well as those, suffering from neurological or psychiatric diseases or with first degree relatives with these disorders were excluded.

Informed Consent

Informed consent was obtained from all participants, and the study was approved by the Ethics Committee of the Ludwig-Maximilians-University Munich and carried out in accordance to the Declarations of Helsinki.

Genotyping

Genomic DNA was extracted from peripheral white blood cells using the QIAamp DNA Maxi Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol, and dissolved in nuclease free water. Using picogreen (Invitrogen, Karlsruhe, Germany) the DNA concentration was measured and subsequently adjusted to 50 ng/μl.

Genotyping of rs12979860 was performed using the MassARRAY platform (Sequenom, San Diego, CA) as described previously (34) with minor adaptations. PCR primer and extension primer were designed using the Assay Designer 4.0 software (Sequenom, San Diego, CA). 12.5 ng of genomic DNA, 0.5 mM dNTP (ABgene, Hamburg, Germany), 100 nM PCR primer (forward: ACGTTGGATGAGCGCGGAGTGCAA TTCAAC, reverse: ACGTTGGATGTCGTGCCTGTCGTGTAC TGA) (Metabion, Martinsried, Germany), 1.625 mM $MgCl_2$, and 0.5 U HotStar Taq-polymerase (Qiagen, Hilden, Germany) were used to perform the initial PCR (initial denaturation at 95°C for 5 min; 45 cycles with 20 s at 95°C, 30 s at 56°C, 1 min at 72°C; final elongation 3 min at 72°C). Following a shrimp alkaline phosphatase treatment, the iPLEX reaction mix containing the extension primer (TGCAATTCAACCCTGGTTC) (Metabion, Martinsried, Germany), was added. PCR reaction was carried out with the following parameters: initial denaturation at 95°C for 30 s, 40 cycles with 5 s at 94°C and 5 cycles at 52°C for 5 s, 80°C for 5 s, final elongation at 72°C for 3 min. After desalting the extension products, samples were spotted on SpecroCHIPs GenII (Sequenom, San Diego, CA) and analyzed with the MassARRAY MALDI-TOF mass spectrometer. Allele-specific extension products and resulting genotypes were identified by Typer 3.4 Software (Sequenom, San Diego, CA).

Quality Control

The following quality criteria were applied to confirm reliable genotypes: compliance with the Hardy-Weinberg equilibrium (HWE) per genotyping unit containing 376 samples, in the control and combined sample ($p \geq 0.05$), sample callrate > 80% and SNP callrate > 95%. Deviation from the HWE was accepted for cases, if controls on the same genotyping unit were in compliance with the HWE. In addition, the allele frequency of the genotyped control group was compared with genetic datasets of populations from Europe, provided by 1,000 Genomes Project Phase 3 on Ensembl (35) and showed a similar distribution (T-allele: 33.9% in the genotyped compared to 30.9% in the European sample).

Statistical Analyses

Group differences in sex and age were calculated using exact Fisher's exact test and Wilcoxon rank sum test. Deviations of the genotype frequency from HWE in the patient, control and combined samples were analyzed using Pearson's chi-squared test. After quality inspection, a logistic regression was calculated using plink 1.7 (36, 37). Additive (additive effect of increasing amounts of the minor allele), dominant (homozygous major allele carriers vs. carriers of the minor allele) and recessive genotype models (homozygous minor allele carriers vs. carriers of the major allele) corrected for age and sex were used to check for an association of rs12979860 genotypes with VN. An a posteriori power calculation was performed using the software Quanto (38) with the following parameters: unmatched case-control study (1:11.75), minor allele frequency of 0.35 (total sample), additive and dominant inheritance models, incidence rate of 0.0155% (as no data on prevalence rates are available) and significance level of $p < 0.05$.

RESULTS

In this study 151 patients with unilateral VN and 1,775 healthy controls were included in the analysis. The mean age of patients with VN (64 females, 42%) was 55.4 ± 14.8 years and the mean age of controls (910 females, 51%) was 55.1 ± 13.4 years, sex showing a slight statistical difference between groups (sex: $p = 0.04$, age: $p = 0.91$).

The T allele of rs12979860 located on chromosome 19q13.2 within the first intron of *IFNL4*, depicted the minor allele with a frequency of 43.4% in cases and 33.9% in healthy controls (**Table 1**). The control group and the combined sample were in Hardy-Weinberg equilibrium (controls: $\chi^2 = 0.05$, $p = 0.82$ and combined sample: $\chi^2 = 0.88$, $p = 0.35$), whereas the case group showed a slight deviation ($\chi^2 = 7.77$, $p = 0.005$).

For association analyses in this case-control study, additive, recessive and dominant logistic regression models corrected for age and sex were calculated. The results are summarized in **Table 2**. The additive model revealed a significant association of rs12979860 with VN ($p = 9.23 \times 10^{-4}$, OR = 1.51, 95% CI = 1.18–1.92), with the T allele being more prevalent in the VN group compared to the control group. A significant result was also found in the calculation of the dominant model (carriers of the minor allele TT + CT vs. homozygote carriers of the major allele CC). This resulted in an OR of 2.15 (95% CI = 1.48–3.13) for carriers of the T allele and compared to healthy controls, the proportion of T allele carriers was higher in the VN group. Using the recessive model, no significant association was found. Based on the results of our study, a power calculation was performed, which yielded a statistical power of 92.17% for the additive model (OR = 1.51) and 98.60% for the dominant model (OR = 2.15).

TABLE 1 | Allele and genotype frequencies of rs12979860.

	Allele		Genotype		
Sample	**C *n* (%)**	**T *n* (%)**	**CC *n* (%)**	**CT *n* (%)**	**TT *n* (%)**
VN	171 (56.6)	131 (43.4)	40 (26.4)	91 (60.2)	20 (13.2)
Controls	2,346 (66.3)	1,204 (33.9)	773 (43.5)	800 (45.1)	202 (11.4)
Combined	2,517 (65.3)	1,335 (34.7)	813 (42.2)	891 (46.3)	222 (11.5)

TABLE 2 | Logistic regression analysis.

Model	***P*-value**	**OR (95% CI)**	**SE**
Additive	9.23×10^{-4}	1.51 (1.18–1.92)	0.12
Dominant	5.86×10^{-5}	2.15 (1.48–3.13)	0.19
Recessive	5.29×10^{-1}	1.17 (0.72–1.92)	0.25

OR, Odds ratio; CI, confidence interval; SE, standard error.

DISCUSSION

In this case-control study the genotype frequency of rs12979860 located in the first intron of the *IFNL4* gene on chromosome 19q13.2 and its association with VN were analyzed and a significantly higher frequency of the T-allele was detected in VN patients compared to healthy subjects. Increased ORs (additive model: OR = 1.51, dominant model: OR = 2.15) were obtained in the calculation of both the additive and the dominant models. However, the main limitation of the study is the small size of the case sample, which could lead to the observed deviation from the HWE, though the high MAF of the variation (34.7% in the combined sample) is less prone to be influenced by individual genotype changes than a low MAF variation, thereby arguing in favor of an association effect. The underlying mechanisms leading to the development of VN still need to be uncovered. According to the virus hypothesis, viral pathology induced by neurotropic viruses such as HSV-1 is thought to be the most likely cause of VN (29). This hypothesis is supported by a recent GWAS in VN, in which 3 loci were detected containing genes that are also involved in viral processes (14). Furthermore, the results obtained in this candidate gene study showing an increased risk of VN for carriers of the rs12979860 T allele support the observation that this allele is associated with increased clinical severity and recurrence rates of herpes labialis in a dose dependent manner (16). In the same study, it was shown that the replication of HSV-1 is inhibited by the induction of a type III IFN response. Interestingly, reduced IFN-λ levels were found in patients with recurrent herpes labialis compared to seropositive controls without a history of recurrence in a study by (21).

The unfavorable effect of the rs12979860 T allele has been further demonstrated by an association with virus persistence (17) and a diminished response to antiviral therapy with pegylated IFN-λ and ribavirin in patients with HCV (19, 39). Remarkably, a HCV treatment study conducted by Rallón et al. (40) showed reduced IFN- λ3 levels in T allele carriers, while rs12979860-C allele carriers were reported to have higher IFN-λ3 plasma levels (41).

Other studies demonstrated a protective effect of the rs12979860 T allele, thereby representing conflicting results on the allele effect, e.g., in studies on the replication of the cytomegalovirus in patients with solid-organ transplants and allogenic stem cell transplants. The sample size in these studies, however, was very small (42, 43).

Functional consequences of the specific alleles of rs12979860 are still under exploration. In addition to a potential role in the promoter of the *IFNL3* gene due to its position in the 5' region of the gene, rs12979860 also shows a strong LD to rs368234815, a dinucleotide insertion/deletion variant within the first exon of *IFLN4*. Unlike the TT variant, which is subjected to degradation by mRNA by nonsense mediated decay, the ΔG allele induces a frameshift which enables expression of the *INFL4* gene (22). The ΔG variant correlates perfectly with the T allele of rs12979860 in Asians ($r^2 = 1.0$) and is well correlated in Europeans ($r^2 > 0.9$), but only moderately in Africans ($r^2 \sim 0.7$). Thus, the ability to express IFN- λ4, which is restricted to carriers of the ΔG variant, might explain the strong association of rs12979860 with impaired HCV clearance in Europeans, while Africans exhibit a higher association with rs368234815 (22).

Though knowledge regarding the physiological function of IFN- λ4 is still sparse, significant differences to the other type III IFNs have been described. Compared to IFN- λ3, IFN- λ4 seems to act faster during acute antiviral response, but at the same time induces the expression of genes that inhibit IFN response. For example, the expression of *USP18*, which is an inhibitor of antiviral activity of IFN- λ, was elevated in liver biopsies of HCV patients who carried the ΔG allele and were thus capable of producing IFN- λ4 (28). In addition, IFN- λ4 was shown to induce the expression of *SOCS1*, an inhibitor of anti-HCV activity of IFNs. These results are supported by the investigation of another SNP in the second exon of the *IFNL4* gene. Rs117648444 causes a substitution of an amino acid, turning IFN- λ4 into a protein variant with reduced activity, named IFN- λ4-S70. Compared with the fully active IFN- λ4-P70 variant, humans encoding the impaired IFN- λ4-S70 variant display better spontaneous HCV clearance rates and overall

better treatment response rates (44). These findings suggest that the presence, rather than absence of IFN- λ4 could be the relevant disadvantage in viral defense (45). This is supported by the fact that the ΔG variant seems to be undergoing a negative selection, since 95% of Africans, but only about 54% of Europeans and 13% of Asians, carry at least one ΔG variant (23).

Besides the favorable effects of the CC genotype (rs12979860) on HCV clearance rates, this genotype was also associated with a higher rate of hepatic inflammation and fibrosis in patients with chronic HCV infection and with a higher frequency of pulmonary fibrosis in patients with systemic sclerosis, accompanied by increased IFN- λ3 activity (46, 47). A high IFN-λ3 activity thus seems to be an advantage for virus elimination, but also to mediate chronic inflammatory processes. The results of this study may therefore suggest that VN is more likely to be caused by a reactivation of HSV-1 than by inflammation in response to the virus.

In summary, the underlying pathological mechanisms for VN are not yet known, but a viral genesis is discussed, with HSV-1 being the most likely candidate (29). In an earlier study, an association of the rs12979860 T allele with the severity of recurrent herpes labialis was demonstrated (16), establishing a link to HSV-1 infection. Since the T allele of rs12979860 and the functional ΔG variant rs368234815 are in strong LD, the expression of IFN- λ4, which depends on the presence of the ΔG variant, could contribute to the differing antiviral responses between IFN- λ4 and other type III IFNs. However, in addition to the established impact of IFN- λ4 on HCV-infection further studies are needed to confirm whether an influence on HSV-1 infections also occurs. This would further strengthen the viral hypothesis of the latent HSV-1 infection for VN. Although the present study comprises a relatively small sample size and replication is necessary, these results are consistent with previous studies on rs12979860.

DATA AVAILABILITY STATEMENT

The authors acknowledge that the data presented in this study is available upon request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Ludwig-Maximilians-University, Munich, Germany. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DR, MS, and IG were involved in the conception and design of study. MH, AH, and SM participated in the acquisition and analysis of the data. BK was responsible for the statistical analysis. All authors contributed to the article and approved the submitted version.

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TRPM7 as a Candidate Gene for Vestibular Migraine

*Eun Hye Oh[1], Jin-Hong Shin[1], Jae Wook Cho[1], Seo-Young Choi[2], Kwang-Dong Choi[2] and Jae-Hwan Choi[1]**

[1] Department of Neurology, Pusan National University School of Medicine, Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, South Korea, [2] Department of Neurology, Pusan National University Hospital, Pusan National University School of Medicine and Biomedical Research Institute, Busan, South Korea

Objectives: Vestibular migraine (VM) is a common vestibular disorder, and familial aggregation of VM with autosomal-dominant inheritance has been described, which supports a genetic background. This study aimed to describe the clinical phenotype of a family with VM, and identify a candidate gene for VM.

Methods: We recruited six individuals (four affected and two unaffected) from three consecutive generations of a Korean family with VM, and performed whole-exome sequencing to search for candidate genes.

Results: All affected individuals presented with recurrent vertigo, headache, and nausea/vomiting that fulfilled the diagnostic criteria of VM. Two individuals also experienced transient hemiparesis or dysarthria during the episodes. The symptoms were triggered by physical or emotional stress. Interictal examinations showed uni- or bi-directional horizontal gaze-evoked nystagmus in three of the individuals. They had no causative mutations in genes causing familial hemiplegic migraine or episodic ataxia. Through whole-exome sequencing from three affected individuals, we identified a nonsense mutation c.3526C>T in *TRPM7* that encodes a cation channel selective to Ca^{2+} and Mg^{2+}.

Conclusions: Alterations in intracellular Ca^{2+} and Mg^{2+} homeostasis by *TRPM7* mutation may contribute to the development of the VM phenotype. Our result suggest that *TRPM7* is a novel candidate gene for VM.

Keywords: vestibular migraine, genetics, whole-exome sequencing, TRPM7 channel, ion homeostasis

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***Correspondence:**
Jae-Hwan Choi
rachelbolan@hanmail.net

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INTRODUCTION

Vestibular migraine (VM) is one of the most common vestibular disorders, affecting around 1% of the general population (1). It is characterized by recurrent attacks of vestibular symptoms, a current or previous history of migraine, and the existence of one or more migraine features during the vestibular episodes. Recently, VM has been included as a diagnostic category in the latest International Classification of Headache Disorders (ICHD) criteria (2).

Most cases of VM are considered sporadic, but familial aggregation of VM with autosomal-dominant inheritance has been described in several families, supporting a genetic background for the condition (3, 4). Genome-wide association studies (GWASs) have identified numerous single-nucleotide polymorphisms (SNPs) associated

with a genetic predisposition for migraine with or without aura (5, 6). Familial hemiplegic migraine (FHM), which is a subtype of migraine with aura, is caused by mutations in *CACNA1A*, *ATP1A2*, and *SCN1A* (7). However, no candidate gene has been validated in VM, although several genetic loci such as chromosomes 5q35, 9q13-q22, 11q, and 22q12 are known (8–11). Furthermore, previous studies have not detected pathogenic mutations in FHM or episodic ataxia (EA) genes in VM patients (12, 13).

Transient receptor potential (TRP) channels are a family of cation channels expressed mostly on the cell (14, 15). They are divided into seven subfamilies including TRPC, TRPV, TRPM, TRPA, TRPN, TRPP, and TRPML, which mediate sensory transduction such as pain, touch, hearing, and thermal sensation. In addition, TRP channels enable individual cells to respond to changes in their local environment. These ion channels have a relatively non-selective permeability to cations including Ca^{2+} and Mg^{2+}, and modulate ion entry driving forces. TRP channels have been linked to neurodegenerative disorders, kidney disease, and cancers by altering intracellular ion homeostasis (15–18). Interestingly, they have been repeatedly hypothesized to contribute to migraine via the activation of meningeal nociceptors or the release of calcitonin gene-related peptide (CGRP) (19–22).

The present study investigated the clinical phenotype of a Korean family with VM showing autosomal-dominant inheritance. Here we report one novel mutation in *TRPM7* that may explain the VM phenotype in the family.

MATERIALS AND METHODS

Subjects

Six individuals from three consecutive generations of a Korean family with VM (four affected and two unaffected individuals) were recruited at the Dizziness Clinic of Pusan National University Yangsan Hospital (**Figure 1**). VM was diagnosed based on the criteria of the Bárány Society (1). All individuals underwent full neurological and neuro-otological examinations by the author (J-HC), and the four affected individuals received brain magnetic resonance imaging to exclude other possible causes of their symptoms.

All experiments followed the tenets of the Declaration of Helsinki, and informed consents were obtained from the participants after the nature and possible consequences of this study had been explained to them. This study was approved by the institutional review boards of Pusan National University Hospital.

Whole-Exome Sequencing and Data Analysis

To systematically search for candidate genes, we applied trio exome sequencing to blood samples from three of the affected individuals (II-5, II-8, and III-3). Genomic DNA was extracted from the blood samples, and whole-exome sequencing was conducted using the SureSelect Focused Exome Kit (Agilent, Technologies, Santa Clara, CA, USA). Qualified genomic DNA samples were randomly fragmented by Covaris, followed by adapter ligation, purification, hybridization, and PCR. Captured libraries were analyzed using a bioanalyzer (Agilent 2100, Agilent Technologies) to estimate the quality, and they were loaded onto a sequencing system (Illumina HiSeq 2500, Theragen Etex Bio Institute, Suwon, Korea). Raw image files were processed for base-calling using HCS software (version 1.4.8) with default parameters, and the sequences of each individual were generated as 100-bp paired-end reads. Sequence reads were aligned to the human reference genome sequence (GRCh37.3, hg19) using the Burrows-Wheeler Aligner (version 0.7.12). PCR duplicate reads were marked and removed using Picard tools (version 1.92). The Genome Analysis Toolkit (version 2.3-9) was used for indel realignment and base recalibration. Variation annotation and interpretation analysis were performed using SnpEff (version 4.2).

For candidate gene screening, we first filtered heterozygous single-nucleotide variants (SNVs) and insertions/deletions (indels) shared by all affected individuals (**Figure 2**). Then, variants causing non-synonymous amino acid substitutions, stop codons, indels in coding regions, and changes to splice-site sequences at exon-intron boundaries were included. Common

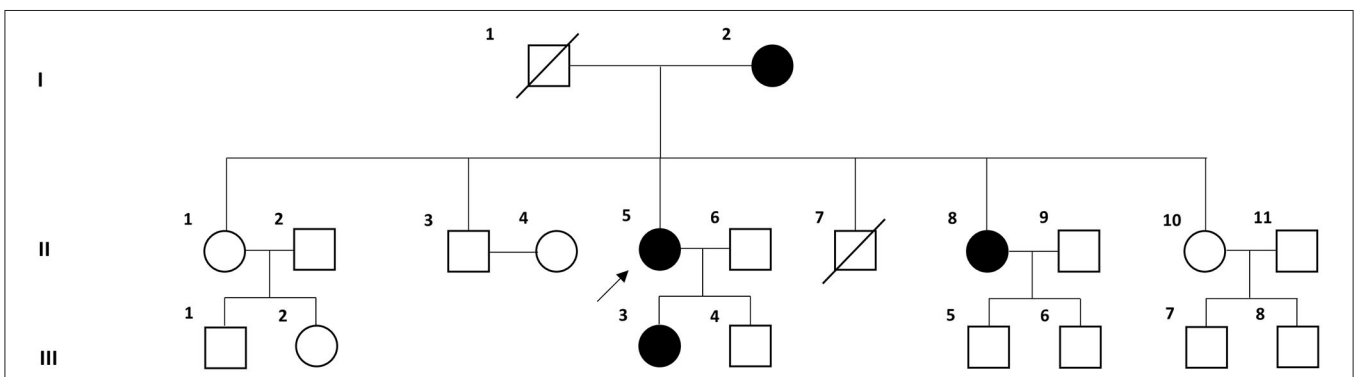

FIGURE 1 | Pedigree of the Korean family with vestibular migraine. Solid symbols (squares for males and circles for females) indicate clinically affected individuals (open symbols are unaffected individuals and slashed symbols are deceased individuals). The proband is indicated by an arrow.

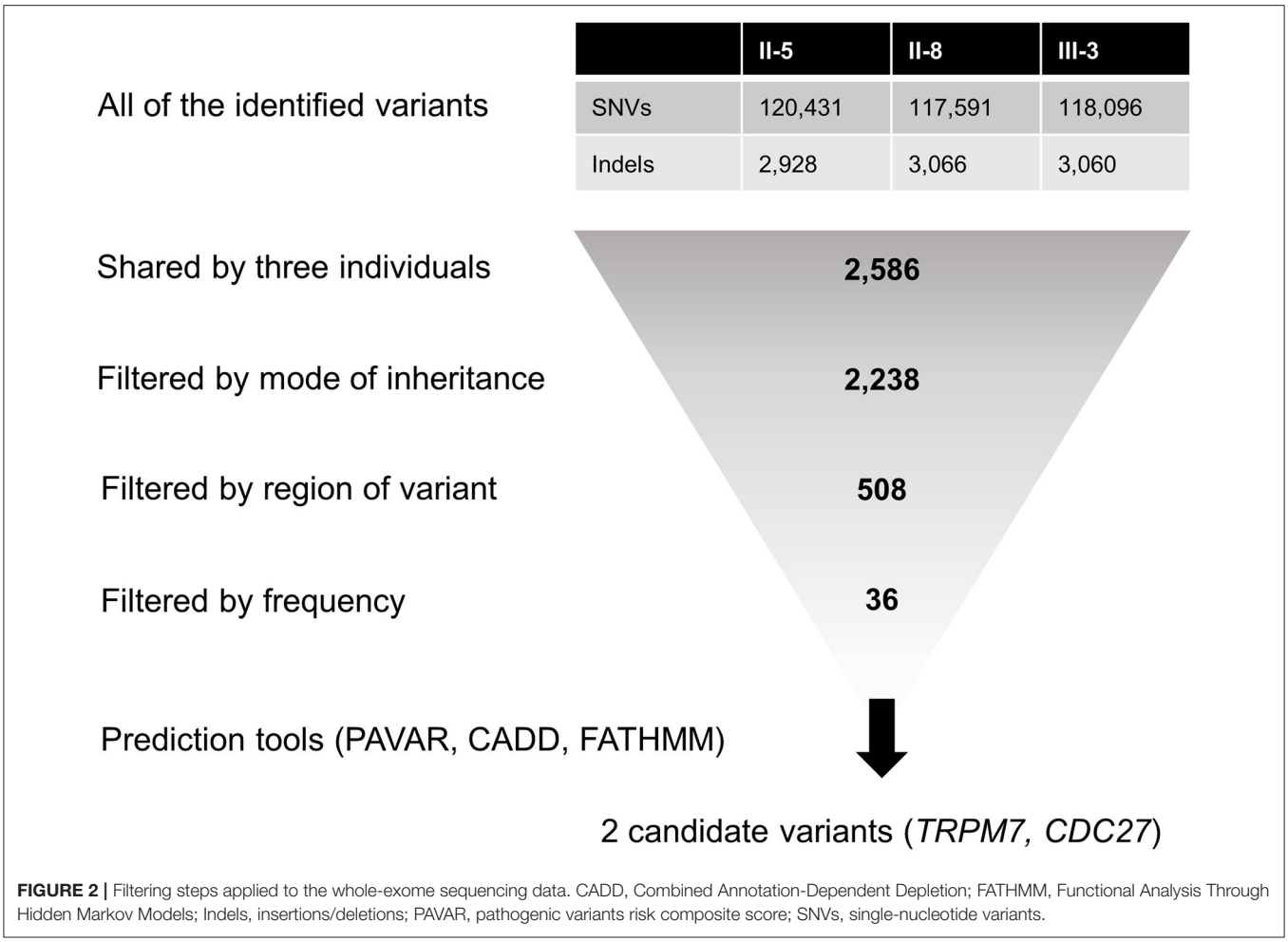

FIGURE 2 | Filtering steps applied to the whole-exome sequencing data. CADD, Combined Annotation-Dependent Depletion; FATHMM, Functional Analysis Through Hidden Markov Models; Indels, insertions/deletions; PAVAR, pathogenic variants risk composite score; SNVs, single-nucleotide variants.

variants with a minor allele frequency (MAF) of >0.001 in the dbSNP147, the 1,000 Genomes Project, the NHLBI GO Exome Sequencing Project (ESP), and the Genome Aggregation Database (gnomAD) were excluded. Since the frequency of variants may differ between racial groups, we further filtered rare variants with a MAF of <0.001 in the Korean Reference Genome Database (KRGDB). Candidate variants were identified and prioritized by performing functional annotation using the following tools: (1) the pathogenic variants risk composite score (PAVAR score), which is a seven-point scoring system based on annotations using SIFT (Sort Intolerant From Tolerant), PolyPhen2 (Polymorphism Phenotyping version 2), Grantham's Matrix, GERP+ (Genomic Evolutionary Rate Profiling), MutationTaster, PhastCons and PhyloP, (2) Combined Annotation-Dependent Depletion (CADD), and (3) Functional Analysis Through Hidden Markov Models (FATHMM). Variants with PAVAR score ≥5, CADD score ≥20, and FATHMM score ≤-1.5 were considered to be potentially pathogenic (23). Finally, candidate variants were interpreted according to the standards and guidelines recommended by the American College of Medical Genetics and Genomics (ACMG) (24). The gene expression patterns were analyzed using public databases such as the Genotype-Tissue Expression (GTEx), BioGPS, and Serial Analysis of Gene Expression (SAGE), and the gene function was assessed using the HuGE Navigator, the GeneCards Human gene database, the Online Mendelian Inheritance in Man database, and PubMed. The candidate variants were validated by Sanger DNA sequencing, and were screened in another individuals within the family and 100 normal controls.

RESULTS

Clinical Phenotype

The clinical characteristics of the family are summarized in **Table 1**. All affected individuals presented with rotatory vertigo, headache, and nausea/vomiting lasting for several hours to days. All had a history of migraine with or without visual aura, and showed one or more migraine features during the vestibular episodes. Two individuals (I-2 and II-8) experienced transient hemiparesis during the attacks, one (II-8) of whom also had dysarthria and facial dysesthesia on the same side as the hemiparesis. The symptoms were triggered by physical or emotional stress. Two individuals also had auditory symptoms such as tinnitus (II-5) and ear fullness (II-8), but air-conduction audiograms showed normal hearing functions (**Supplementary Figure 1**). One individual (II-8) had a past

TABLE 1 | Clinical characteristics of the affected family members.

Patient no.	Sex/ age	Age at onset	Duration	Ictal symptoms	Interictal signs	Migraine features	Additional symptoms
I-2	F/86	50	Hours-days	Vertigo, headache, nausea/vomiting, hemiplegia	Uni-directional GEN	One sided location, pulsatile quality, moderate pain intensity, nausea	(–)
II-5	F/54	53	Hours	Vertigo, headache, nausea/vomiting	Bi-directional GEN	One sided location, pulsatile quality, moderate pain intensity, photophobia, nausea	Tinnitus
II-8	F/48	30	Hours-days	Vertigo, headache, nausea/vomiting, hemiplegia, dysarthria, facial dysesthesia	(–)	One sided location, pulsatile quality	Seizure, ear fullness
III-3	F/30	19	Hours	Vertigo, headache, nausea/vomiting	Bi-directional GEN	Visual aura, moderate or severe pain intensity, aggravation by physical activity, nausea/vomiting, photophobia/phonophobia	(–)

F, female; GEN, gaze-evoked nystagmus.

TABLE 2 | Candidate variants identified using bioinformatics tools after applying filtering and prioritization processes.

Chr	Position	Gene	cDNA	Protein	Variant effect	PAVAR score	CADD	FATHMM	ACMG classification	MAF
15	50885896	*TRPM7*	c.3526C>T	p.Gln1176*	Nonsense	7	38	(–)	Pathogenic (PVS1,PS3,PM2, PP1,PP3,PP4)	NR
17	45258960	*CDC27*	c.71C>G	p.Ala24Gly	Missense	6	32	−3.23	Uncertain significance (PM2,PP1,PP3,PP4)	NR

Transcript ID: TRPM7, NM_017672.5; CDC27, NM_001114091.2.
Evidence code descriptions according to the ACMG classification: PM, pathogenic moderate; PP, pathogenic supporting; PS, pathogenic strong; PVS, pathogenic very strong. ACMG, American College of Medical Genetics and Genomics; CADD, Combined Annotation-Dependent Depletion; Chr, chromosome, FATHMM, Functional Analysis Through Hidden Markov Models; MAF, minor allele frequency; NR, not reported; PAVAR, pathogenic variants risk composite score.

history of recurrent seizure during her childhood. Between the vestibular episodes, three individuals (I-2, II-5, and III-3) showed uni- or bi-directional horizontal gaze-evoked nystagmus. The other neurological and neuro-otological examinations were unremarkable. Three individuals who received treatments showed a good response to flunarizine (II-5 and II-8) or propranolol (III-3).

Genetic Analysis

An average of 5.49 billion bases were generated per individual, with an average sequencing depth of approximately 63 in the target region, achieving the high quality of the sequencing (**Supplementary Table 1**). An initial screening of genes causing FHM and EA revealed no causative mutation in *CACNA1A*, *ATP1A2*, *SCN1A*, *KCNA1*, *CACNB4*, or *SLC1A3*.

The number of SNVs and indels identified per individual ranged from 120,657 to 123,359. Among them, a total of 2,586 variants were shared by all three individuals, and 508 of them were heterozygous non-synonymous missense variants, stop codons, coding indels, and splice-site variants at exon-intron boundaries (**Figure 2**). Next, 36 rare variants were filtered after excluding common variants with a MAF of >0.001 in public databases including gnomAD and KRGDB (**Supplementary Table 2**), but only two candidate variants in *CDC27* and *TRPM7* were prioritized by the functional annotations (**Table 2**). Both genes were found to be widely expressed in the brain including the cerebellum in public databases such as GTEx, BioGPS, and SAGE (**Supplementary Figure 2**).

The *CDC27* variant was a missense mutation c.71C>G of exon 2, which results in the amino acid substitution of alanine by glycine at codon 24. This gene participates in regulation of the cell cycle by encoding the anaphase-promoting complex, and is known to be associated with breast and prostate cancers. However, this variant was classified as "uncertain significance" in ACMG guidelines based on only one moderate (PM2) and three supporting (PP1, PP3, PP4) pathogenic criterion. Furthermore, a literature review did not reveal any evidence for a relationship between the *CDC27* and our family's phenotypes including migraine, vertigo, vestibular or ataxic disorders. Thus, this variant was excluded as a candidate gene for VM.

The *TRPM7* variant was a nonsense mutation c.3526C>T of exon 25 that cause a premature stop codon and loss-of-function in the protein. This variant was also present in

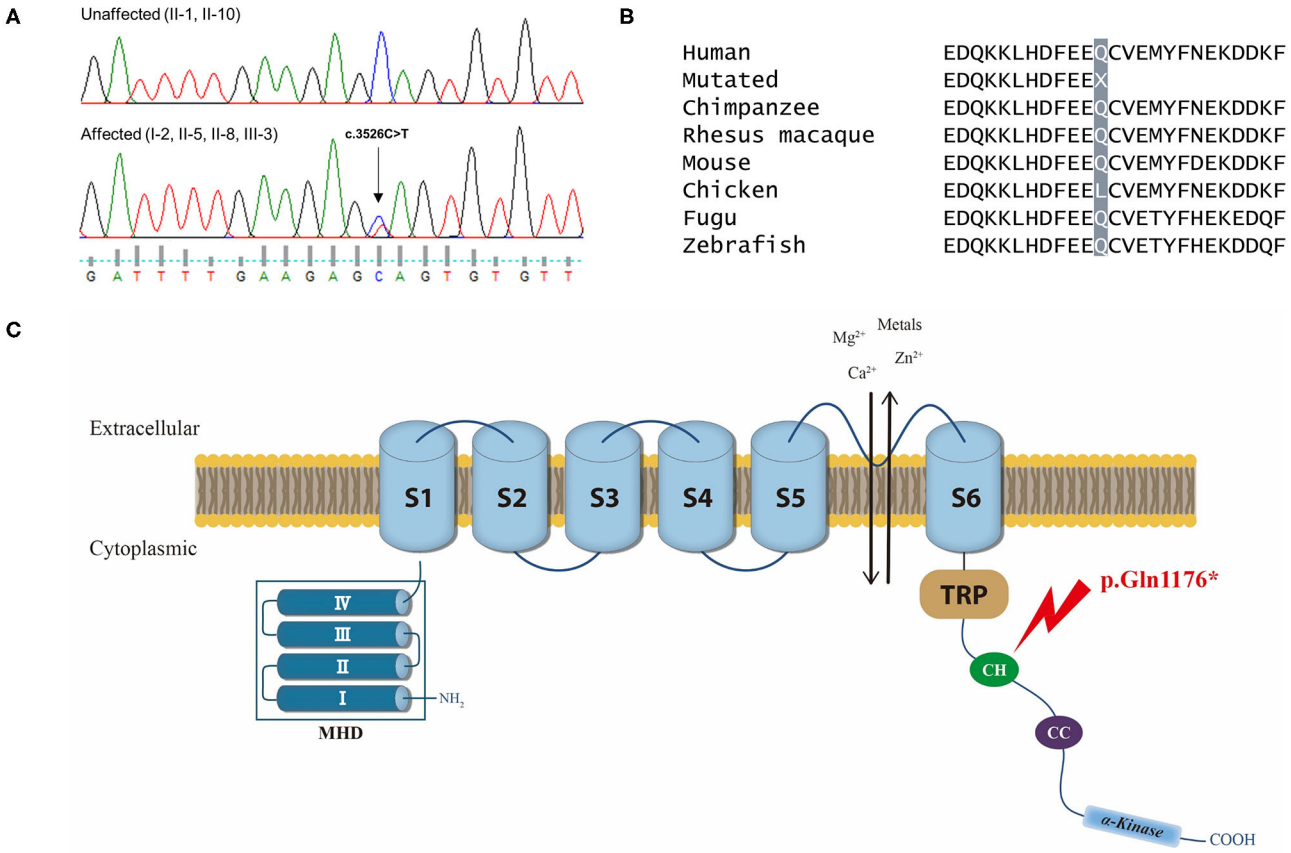

FIGURE 3 | (A) Chromatograms of the affected individuals showing a heterozygous nonsense mutation (c.3526C>T) in exon 25 of *TRPM7*, which would result in a premature stop codon (p.Gln1176*). **(B)** Conservation of the mutated residue (Gln, Q) of a "connecting helix" (CH) in the C-terminus, highlighted in gray. **(C)** Schematic representation of the TRPM7 channel and localization of the mutation. The TRPM7 channel comprises a tetrameric complex with each subunit containing six-transmembrane segments (S1–S6) and a pore-forming loop between S5 and S6. Its cytoplasmic N-terminus has four regions of TRPM subfamily homology domain (MHD), whereas the C-terminus contains a TRP box of highly conserved residues, a coiled-coil (CC) domain, and an atypical serine/threonine protein kinase domain. The mutation (p.Gln1176*) is located in a CH that links the transmembrane segment and the CC domain in the C-terminus (arrow).

another affected individual (I-2), while it was absent in the unaffected individuals (II-1 and II-10), 100 normal controls, gnomAD and KRGDB (**Figures 3A,B**). By applying ACMG guidelines, the variant was considered "pathogenic" based on one very strong (PVC1), one strong (PS3), one moderate (PM2), and three supporting (PP1, PP3, PP4) pathogenic criterion. The TRPM7 channel belongs to the melastatin subfamily of TRP channels, and it plays a crucial role in maintaining intracellular Ca^{2+} and Mg^{2+} homeostasis (**Figure 3C**). This has led to proposals that genetic variations in *TRPM7* influence the susceptibility to neurodegenerative diseases (15, 16). In addition, SNVs within some TRP genes have been linked to migraine susceptibility (5, 25–27). Therefore, the *TRPM7* variant may be a causal variant for explaining the phenotypes in the present family.

DISCUSSION

This study identified a nonsense mutation in *TRPM7* in a Korean family with VM. This variant may cause a premature stop codon and loss-of-function in the protein, thus contributing to the development the VM phenotype by altering the homeostasis of intracellular ions.

Several hypotheses have been proposed for explaining the pathophysiology of VM, which remains unclear. The genetic susceptibility of migraine suggests ionic channelopathy involved in glutamate homeostasis as the underlying pathophysiology of VM (28, 29). Indeed, there is accumulating evidence that Ca^{2+} channels could be involved in migraine and VM pathophysiology. Mutations in *CACNA1A*, which encodes Cav2.1, the α1A subunit of the P/Q-type voltage-gated Ca^{2+} channel, cause three neurological channelopathies: FHM type 1, EA type 2 (EA2), and spinocerebellar ataxia type 6 (30). Patients with FHM or EA2 often experience paroxysmal vertigo, and more than half of EA2 patients have migraine that meets the ICHD criteria. Some members of the present family also presented with hemiplegia and dysarthria during their episodes. Nevertheless, previous studies failed to detect mutations in patients with VM in the genes causing FHM or EA, such as *CACNA1A*, *ATP1A2*,

SCN1A, and *CACNB4* (12, 13), which is consistent with the present study.

TRPM7 reported here encodes the cation selective ion channel that is highly permeable to Ca^{2+}, Mg^{2+}, and metal ions such as Zn^{2+} (16). This channel comprises a tetrameric complex, with each subunit containing six-transmembrane segments (S1–S6) and a pore-forming loop between S5 and S6 [**Figure 3C**; (31)]. Its C-terminus contains a TRP box of highly conserved residues, a coiled-coil (CC) domain, and an atypical serine/threonine protein kinase domain (32). The *TRPM7* mutation identified in the present study is located in a long "connecting helix" that links the transmembrane segment and the CC domain in the C-terminus, thus predicting truncation of the C-terminus including the CC and kinase domains (32). These domains play an important role in the tetrameric assembly of the channel or in regulating channel function by Mg^{2+} nucleotides (31–34). Therefore, the mutation identified in the present study seems to affect TRPM7 channel activity via structural instability or functional impairment. This hypothesis is supported by a previous animal study which generated knockout mice with the deletion of TRPM7 kinase domain (35). These mice carried truncated mutation quite similar to the mutation described in this study. Although homozygous knockout mice caused embryonic lethality, heterozygous mice were viable, but showed abnormality in the regulation of Mg^{2+} homeostasis and alterations in TRPM7 channel properties. These mice exhibited abnormal behaviors such as clasping, tremor, seizure, and violent sudden leaps as a reaction to the light and noise, similar to photophobia and phonophobia seen in VM. Furthermore, one of the affected individuals in this family had a history of recurrent seizure.

Ca^{2+} and Mg^{2+} play important roles in regulating various neuronal functions. In particular, they exert opposite effects in the signaling of the excitatory neurotransmitter glutamate: glutamate release is triggered by an influx of Ca^{2+}, whereas Mg^{2+} inhibits glutamate release by antagonizing Ca^{2+} (36). Thus, a tight balance between Ca^{2+} and Mg^{2+} is needed in order to maintain the proper excitability of neurons. Since TRPM7 channels that are abundantly expressed in neuronal cells are highly selective to Ca^{2+} and Mg^{2+} (37, 38), mutation in *TRPM7* may cause alterations in Ca^{2+} and Mg^{2+} homeostasis and neuronal excitability. Migrainous headache is the consequence of cortical spreading depression evoked by glutamate release and N-methyl-D-aspartate (NMDA) receptor activation in the brain. These processes have been linked to low Mg^{2+} concentrations, which contribute to the hyperexcitability of the NMDA receptor (36). A beneficial effect of Mg^{2+} supplementation has also been reported in migraine patients (39). In addition, low Mg^{2+} concentrations increase the amount of substance P released, which is a neuroinflammatory mediator (40). Since the TRPM7 channel is an important Mg^{2+} transporter, it may contribute to VM attacks by affecting intracellular Ca^{2+} and Mg^{2+} homeostasis.

Recently, several TRP channels have been linked to migraine pathophysiology, including TRPV1, TRPV4, TRPM8, and TRPA1 (19). These channels are expressed on trigeminal sensory neurons that innervate the meninges (20, 41). The activation of TRP channels promotes the excitation of nociceptive afferent fibers and potentially leads to pain and allodynia (19, 20). In addition, it can elicit the release of CGRP, causing vasodilation and neurogenic inflammation (19, 42, 43). Several SNPs in *TRPV1*, *TRPV3*, and *TRPM8* were found to be associated with migraine susceptibility in meta-analyses of observational studies and GWAS (5, 25–27). Based on these findings, TRP channels have been proposed as a therapeutic target in migraine (19, 21, 22, 44). More studies are needed to better explore the potential role for these channels including *TRPM7* in migraine pathophysiology.

This study was subjective to some potential limitations. We did not perform functional study determining pathogenicity of the candidate variants. Despite the rarity and putative pathogenicity in functional annotations, establishing the pathogenicity of the variants may be difficult without a functional assay. However, previous functional studies demonstrated that the deletion of TRPM7 kinase domain reduced channel activity and increased its sensitivity to Mg^{2+} inhibition (33–35). Another limitation is difficulty in detecting copy number variations (CNVs) through whole-exome sequencing. CNV analysis can aid the detection of large deletions or duplications of causative genes, but whole-genome sequencing may be more suitable than whole-exome sequencing for CNV analysis. Finally, we used the hg19 as the reference genome for the reads mapping instead of hg38.

In summary, we have presented the clinical characteristics of a Korean family with VM. Whole-exome sequencing identified a potential disease-causing variant in *TRPM7*, which may cause alterations in intracellular Ca^{2+} and Mg^{2+} homeostasis and neuronal excitability. Our results highlight *TRPM7* as a novel candidate gene for VM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Pusan National University Yangsan Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EO conducted the experiments and interpretation of the data, and wrote the manuscript. J-HS, JC, S-YC, and K-DC contributed to the interpretation and analysis of data. J-HC conducted the design and conceptualization of the study, interpretaion of the data, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.595042/full#supplementary-material

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Presbyvestibulopathy, Comorbidities, and Perception of Disability: A Cross-Sectional Study

Andrés Soto-Varela[1,2]*, *Marcos Rossi-Izquierdo*[3], *María del-Río-Valeiras*[4], *Isabel Vaamonde-Sánchez-Andrade*[4], *Ana Faraldo-García*[4], *Antonio Lirola-Delgado*[4] and *Sofía Santos-Pérez*[1,2]

[1] Division of Neurotology, Department of Otorhinolaryngology, Complexo Hospitalario Universitario, Santiago de Compostela, Spain, [2] Department of Surgery and Medical-Surgical Specialities, University of Santiago de Compostela, Santiago de Compostela, Spain, [3] Department of Otorhinolaryngology, University Hospital Lucus Augusti, Lugo, Spain, [4] Department of Otorhinolaryngology, Complexo Hospitalario Universitario, Santiago de Compostela, Spain

Objective: To assess the perception of disability in patients with presbyvestibulopathy and to determine the factors (demographic, balance test scores, and comorbidities) that determine higher levels of disability.

Material and Methods: This was a cross-sectional study conducted in a tertiary university hospital. There were 103 patients who fulfilled the diagnostic criteria for presbyvestibulopathy and were included. Dizziness Handicap Inventory (DHI) score was the main variable used to quantify disability. Influence on DHI score, sex, age, time of evolution, equilibriometric parameters (posturographic scores and timed up and go test), history of falls, comorbidities (high blood pressure, diabetes, and dyslipidemia), psychotropic drug use, tobacco or alcohol use, living environment (urban or rural), and active lifestyle were analyzed.

Results: Most of the DHI scores showed a moderate (46 patients, 44.7%) or severe (39 participants, 37.9%) handicap. DHI scores were higher in women (59.8 vs. 36.1, $p < 0.001$), patients with obesity (58.92 vs. 48.68; $p = 0.019$), benzodiazepine (59.9 vs. 49.1, $p = 0.008$) or other psychotropic drug (60.7 vs. 49.2, $p = 0.017$) users, and fallers (57.1 vs. 47.3, $p = 0.048$). There was also a significant positive correlation between DHI score, time (Rho coefficient: 0.371, $p < 0.001$), and steps (Rho coefficient: 0.284, $p = 0.004$) used in the TUG and with the short FES-I questionnaire (a shortened version of the Falls Efficacy Scale-International) score (Rho coefficient: 0.695, $p < 0.001$). DHI scores were lower in alcohol consumers than in non-drinkers (46.6 vs. 56, $p = 0.048$). No significant correlation was found between DHI scores and age, time of evolution, posturographic scores, comorbidities, environment (rural or urban), or active lifestyle.

Conclusion: Most patients with presbyvestibulopathy show an important subjective perception of disability in relation to their symptoms. This perception is substantially higher in women than in men. The most influential factors are difficulties in walking, fear of falling, and obesity.

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Keywords: presbyvestibulopathy, comorbidities, disability, handicap, dizziness handicap inventory, DHI

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Tien-Chen Liu,
National Taiwan University, Taiwan

Reviewed by:
Habib Georges Rizk,
Medical University of South Carolina, United States
Tjasse Bruintjes,
Gelre Hospitals, Netherlands
Giuseppe Chiarella,
University of Catanzaro, Italy

***Correspondence:**
Andrés Soto-Varela
andres.soto@usc.es

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INTRODUCTION

Vestibular symptoms in the elderly are common and may result in reduced quality of life of these individuals (1). In addition, their consequences (primarily limiting mobility and increasing the risk of falls) are especially serious in this age group, leading to social isolation, and to direct morbidity and mortality (derived from eventual fractures caused by falls) (2). Health care for these patients is a major challenge for public healthcare systems (3).

The causes of vestibular symptoms in the elderly are varied (4, 5). Some highly prevalent clinical disorders (such as benign paroxysmal positional vertigo, BPPV) are more common in the elderly than in younger adults. Exposure to drugs or other vestibulotoxic substances is more likely and has a cumulative effect on people who are older because aging increases the likelihood of exposure to these substances. Various medications frequently used in older people (such as benzodiazepines and other central nervous system depressants) may slow vestibular reflexes. The existence of diseases that affect other systems (visual, locomotor, neurological, and cardiovascular, among others) may give rise to symptoms of dizziness or instability, which trigger (or potentiate) strictly vestibular symptoms (6). Lastly, aging itself may cause histologically demonstrable damage to vestibular receptors and organs (7–9) which may be responsible for the symptoms of dizziness, imbalance, or instability frequently reported by elderly people.

The physiological deterioration associated with aging has been referred to by various names in recent decades (including presbystasis, presbyvertigo, presbyequilibrium, and geriatric dizziness) (2). However, until 2019, there was no consensus on its description, and its existence was not generally accepted. The publication of the diagnostic criteria for presbyvestibulopathy by the Bárány Society in that year (10) has enabled an adequate characterization and homogenization of these patients, which has facilitated their study. Presbyvestibulopathy is almost invariably associated with other functional disorders in these patients, due to either aging itself (such as presbyopia or presbycusis) or the coexistence of other diseases (locomotor, neurological, and cardiovascular, among others). Therefore, it is not easy to assess how much of the disability that elderly people perceive regarding vestibular symptoms directly results from the aging of this system and how much of it results from other superimposed factors (comorbidities, use of central nervous system depressant drugs, the living environment of the patients and their level of physical activity, among others).

Different instruments can be used to measure disability caused by vestibular symptoms. The most commonly used instrument is the Dizziness Handicap Inventory (DHI) (11), developed in 1990 by Jacobson and Newman. Validated in different languages (including Spanish) (12), the DHI includes 25 questions divided into three groups (9 referred to the functional scale, 9 to the emotional scale, and 7 to the physical scale). Each of these 25 questions has three possible answers, which are scored as follows: "yes" (4 points), "sometimes" (2 points), and "no" (0 points). A score of 100 would indicate an absolute perception of disability, whereas a score of 0 would indicate that the subject does not perceive any disability. Total scores lower than 30 indicate mild disability, from 31 to 60 moderate disability, and higher than 60 severe disability (13).

The objective of this study was to evaluate the perception of disability in a sample of patients with presbyvestibulopathy who live in the community and to identify its determining factors (demographic, balance, comorbidities, and drugs, among others).

MATERIALS AND METHODS

This study was part of a clinical trial funded by the project PI1500329, integrated into the Spanish State Plan for R + D + I and funded by the Instituto de Investigación en Salud Carlos III- ISCIII -Subdirección general de Evaluación y Fomento de la Investigación and the Fondo Europeo de Desarrollo regional (FEDER). This clinical trial, the full protocol for which has already been published (14), aims to determine whether vestibular rehabilitation is useful in elderly patients with instability for improving their balance and reducing their risk of falling.

Study Design

This was an observational cross-sectional study, conducted at the Otoneurology Unit of a tertiary hospital.

Study Population: Inclusion and Exclusion Criteria

The total sample of the previously mentioned clinical trial was the initial population, consisting of individuals older than 65 years, with postural instability, who lived in the community (not institutionalized) and who met at least two of the following inclusion criteria:

(a) Having suffered at least one fall in the last 12 months.
(b) Taking more than 15 s or requiring a walking aid to complete the "timed up and go" test (15) (specific normality threshold calculated in previous studies).
(c) Having a mean balance percentage in the sensory organization test (SOT) of dynamic posturography (PD) $< 68\%$.
(d) Having suffered at least one fall in the SOT of PD.
(e) Scoring 60% or higher in the Vertiguard geriatric Standard Balance Deficit Test (gSBDT).

The following exclusion criteria were used:

(a) Cognitive impairment or reduced cultural level, which prevented the patient from understanding the examinations and from giving their informed consent. All patients underwent a medical history, including questions about their symptoms. Specifically, those who were found to have difficulties in understanding the DHI items were excluded.
(b) Organic diseases, which prevented standing, which was necessary for balance assessment.
(c) Balance disorders caused exclusively by diseases other than age (neurological and vestibular, among others).

In all cases, imbalance was the symptom for which they consulted. A part of the patients was referred from Primary Care to the Otoneurology Unit; the rest were referred from

the Department of Neurology. From this initial population (180 patients), the subjects who met the following diagnostic criteria of presbyvestibulopathy (10) were selected:

A. Chronic vestibular syndrome (at least 3 months duration) with at least 2 of the following symptoms:
   1. Postural imbalance or unsteadiness
   2. Gait disturbance
   3. Chronic dizziness
   4. Recurrent falls.

B. Mild bilateral peripheral vestibular hypofunction documented by at least 1 of the following:
   1. VOR gain measured by video-HIT between 0.6 and 0.8 bilaterally
   2. VOR gain between 0.1 and 0.3 upon sinusoidal stimulation on a rotatory chair (0.1 Hz, Vmax = 50–60○/s)
   3. Reduced caloric response (sum of bithermal maximum peak SPV on each side between 6 and 25○/s).

C. Age ≥60 years
D. Not better accounted for by another disease or Disorder

In total, 103 individuals met these criteria, forming the study population.

Sample Size Estimation

To assess whether the sample size was sufficient to draw statistically significant conclusions, an estimate was performed, using the mean DHI score as a reference. From a previous study in our research group (16), the estimated standard deviation for this value was 18. A difference in score between study groups of 10 points was considered relevant. With a 95% confidence level (1-$\acute{\alpha}$) and a 0.5 probability of type II error (β), for a bilateral hypothesis test, 84 subjects were deemed necessary. Therefore, the available sample (103 individuals) was considered sufficient to establish the existence of significant differences.

Samples

The sample consisted of 103 patients with postural instability, who met the inclusion criteria. The mean age was 78.19 ± 5.72 years, with a minimum of 65.17 and a maximum of 92.31 years. Of the study population 77 patients were women (74.8%) and 26 were men (25.2%); the female/male ratio was 2.96/1.

Method

A clinical history and a complete vestibular evaluation were performed to detect the causes of vestibular symptoms different from aging; This evaluation also served to confirm that the patients met the diagnostic criteria for presbyvestibulopathy. The examination included:

- Detection (or absence) of spontaneous nystagmus using Frenzel glasses: its presence was an exclusion criterion.
- Positional tests to detect benign paroxysmal positional vertigo (its presence did not necessarily mean the exclusion of the patient from the study; the patient was included in the study protocol when, once BPPV had resolved, symptoms and exploratory data consistent with presbyvestibulopathy persisted).
- Evaluation of the vestibulo-ocular reflex, through clinical (mainly, the cephalic impulse test) and instrumental (video Head Impulse Test and/or bithermal caloric tests) tests:
  - video Head Impulse Test (vHIT): A portable video-oculography system (vHIT, GN Otometrics, Denmark), a high-speed infrared camera (250 Hz), and an accelerometer were used to measure movements of the right eye and head, during the cephalic impulses, in the horizontal plane. The head speed ranged from 150 to 240°/s, with amplitudes ranging from 15 to 20° from the center to the lateral position. Twenty records were collected and processed on each side, evaluating gain and the presence or absence of refixation saccades.
  - Bithermal caloric testing (videonystagmograph HIS model, France) using water, with the following sequence: irrigation of left ear at 44°C, irrigation of right ear at 44°C, irrigation of left ear at 30°C, and irrigation of right ear at 30°C. The reflectance of each of the two ears was evaluated (sum of the mean speed of the slow phase of the nystagmus, at the maximum peak response, of the two stimulations of each ear).
- All the patients underwent a clinical neurological examination. When necessary (due to suspicion of neurological disease that could cause relevant symptoms), imaging (brain magnetic resonance imaging) was performed.

To evaluate balance (necessary to determine whether the patients met the inclusion criteria of the initial research project, from which the sample of the present study was obtained), the following tests were performed:

(a) A modified version of the Timed Up and Go (mTUG) test (15): the patient, sitting in a chair, must stand up without using their hand to push up, walk 3 m, turn around, walk around the chair, and sit down again. The time spent and the number of steps necessary to complete the test are quantified.

(b) The computerized dynamic posturography—sensory organization test (CDP-SOT) (Neurocom Smart Equitest platform): The SOT included quantitation of the patient's center of gravity (COG) displacements in 6 different sensorial information conditions as follows:

- 1: fixed surface and visual surround, with eyes open.
- 2: fixed surface, with eyes closed.
- 3: fixed surface and moving visual surround, with eyes open.
- 4: moving surface and fixed visual surround, with eyes open.
- 5: moving surface, with eyes closed.
- 6: moving surface and visual surround, with eyes open. Each of the six conditions was repeated three consecutive times, with the participants completing a total of 18 trials. The time established for each of these trials was 20 s.

(c) Limits of stability in the CDP (CDP-LOS): Following visual feedback (movement of a pictogram representing the subject's COP on a TV monitor), the patient had to voluntarily move

his or her COP without moving his or her feet on the platform, to reach eight points around him/her. These points represented 100% of the displacement limit of the subject's COP, according to height and age.

(d) Balance record study using the mobile Vertiguard system (Vesticure GmbH, Germany): The following 14 tests were performed, and the analysis of the results represented the gSBDT:

- Standing still (SS), with eyes open, on a normal surface (NS).
- SS, with eyes closed, on a NS.
- SS, one leg, eyes open, NS
- Making 8 steps in tandem, with eyes open, on a NS.
- SS, with eyes open, on a foam surface (FS).
- SS, with eyes closed, on a FS.
- Making 8 steps in tandem, with eyes open, on a FS.
- Walking 3 m, with eyes open.
- Walking 3 m, with eyes open, while turning the head from side to side.
- Walking 3 m, with eyes open, while moving the head up and down.
- Walking 3 m, with eyes closed.
- Walking over 4 barriers (height: 26 cm; distance between barriers: 1 m).
- Sitting down on a chair.
- Getting up from a chair.

(e) A questionnaire assessing the perception of disability in relation to postural instability: the Dizziness Handicap Inventory (DHI), validated in Spanish (12), and previously explained in the introduction of this manuscript.

(f) A questionnaire assessing the fear of falling: a shortened version of the Falls Efficacy Scale-International to assess fear of falling (Short FES-I) (17). It evaluates fear of falling while performing 7 everyday activities. Each question has 4 possible answers scored as follows: "not at all concerned" (0 points), "somewhat concerned" (1 point), "quite concerned" (2 points), and "very concerned" (3 points). The highest score (greatest fear of falling) is 21, and the lowest is 0.

(g) The patients were directly asked whether they had suffered a fall in the previous 12 months and, if the answer was yes, the number of falls during this time.

The balance tests (mTUG, CDP, and Vertiguard) were carried out by trained personnel in vestibular assessment. In all of them, before performing the test, the patient received a detailed explanation and an initial training record was made. Next, the tests were carried out according to the protocol followed in our clinic: three trials in each task for the CDP-SOT and one each for the mTUG, CDP-LOS, and gSBDT Vertiguard. The questionnaires were delivered in writing to the patient (after an explanation by the researcher), who answered them on their own or with the help of a family member.

Study Variables

The main variable was the DHI score, considered a continuous (score) and discontinuous (mild disability, 30 points or lower; moderate disability, from 31 to 60 points; and severe disability, 60 points or higher) variable (13). The scores of each DHI scale (functional, emotional, and physical) were considered secondary variables.

The relationships of the DHI score with the following variables were analyzed:

a) Sex.
b) Age.
c) Age at onset of symptoms.
d) Time of symptom progression (in months).
e) Body mass index: weight (in kg)/ height^2 (in meters), according to which the participants were divided into obese (BMI $\geq$ 30) and non-obese (BMI <30).
f) Falls in the previous 12 months: number of falls, dividing patients into fallers (at least one fall) vs. non-fallers (no falls), and also dividing them into recurrent fallers (more than one fall) vs. non-recurrent fallers (no or one fall) (10).
g) mTUG: time and steps necessary to complete the test.
h) CDP-SOT:

- The equilibrium score for each condition (the arithmetic mean of the three entries for each condition).
- The composite equilibrium score, which was calculated as the weighted average of the 18 SOT scores.
- The effectiveness of somatosensory input use, which was a percentage value from the application of the following formula: (average score of condition 2/average score of condition 1) $\times$ 100.
- The effectiveness of visual input use, which was calculated using the following formula: (average score of condition 4/average score of condition 1) $\times$ 100.
- The effectiveness of vestibular input use, which was assessed using the following calculation: (average score of condition 5/average score of condition 1) $\times$ 100.
- The ability to assume erroneous visual input, a score was assigned using the following calculation made using the values determined by the conditions: [(2+5)/(3+6)] $\times$ 100.

i) CDP-LOS:

- Maximum excursion (ME): measurement of the maximum COP excursion, with respect to 100% of the theoretical limit of stability (as a percentage).
- Endpoint excursion (EE): measure of the distance achieved toward a target on the initial movement (as a percentage).
- Directional control: comparison between movement in the direction of the target vs. movement away from that direction, as a percentage. A value of 100% would be a straight line from COP to the intended target.

j) Short FES-I score.
k) Association with BPPV (with diagnosis confirmed by positional tests).
l) Association with comorbidities (detected by directly asking patients and by consulting their electronic medical records):

- Obesity (calculated according to the BMI).
- Heart disease (primarily hypertensive and/or ischemic heart disease).

- Diabetes mellitus.
- Neurological disease (primarily ischemia or Parkinson's disease).

m) Use of psychotropic drugs (benzodiazepines and other psychotropic drugs), by directly asking patients and by consulting their electronic medical records.
n) Consumption of alcohol, by directly asking the patients.
o) Development of an active (with the ability to walk without assistance and to be independent to perform the basic activities of daily life) or inactive (participants who need help walking and/or performing basic activities of daily life) lifestyle.
p) Living environment (rural vs. urban). The environment was defined according to legal and administrative criteria. In Spain, a rural environment is defined as that whose population is <5,000 inhabitants. The demographic data of each population were retrieved from the population records available in the National Institute of Statistics database (Instituto Nacional de Estadística—INE).

Statistical Analysis

Fisher's exact test was used to analyze the relationship between nominal variables, in 2 × 2 contingency tables, calculating the odds ratio and 95% confidence intervals. The Chi-squared test was used to analyze the relationship between nominal variables and the DHI score (as a categorical variable). The Kolmogorov-Smirnov test was used to determine whether the continuous variables followed a normal distribution. When this test confirmed the hypothesis of normality, a Student's *t*-test was used to relate them to the nominal variables. Conversely, when the continuous variables did not follow a normal distribution, a non-parametric Mann-Whitney *U*-test was used to examine these relationships. To assess the effect of the variables sex (males and females), associated neurological disease, Benzodiazepine use, use of other psychotropic drugs, and alcohol consumption (yes vs. no) on DHI, generalized linear models (GLM) were used. The Akaike Information Criteria (AIC) corrected for finite samples was used as goodness-of-fit test of the models, and the Wald test was used to compare model effects. Finally, to correlate continuous variables with each other, Spearman's Rho correlation test was used. The level of statistical significance for all tests was set at $p < 0.05$.

The SPSS 15.0 software for Windows was used for the statistical analyses.

Ethical Aspects

The protocol was approved by the Independent Ethics Committee of Galicia (protocol No. 2014/411). The study was conducted in accordance with the ICH Good Clinical Practices, the Declaration of Helsinki, and Law 14/2007 of 3 July on Biomedical Research. All the patients signed a written informed consent form to participate in the study.

TABLE 1 | Distribution of history of falls, comorbidities, psychotropic drug use, tobacco or alcohol use, living environment, and active lifestyle.

Variable	Yes	No
Faller	70 (67.96%)	33 (32.04%)
Recurrent faller	51 (49.51%)	52 (50.49%)
BPPV	5 (4.85%)	98 (95.15%)
Heart disease (hypertensive and/or ischemic)	66 (64.08%)	37 (35.92%)
Diabetes mellitus	25 (24.27%)	78 (75.73%)
Dyslipidemia	58 (56.31%)	45 (43.69%)
Associated neurological disease	17 (16.50%)	86 (83.50%)
Benzodiazepine use	46 (44.66%)	57 (55.34%)
Consumption of other psychotropic drugs	43 (41.75%)	60 (58.25%)
Tobacco use	4 (3.88%)	99 (96.12%)
Alcohol consumption	23 (22.33)	80 (77.67%)
Active lifestyle	98 (95.15%)	5 (4.85%)
Rural environment	68 (66.02%)	35 (33.98%)

RESULTS

The mean DHI score of the sample was 53.65 ± 22.28. The mean score on the functional scale was 22.37 ± 9.35, the emotional scale was 14.78 ± 8.52, and the physical scale was 16.50 ± 7.71. With respect to the total DHI score, 18 patients (17.5%) had a perception of mild disability, 46 (44.7%) of moderate disability, and 39 (37.9%) of severe disability.

Regarding the demographic and clinical variables, the mean BMI was 29.88 kg/m^2 ± 4.24, the mean age at onset of symptoms was 75.03 ± 6.16 years, and the mean time of symptom progression was 3.17 ± 3.15 years. The mean number of falls suffered in the previous 12 months was 8.36 ± 36.78. The distribution of the other variables is outlined in **Table 1**.

The DHI score was affected by the sex of the patients (higher mean score in women than in men: 59.77 vs. 36.08; $p = 4.06\ e^{-7}$, Student's *t*-test). Conversely, no correlation of the DHI score with the age of the patient ($p = 0.824$, coefficient value = −0.022), age of symptom onset ($p = 0.596$, coefficient value = −0.053) or the time of progression of these symptoms ($p = 0.348$, coefficient value = 0.093) was detected by Spearman's Rho correlation.

Correlations were detected between the DHI score and the number of falls suffered in the last year ($p = 0.009$, coefficient value = 0.255; Spearman's Rho correlation), between being a faller or non-faller (higher mean DHI score in fallers than in non-fallers: 57.13 vs. 47.33; $p = 0.048$, Student's *t*-test), and between being a recurrent or non-recurrent faller (higher mean score in recurrent-fallers: 59.18 vs. 48.23, $p = 0.012$, Student's *t*-test).

Regarding the balance evaluation, a Spearman's Rho correlation showed no relationship between the DHI score and most measures of dynamic posturography. As shown in **Table 2**, only the score of condition 2 of the sensory organization test showed some correlation with the DHI score. In turn, the TUG values were significantly correlated with both the time ($p = 0.0001$, coefficient value = 0.371) and the number of steps necessary to complete the test ($p = 0.004$, coefficient value = 0.284). Fear of falling, measured using the short FES-I

TABLE 2 | Correlation between DHI score and most relevant CDP scores (Spearman's Rho correlation).

	Parameter	Coefficient value	*P*-value
CDP sensory organization test	Overall average balance	−0.142	0.152
	Condition 2	−0.218	0.027
	Condition 5	−0.031	0.754
	Somatosensory input	−0.131	0.186
	Visual input	−0.153	0.122
	Vestibular input	−0.026	0.795
	Visual preference	0.003	0.976
CDP limits of stability	Endpoint excursion	−0.083	0.404
	Maximum excursion	−0.073	0.461
	Directional control	−0.085	0.392

TABLE 3 | Multivariate analysis evaluating how sex, obesity, absence of neurological disease and benzodiazepine use, use of other psychotropic drugs, and alcohol consumption, influence DHI score.

Variable	Coefficient (95% CI)	Wald's Chi square	*P*-value
Sex (female)	19.273 (9.459; 29.086)	14.816	0.0001
Associated neurological disease (no)	7.035 (−3.110; 17.179)	1.847	0.174
Benzodiazepine use (no)	−4.719 (−12.242; 2.804)	1.511	0.219
Consumption of other psychotropic drugs (no)	−7.424 (−15.910; −0.062)	3.778	0.052
Alcohol consumption (no)	−1.264 (−10.862; 8.335)	0.067	0.796
Obesity	8.468 (1.308; 15.629)	5.373	0.020
Intersection	37.176 (23.514; 50.838)	28.443	$9.65\ e^{-8}$

questionnaire, was strongly correlated with the DHI score ($p = 3.95\ e^{-16}$, coefficient value = 0.695).

No relationship was detected between the DHI score and any of the following study variables: presence of BPPV ($p = 0.381$, Mann–Whitney *U*-test), hypertensive and/or ischemic heart disease ($p = 0.791$, Student's *t*-test), diabetes mellitus ($p = 0.798$, Student's *t*-test), practicing physical activity ($p = 0.275$, Student's *t*-test), or living in a rural or urban environment ($p = 0.142$, Student's *t*-test).

Higher DHI scores were related to obesity (higher mean score in obese patients, 58.92 vs. 48.68; $p = 0.019$, Student's *t*-test), absence of associated neurological disorders (higher mean score in those without associated neurological disorders than in those with such disorders, 55.84 vs. 43.88; $p = 0.016$, Student's *t*-test), benzodiazepine use (49.07 mean in non-users vs. 59.91 in users; $p = 0.008$, Student's *t*-test), use of other psychotropic drugs (49.23 mean score in non-users vs. 60.71 in users; $p = 0.009$, Mann–Whitney *U*-test) and absence of alcohol consumption (46.64 mean score in alcohol drinkers vs. 55.98 in teetotalers; $p = 0.048$, Student's *t*-test). Some of these variables were affected by sex. Associated neurological disorders were more frequent in men (34.6%) than in women (10.4%), with $p = 0.007$ [Fisher's exact test; OR = 4.566, 95% CI (1.535; 13.585)]. Benzodiazepine use was much less frequent in men than in women [19.2 vs. 53.2%; $p = 0.002$, Fisher's exact test; OR = 0.209, 95% CI (0.071; 0.611)]. Lastly, 53.8% of the men consumed alcohol often, whereas only 11.7% of the women do so [$p = 3.24\ e^{-5}$, Fisher's exact test; OR = 8.815, 95% CI (3.121; 24.894)].

Multivariate analysis including sex, obesity, absence of neurological disease and benzodiazepine use, use of other psychotropic drugs, and alcohol consumption (**Table 3** presents the final generalized linear model) shows that sex, followed by obesity, was the variable that most significantly associated with an increase in DHI score. Female sex was associated with an increase in DHI by 20.29 points (95% CI 10.27; 30.32) compared to the male sex, whereas being obese increased the DHI score by 8.47 points (95% CI 1.31; 15.629) in comparison with those who were not obese.

Lastly, the relationships between the scores of the three DHI scales (functional, emotional, and physical) and the variables that affected the total DHI score were analyzed. As shown in **Table 4**, the emotional scale was the most strongly affected by the study variables because its relationships were significant with all of them.

DISCUSSION

Symptoms related to aging of the vestibular system (presbyvestibulopathy) significantly limit the quality of life of the elderly due to their associated discomfort, reduced mobility, isolation, and morbidity (1). They are typically associated with aging related alterations in other sensory systems, such as presbyopia or presbycusis, which have been associated with an increased risk of falls, depression, and mortality (18–20). However, in elderly people, presbyvestibulopathy symptoms do not appear in isolation but typically occur in complex clinical and socio-family contexts. They are usually associated with comorbidities, which cause disability. Drugs that affect the central nervous system are frequently used at these ages, and their effect may be aggravated by the intake of toxic substances (such as alcohol). An individual's living environment may also influence the subjective perception of the disability that results from vestibular symptoms. Identifying the key elements that affect this disability, in the usually heterogenous clinical context of these patients, would make it possible to preferentially target them and improve the subjective perception of their own ability, their relationships with their family, and their social environment.

It is first important to realize that the perception of disability is high among patients with presbyvestibulopathy. Although not specifically referring to this condition, previous studies have already reported that elderly patients with dizziness present with decreased quality of life (21, 22). In our study, 82.6% of the individuals showed DHI scores that revealed moderate or severe disability. The limitation caused by presbyvestibulopathy is, therefore, important for the life of these patients, which further underscores the need to identify the factors that condition this disease and to appropriately target them. Moreover, the

TABLE 4 | Relationships between the scores of the three DHI scales (functional, emotional, and physical) and the variables that affected the total DHI score.

Variables	Scales		
	Functional	**Emotional**	**Physical**
Sex	***p* = 3.49 e^{-5}** (Mann–Whitney *U*- test)	***p* = 8.95 e^{-6}** (Student's *t*-test)	***p* = 0.0004** (Mann–Whitney *U*-test)
Number of falls (previous year)	$p = 0.144$, coefficient value = 0.145 (Spearman's Rho correlation)	***p* = 0.004, coefficient value = 0.283** (Spearman's Rho correlation)	***p* = 0.016, coefficient value = 0.237** (Spearman's Rho correlation)
Faller vs. non-faller	$p = 0.519$ (Mann–Whitney *U*-test)	***p* = 0.008** (Student's *t*-test)	$P = 0.304$ (Mann–Whitney *U*-test)
Recurrent faller vs. no recurrent faller	$P = 0.113$ (Student's *t*-test)	***p* = 0.010** (Student's *t*-test)	***p* = 0.044** (Mann–Whitney *U*-test)
TUG (time)	***p* = 8.48 e^{-5} coefficient value = 0.377** (Spearman's Rho correlation)	***p* = 0.005, coefficient value = 0.272** (Spearman's Rho correlation)	***p* = 0.002, coefficient value = 0.300** (Spearman's Rho correlation)
TUG (steps)	***p* = 0.002 coefficient value = 0.296** (Spearman's Rho correlation)	***p* = 0.022, coefficient value = 0.225** (Spearman's Rho correlation)	***p* = 0.045, coefficient value = 0.198** (Spearman's Rho correlation)
Short FES-I score	***p* = 1.12 e^{-12} coefficient value = 0.629** (Spearman's Rho correlation)	***p* = 4.70 e^{-13}, coefficient value = 0.637** (Spearman's Rho correlation)	***p* = 4.86 e^{-9}, coefficient value = 0.537** (Spearman's Rho correlation)
Neurological disease	$P = 0.055$ (Mann–Whitney *U*-test)	***p* = 0.010** (Student's *t*-test)	$p = 0.059$ (Mann–Whitney *U*-test)
Benzodiazepines	$P = 0.072$ (Mann–Whitney *U*-test)	***p* = 0.002** (Mann–Whitney *U*-test)	***p* = 0.032** (Mann–Whitney *U*-test)
Psychotropic drugs	***p* = 0.017** (Student's *t*-test)	***p* = 0.006** (Student's *t*-test)	$p = 0.317$ (Student's *t*-test)
Alcohol consumption	***p* = 0.041** (Student's *t*-test)	***p* = 0.013** (Student's t-test)	$p = 0.753$ (Mann–Whitney *U*-test)

The bold values are statitistically significant.

perception of disability is much higher in women than in men, an effect which is not affected by age. This may be explained by the differences that persist between the sexes in terms of work activities and responsibility for housework. In our society, men carry out their work essentially outside the home and take on less household chores. When they reach 65 years (the age around which retirement typically occurs), their physical activity decreases substantially. Therefore, they perceive a lower disability due to vestibular symptoms. Conversely, retired women usually continue to lead a much more active life (housework and taking care of grandchildren, among other activities). Thus, the same vestibular symptoms may imply a greater disability in their day-to-day life, compared to men. This gender difference in roles has decreased significantly in recent decades, but it is still evident in the elderly population today.

Regarding the factors that are most directly related to vestibular symptoms, falls show the clearest relationship with the perception of disability. The number of falls suffered in the previous 12 months, the fact of having fallen infrequently or, particularly, frequently, and the fear of falling (measured using the short FES-I questionnaire), are all parameters related to a higher DHI score. This relationship between falls and DHI has been previously reported in a group of elderly patients with postural instability (albeit without meeting the presbyvestibulopathy criteria and which had not yet been published at that time) (23). Therefore, all actions aimed at reducing (or avoiding) falls and the fear of falling may lead to a significant decrease in the perception of disability. They will also make it possible to break the vicious cycle of falling-fear and falling-disability-decreased mobility-increased risk of falling. Along the same lines, poor TUG scores (in both time and steps necessary to complete the test) are strongly correlated with DHI score. Therefore, walking difficulties increase the perception of disability and, as such, improving this mobility is essential to increase feelings of security.

However, the dynamic posturography results are unrelated to the perception of disability in patients with presbyvestibulopathy. Regarding this possible relationship, although not specifically referring to elderly patients, discordant results have been published in the literature. Some authors find no relationship between DHI and posturography (24), whereas others report low (25) or moderate (26) correlations. In elderly patients with postural instability (without meeting presbyvestibulopathy criteria, albeit not yet published at the time), a relationship was found between DHI and the scores of a mobile posturographic system (Sway Star), but not between DHI and computerized dynamic posturography (23). This lack of relationship detected in our sample may be due to the fact that, although dynamic posturography is a good method for assessing and quantifying balance, it does not assess stability when walking. Falls in elderly individuals (the key parameter related to DHI score) do not usually occur in static situations but when walking or moving body position, thereby accounting for this lack of correlation between posturography and perception of disability in the elderly with presbyvestibulopathy.

The relationship (or lack thereof) between the DHI score and most comorbidities analyzed in this study stands out. The DHI score is indeed related to obesity, with worse scores in individuals with presbyvestibulopathy and BMI >30. The relationship between obesity and worsening of balance in elderly people with postural instability has already been published (albeit without meeting presbyvestibulopathy diagnostic criteria, which had not yet been defined) (27). Moreover, the existence of another associated vestibular disease (in this case, BPPV), heart disease, dyslipidemia, or diabetes has no effect on the DHI score. Although this questionnaire aims to measure the perception of disability regarding vestibular symptoms, the

accumulation of diseases should heighten the perception of disability, but this does not occur in our sample, perhaps because, when properly treated, these comorbidities do not necessarily imply an increased perception of disability. Surprisingly, the presence of associated neurological diseases (Parkinson's disease and history of ischemic heart disease, among other conditions) were associated with lower DHI scores (when the opposite was expected). This may be because the neurological damage masks presbyvestibulopathy symptoms, which go more unnoticed by the patient. As a result, patients may blame other conditions for the eventual disability.

The association between the use of benzodiazepines and other psychotropic drugs and worse DHI scores was expected. Beyond the underlying disease that determines the use of these drugs (which can increase the perception of disability), central nervous system depressant drugs slow vestibular reflexes, aggravating presbyvestibulopathy symptoms. Therefore, whenever possible, the use of these drugs should be limited in elderly patients with presbyvestibulopathy because they worsen the perception of their functional capacity. The lower scores in patients who consume alcohol may be due to their lower awareness of their limitations considering the effects of alcohol on the central nervous system. Tobacco use had no effect on DHI scores, which is not relevant because almost the entire sample (96%) consisted of non-smokers (likely due to the predominance of women, among whom smoking is less prevalent than among men).

In turn, living in a rural or urban environment had no effect on the perception of disability among these subjects. Considering their greater variety of sensory stimuli that may worsen the symptoms of presbyvestibulopathy, urban settings were expected to heighten the perception of disability, but we did not observe this difference in our sample. In fact, previous studies on elderly patients with postural instability (not necessarily due to presbyvestibulopathy) have also failed to find a relationship between DHI score and living in a rural or urban environment (28). The lack of effect of physical activity must be interpreted with great caution. In total, 95% of our patients maintained an active lifestyle (since our sample was chosen from a population of patients who were candidates for vestibular rehabilitation and who tolerated standing, which was one of the inclusion criteria). It would be interesting to analyze whether the perception of disability influences in any way the level of physical activity of the patients (in this study, we have divided them into active vs. inactive lifestyle, but the different degrees of physical activity have not been analyzed).

Questions regarding the emotional scale showed the highest score in relation to the study variables. Disability is, therefore, a problem more related to a subjective perception of limitation than to an actual physical and/or functional disability. This aspect must be considered in the therapeutic strategies that are designed to reduce this disability because emotional care for these patients could be essential in improving the perception of their own abilities.

The characteristics of our sample involve some limitations that force us to be cautious in generalizing the conclusions. All patients in our series were at least 65 years of age, whereas the diagnostic criteria for presbyvestibulopathy includes individuals 60 years and older. Subjects between 60 and 65 years of age are still of working age, so a relevant factor in their perception of disability may eventually be the effect of vestibular symptoms on their ability to work. In our sample, given the age of the patients, most patients were already retired, so this parameter was not analyzed in this study.

Another limitation refers to the quantification of falls in the previous 12 months. These data were collected by directly asking the patients and may therefore have a memory bias. The patients are likely to remember exactly whether they have fallen or not (fallers vs. non-fallers) and even whether they have fallen more than once (recurrent fallers vs. non-recurrent fallers), but it is more difficult to reliably pinpoint the exact number of falls.

A third limitation refers to the absence of an assessment for an anxiety disorder. It would have been interesting to have done it, especially after having detected the highest scores on the emotional subscale of the DHI. We have used benzodiazepine consumption (which is associated with higher DHI scores) as an indirect way of measuring anxiety, but a specific questionnaire quantifying it had been useful.

Finally, a fourth limitation refers to the absence of a systematic evaluation of hearing and vision in these patients. Most (not 100%) underwent pure tone audiometry; vision assessment was performed in a smaller percentage of patients. This is the reason why these variables, which have been related to the decrease in the quality of life of the elderly, have not been analyzed in this study.

Nonetheless, our results clearly show that most patients with presbyvestibulopathy (at least, those older than 65 years) perceive a moderate-to-severe disability regarding their vestibular symptoms and that the factors most strongly related to this perception are female sex, falls (the fact of having fallen, especially repeatedly, the number of falls, and the fear of falling), and mobility difficulties (measured using the TUG test), together with obesity.

In conclusion, we consider that an adequate weight control in these patients, as well as clinical intervention through vestibular rehabilitation programs for reducing falls (and the fear of suffering them) and improving mobility, will lead to a lower perception of disability and to an improved quality of life in elderly patients with presbyvestibulopathy.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Independent Ethics Committee of Galicia (protocol 2014/411). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AS-V, MR-I, Md-R-V, AF-G, IV-S-A, Md-R-V, AL-D, and SS-P have contributed to the conception and design of this manuscript, revised it critically, approved the final version, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. AS-V has designed the protocol of the study. AF-G, IV-S-A, AL-D, and SS-P have performed the clinical and posturographic examination. Md-RV has collected and analyzed the data. MR-I has developed the statistical analysis. AS-V has written the manuscript. SS-P has revised critically the manuscript. All authors contributed to the article and approved the submitted version.

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Identifying Training, Diagnostic and Therapeutic Needs From a Comparison in the Distribution of Vestibular Disorders in Primary Care and in a Neurotology Unit

Emilio Domínguez-Durán, Carolina Moreno-de-Jesús, Lucía Prieto-Sánchez-de-Puerta, Irene Mármol-Szombathy and Serafín Sánchez-Gómez*

Unidad de Gestión Clínica de Otorrinolaringología, Hospital Universitario Virgen Macarena, Sevilla, Spain

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***Correspondence:**
Emilio Domínguez-Durán
emilio.dominguez.sspa@juntadeandalucia.es

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Introduction: Several epidemiological studies in Neurotology have been previously carried out in the general population. This approach is useful for learning about the most common disorders in clinical population, but it may fail when one is trying to help professionals to guide their training, to optimize their resources and to decide on the highest-priority research objectives.

Objective: To identify which of the neurotological diseases are most common in two different populations, those who attended a consultation in the Neurotology Unit of a tertiary level hospital and those who did so in Primary Care in order to infer which of them requires more attention in each context and their specific needs.

Methods: All the diagnoses made in Hospital Care between October 15, 2017 and October 14, 2018 were reviewed. These diagnoses were coded and classified into syndromes and diseases. Later, the proportions of each category were compared with the proportions of the neurotological diagnoses made in five Primary Care centers over the same period of time.

Results: BPPV is the most common cause of vestibular symptoms in both contexts. Vestibular migraine, ischemic vestibular symptoms, orthostatic hypotension and side effects of drugs are common in Primary Care, whereas Ménière's disease and undifferentiated episodic vestibular syndrome are common in specialized centers.

Conclusion: The proportion of diagnoses in neurotologic patients is different in the general population and in the specialized center population, and therefore they have different needs. Primary Care professionals would benefit from training on maneuvers for repositioning otoliths, the treatment of headache, the identification of cardiovascular risk factors, the orthostatic hypotension and the side effects of the most commonly used drugs. The professionals who work in specialized centers need strategies for dealing with cases of BPPV associated to other vestibular diseases and refractory cases and their research should focus on the development of new

diagnostic tools for the diagnosis of undifferentiated episodic vestibular syndrome and new therapeutic options for Ménière's disease.

Keywords: vestibular diseases, epidemiology, health resources, primary health care, secondary care

INTRODUCTION

In recent years, the amount of literature on the epidemiology of vestibular disorders has grown and several case series on the epidemiology of vestibular disorders have been published. These series have tried to infer how common each neurotologic disease is in their respective populations by using different methods, such as home interviews (1–3), telephone interviews (4–8), postal community questionnaires (9–12), volunteer subjects in Preventive Medicine Centers (13), general practitioners' reports (14–16) or the records of units specialized in vestibular disorders (17, 18) or other medical records (19, 20). However, the challenges of epidemiology research are not limited to finding out how common each pathology is.

One may think that all the different diseases that were detected in previous studies can be treated in the same way; however, when one examines the data more carefully, one realizes that they cannot [e.g., a single episode of benign paroxysmal positional vertigo (BPPV) that is treated early and does not relapse cannot be viewed in the same way as a severe case of Ménière's disease that does not respond well to treatment]. This is because of the amount of resources that each case will need in terms of first clinical visits, diagnostic tests, treatments and follow-up visits, as these will clearly differ. Therefore, when looking at neurotologic entities, it is not only the level of occurrence that matters, but also the impact that this entity has on both the health system and the patient's life. In this regard, while epidemiological studies that were performed in the general population showed the incidence and the prevalence of the neurotological diseases, those performed in specialized centers indicated which diseases need a greater amount of resources.

Taking into account the above, it can be assumed that professionals who work in Neurotology units and those who work in Primary Care face different situations. In relation to Neurotology units, it seems a priority that their research projects deal with diseases that cause the greatest disability in patients and diseases that consume a greater amount of resources, in order to increase the effectiveness of professionals and the development of new therapeutic tools. In relation to Primary Care, professionals should receive training to make them able to diagnose and treat the most frequent diseases and to identify patients with more serious diseases that require care in specialized units.

This study was planned in order to compare the distribution of neurotologic diagnoses between two samples of the same population: those who attended a consultation in the Neurotology Unit of a tertiary level hospital and those who did so in Primary Care. The objective of this study is to identify which of the neurotological diseases are most common in each context and to infer which of them requires more attention.

MATERIALS AND METHODS

Two samples of patients were compared in order to achieve our stated goal. The first sample was made up of a prospective series of all the patients that sought medical assistance for any type of vertigo, instability or dizziness and that belonged to the clusters of inhabitants of five voluntary Primary Care physicians (PCP) from the Public Health System. They attended either Primary Care Centers or Emergency Departments. Their symptoms needed to have started or been present between October 15, 2017 and October 14, 2018. The patients that made up this sample were diagnosed by a multidisciplinary team that included their PCPs, who were responsible for the most common diagnoses, such as BPPV, orthostatic hypotension and the side effects of frequently prescribed drugs, and also neurotologists, who were responsible for diagnosing borderline cases and making all other diagnoses. Before the start of the study, the PCPs received a 1-month training period in the Neurotology unit of the hospital. This training dealt with the most common neurotological disorders, as well as with the use of the HINTS test to diagnose stroke (21). All cases in which the PCPs had diagnostic doubts were referred to the Neurotology unit. This first sample will hereafter be referred to as the "general population." The general population group includes all the patients of this sample, regardless of where their diagnosis was made.

The second sample was recruited retrospectively and included all the patients that visited the corresponding Neurotology Unit of the referring hospital for the patients from the first series over the same period of time. Those that had already been included in the first sample were excluded in order to avoid duplicate patients. This second sample included all the new patients that attended a consultation for the first time or that went for a follow-up consultation. This second sample will hereafter be referred to as the "specialized center population."

Next, the age and gender of all the patients of both populations, and their diagnoses, were recorded. These diagnoses were classified using the layers I (symptoms and signs) and II (syndromes) proposed in the Overview of the International Classification of Vestibular Disorders (22) and, where possible, they were also classified using the layer III-A (disorders and diseases). Patients who did not meet the criteria for any of the disorders or diseases currently defined by the Bárány Society were diagnosed by placing them into other diagnostic categories as appropriate. Not all of the patients received a definitive diagnosis during the study period; the cut-off date for a definitive diagnosis to be made was July 17, 2020.

Later, the samples were compared in order to study the percentages of the following syndromes and diseases: acute vestibular syndromes, BPPV and other positional vertigos, episodic non-positional vestibular syndrome not attributed to ischemia, vestibular syndromes attributed to ischemia,

unsteadiness and chronic vestibular syndrome (CVS), orthostatic hypotension and the side effects of medication, persistent positional-perceptual dizziness (PPPD) and miscellanea, which was made up of patients that could not be included in any of the previous groups. As some patients could have more than one diagnosis, the aforementioned groups were not mutually exclusive.

Finally, each of the aforementioned syndromes and diseases was divided, where possible, into diagnostic categories so that patients could be classified further: patients presenting with acute vestibular syndrome were divided into groups for vestibular neuritis, diseases of the central nervous system or undiagnosed diseases; patients with BPPV were divided into groups for unresolved or probable BPPV, spontaneously resolved and were also classified based on the number of affected canals; patients with episodic non-positional vestibular syndrome not attributed to ischemia were divided into groups for vestibular migraine, Ménière's disease, other less frequent or undiagnosed diseases; patients with vestibular syndromes attributed to ischemia were divided into groups for strokes and transient ischemic accidents (TIA) and, lastly, patients with unsteadiness and CVS were divided into groups for their respective etiologies where possible.

RESULTS

Seven hundred eighty medical records were examined, 176 were included in the general population and 604 were included in the specialized center population. There was no significant between-groups difference in the percentage of women (68.8 and 62.6%, respectively; Fisher's exact test $p = 0.078$). The age of the patients was not normally distributed, and there was a significant difference between the groups (57 and 65 years, respectively; Mann-Whitney U-test $p < 0.001$).

The results obtained from each of the syndromes and diseases studied are detailed below and the percentages are shown in **Table 1**:

a) Acute vestibular syndrome: seven patients in the general population group and 38 patients in the specialized center population. There was no significant difference in the percentage of patients between populations (Fisher's exact test $p = 0.169$). In the general population group, one patient was diagnosed with vestibular neuritis and the remaining patients were diagnosed as having diseases of the central nervous system; in the specialized center population, 27 patients had vestibular neuritis, five had diseases of the central nervous system and six had an acute vestibular syndromes that were not diagnosed. There was a significant difference in the distributions of patients between groups (χ^2 test $p < 0.001$).

b) BPPV and other positional vertigos: 93 patients in the general population and 284 patients in the specialized center population were diagnosed with one of the variants of BPPV accepted by the Bárány Society (23). Sixty-three percentage of the patients in the general population were diagnosed in Primary Care. There was no significant difference in the proportion of patients diagnosed with BPPV in each of the populations studied (Fisher's exact test $p = 0.101$). In the BPPV group, 23.3% were diagnosed with probable BPPV, spontaneously resolved and 13.8% were diagnosed with lithiasis of multiple canals; there was no significant difference in the percentages for these subcategories between populations (Fisher's exact test $p = 0.214$ and $p = 0.537$, respectively). The number of patients presenting with positional vertigo other than BPPV was compared between groups, but no significant difference was found (Fisher's exact test $p = 1$).

c) Episodic non-positional vestibular syndrome not attributed to ischemia: 32 patients in the general population and 237 patients in the specialized center population presented with some kind of episodic, not positional vestibular syndrome other than those caused by ischemia, but the proportion was significantly higher in the latter group (Fisher's exact test $p < 0.001$). When the different subcategories were analyzed, significant differences were found in the proportions of patients diagnosed with "actual" (not probable) vestibular migraine and definite Ménière's disease (Fisher's exact test $p < 0.001$ for both), and also in the proportions of patients for whom no diagnosis could be made (Fisher's exact test $p = 0.03$).

d) Vestibular syndromes attributed to ischemia: 13 patients in the general population and 19 patients in the specialized center population were included in this group, and the difference between these groups was significant (Fisher's exact test $p = 0.014$). Twenty-five percentage of them suffered from a stroke and 75% of them suffered from one or more TIAs. There was no significant difference in the distribution of these two diagnoses between groups (Fisher's exact test $p = 0.413$).

e) Unsteadiness and chronic vestibular syndrome: 24 patients in the general population and 74 patients in the specialized center population attended a consultation because of unsteadiness; there was no statistically significant difference between the proportions in each group (Fisher's exact test $p = 0.354$). The most common cause of the symptoms in this group was cerebral small vessel disease (CSVD). There was no significant difference in the proportion of CSVD between the two groups when all the patients were considered (Fisher's exact text $p = 0.076$) or when only patients with CVS were selected (Fisher's exact test $p = 0.060$). The second most common cause of symptoms in this group was bilateral vestibulopathy, which was significantly more common in the specialized center population (Fisher's exact test $p = 0.012$). Other causes of CVS, such as uncompensated vestibular deficit, acoustic nerve neurinoma or downbeat nystagmus syndrome, were much less common and there was no significant difference in the distributions between groups (Fisher's exact test $p = 0.241$, $p = 0.681$, and $p = 0.237$, respectively). Some syndromes were so uncommon that it was impossible to compare the populations. These included two cases of cervical myelopathy, one case of mesencephalic disease, one case of myopathy and one case of mechanic unsteadiness in the lower limbs in the general population, as well as two cases of space occupying lesions of the brain, one case of idiopathic intracranial hypertension, one case of neuropathy, one case of multisystemic atrophy, one case of Chiari malformation, one case of recurrent fever associated with unsteadiness and one case of mechanic

TABLE 1 | Between-groups comparison of the percentage of different diseases.

Diagnostic syndrome	Subcategories	General population group (176)	Specialized center group (604)
Acute vestibular syndrome		4.0%	6.3%
	Vestibular neuritis*	14.3%	71.1%
	Central nervous system*	85.7%	13.2%
	Undiagnosed	0%	15.8%
BPPV		52.8%	47.0%
	Probable BPPV resolved spontaneously	26.9%	22.2%
	Lithiasis of multiple canals	14.0%	13.7%
Positional vertigo different from BPPV		0.6%	1.2%
Episodic non-positional vestibular syndrome not attributed to ischemia*		18.2%	39.2%
	Vestibular migraine*	71.9%	32.9%
	Probable vestibular migraine	28.1%	13.9%
	Ménière's disease*	0%	31.6%
	Probable Ménière's disease	0%	4.6%
	Other diagnosed causes	0%	5.2%
	Other undiagnosed causes*	0%	11.8%
Vestibular syndrome attributed to ischemia*		7.4%	3.1%
	Stroke	30.8%	21.1%
	Transient ischemic attack	69.2%	78.9%
Unsteadiness and chronic vestibular syndrome		13.6%	12.3%
	Cerebral small vessel disease	70.8%	50.5%
	Bilateral vestibulopathy*	4.2%	27.0%
	Uncompensated unilateral vestibular deficit	4.2%	12.2%
	Acoustic nerve neurinoma	4.2%	4.1%
	Downbeat nystagmus syndrome	0.0%	6.8%
Orthostatic hypotension and side effects of medication*		18.8%	4.1%
	Orthostatic hypotension	78.8%	60.6%
	Side effects of medication	27.3%	48.0%
PPPD		2.8%	6.3%
Miscellanea*		6.8%	3.3%

The total number of individuals in each group is indicated in brackets. The percentage indicated for each of the subcategories is calculated from the total of patients in each syndrome. The pathologies marked with "" were those in which significant between-groups differences were found. The subcategories in the group unsteadiness and CVS and in the group orthostatic hypotension and side-effects of medication were not collectively exhaustive and in the case of unsteadiness and CVS they were not mutually exclusive either.*

unsteadiness in the lower limbs in the specialized center population. In each group there was one case of CVS that could not be diagnosed. The aforementioned diseases are not mutually exclusive, and more than one disease was diagnosed in 13.2% of patients.

f) Orthostatic hypotension and side effects of medication: 33 patients in the general population and 25 patients in the specialized center population were diagnosed with one of these entities, and the proportion of each was significantly higher in the general population (Fisher's exact test $p < 0.001$). 69.7% of the patients in the general population were diagnosed in Primary Care. Orthostatic hypotension was found in 26 patients in the general population group and in 15 in the specialized center population, while side effects of medication were found in 9 and 12 patients respectively. Antihypertensive drugs were the drugs most commonly associated with these diseases (81.2%).

g) Persistent positional-perceptual dizziness: five patients in the general population group and 38 patients in the specialized center population were diagnosed with PPPD; no significant between-groups difference in the proportion of patients was found (Fisher's exact test $p = 0.051$). 30.2% of these patients were considered primary cases, whereas in the remaining 69.8% they were seen as being secondary to another preexisting neurotologic condition (confidence interval 95% 53.7–82.3%). There was no significant difference in the distribution of primary and secondary cases between the populations (Fisher's exact text $p = 0.518$). The triggers of the

30 secondary cases were BPPV (13 cases), vestibular neuritis (four cases), stroke (one case), TIAs (two cases), vestibular migraine (15 cases), Ménière's disease (one case), myelopathy (one case), CSVD (four cases), uncompensated vestibular deficit (one case) or side effects of medicaments (two cases). The previous triggers are not mutually exclusive and therefore their sum is higher than 30.

h) Miscellanea: 32 patients could not be included in any of the previous categories. These included seven patients with presyncopal dizziness, four patients with altered eye movement, three patients with panic attacks, one patient with anemia, one patient with hyperthyroidism and one patient with myasthenia gravis. In five patients, no diagnosis could be made, and nine patients failed to attend their medical appointment. There was a significant difference in the proportion of the miscellanea group between the populations (Fisher's exact test $p = 0.037$) and this was attributed to the different ways in which missing patients were measured in the two populations.

DISCUSSION

Our results indicate that the populations that present otoneurological symptoms in Primary Care and in Hospital Care are different; at least in our health system. Therefore, in our area, the resources allocated to each area must be different too, and the training given to professionals and the most relevant lines of research must also be specific to that healthcare context. Caution should be exercised when extrapolating our results to other health systems because different relationships between PCPs and neurotology specialists could change the results obtained. Furthermore, an analysis of the subcategories within each diagnostic group has led the authors to propose the following hypotheses:

Vestibular Neuritis: Uncommon but Very Noticeable?

Although the percentage of acute vestibular syndromes can be considered to be similar in both groups, it is interesting to note that diseases of the central nervous system were more common in the general population group. Traditionally, it has been argued that the most common cause of acute vestibular syndrome is vestibular neuritis, but this is not corroborated by our results. On the one hand, this could be due to the fact that the previous series came from specialized centers (24) and emergency departments (25) or it could also be due to the fact that magnetic resonance imaging has a low sensitivity when detecting lesions in the posterior fossa (26), thus leading to the underdiagnosis of small strokes. On the other hand, vestibular neuritis may have been less common in the general population because it is accompanied by an abrupt and intense crisis of vertigo which may mean that patients tend to seek medical attention in hospitals, whereas central nervous system vertigo symptoms can be more subtle, and this might be why they are more common in Primary Care centers. In any case, our results suggest that vestibular neuritis is not as common as was once thought. It is also important to note that no diagnosis could be made in a considerable number of cases (15.8%) in the specialized center population. This is similar to data collected by Yebra-González et al. (27), where 39.2% of acute vertigo was of unclear origin.

Two Different Benign Paroxysmal Positional Vertigos

If one considers the data obtained for BPPV, one realizes that it was the most common disorder in both populations, but one may also wonder why the incidence of BPPV was similar in both groups. We attribute this to the fact that there could have been less cases associated to other neurotologic diseases in the general population group and more cases associated to other neurotologic diseases and refractory to treatment cases in the specialized center group. If the proportion of cases non-associated to other neurotologic diseases was similar in both contexts, then the percentage of spontaneously resolved cases should have been more different. This hypothesis was based on the fact that in the general population group the time required to get a consultation with a general practitioner is 1 week, whereas in the specialized center group this can take up to 4 months. Thus, if one considers the data obtained by Álvarez-Morujo et al. in which they estimate that the percentage of patients whose BPPV spontaneously resolves increases with time (28), the percentage of spontaneously resolved cases in the specialized center should have been significantly higher. A further analysis of this data also showed that the percentage of cases of BPPV associated with acute and episodic vestibular syndromes was significantly higher in the specialized center population (20.1 vs. 10.8%, Fisher's exact test $p = 0.026$), thus supporting this hypothesis. Based on our experience and taking into account that the cases of BPPV treated in Primary Care are simpler, PCPs could benefit from a training in otolith repositioning maneuvers. None of the volunteer PCPs who carried out this study performed these maneuvers before their training in the Neurotology unit; After training, they were able to identify the symptoms of the disease more often, treat the simplest cases, and refer doubtful cases.

Ménière's Disease: Still a Needle in the Haystack

Episodic non-positional vestibular syndrome not attributed to ischemia was more common in the specialized center population than in the general population. A closer look at this group showed that this could be due to the presence of patients diagnosed with Ménière's disease in the specialized center population that were not present in the general population group. This phenomenon was described quite a while ago and it was called "the needle in the haystack" (7). Based on a comparison of our groups, we can conclude that professionals who deal with neurotologic patients in Primary Care would benefit from a better knowledge of how to treat patients suffering from vertigo and headache (16), whereas specialized centers should handle the diagnosis and management of patients suffering from Ménière's disease. Headache treatment is a basic competence of the PCP. Even if the PCP is not prepared to make the diagnosis of vestibular migraine, knowing the association between headache and vestibular symptoms can

help improve the patient's symptoms by prescribing analgesic treatments while requesting an evaluation in a specialized unit. It is important to note that a considerable number of patients in the specialized center population could not be diagnosed, and this was due to difficulties when deciding whether a patient was suffering from vestibular migraine or Ménière's disease. Fortunately, new diagnostic tools that will help to differentiate between these two conditions, such a genetic tests or detection of inflammatory citoquines, are being developed so presumably some of these patients will be able to be classified in the near future (29).

Mild Neurotologic Symptoms Due to Ischemia Are Underdiagnosed

Our Neurotology Unit is the reference unit for a population that is 60 times bigger than the general population sampled for this study; however, the number of patients found to be suffering from ischemic vestibular syndromes was not even two times higher in absolute numbers. This may be due to the fact that when a stroke was diagnosed in an Emergency Room, the patient was sent to the stroke unit, instead of the Neurotology Unit. However, making a diagnosis of TIA accompanied by vestibular symptoms is much more difficult and quite a lot of the patients that suffered from them should have been referred to the Neurootology Unit. This seems to support the hypothesis that ischemic vestibular syndromes are underdiagnosed in our population and therefore more training about the relationship between cardiovascular risk and vestibular symptoms is needed in Primary Care and Emergency Rooms. Based on our own experience, it is possible to train PCPs in the use of the HINTS test. Although this test is not easy to perform, we noted that the training increased the awareness of the PCPs in the possibility of an ischemic etiology in many cases. We believe that it is preferable that PCPs refer doubtful cases of HINTS test and these are evaluated by experienced professionals to confirm the diagnosis.

When Is Cerebral Small Vessel Disease a "Incidental" Finding?

The most common cause of CVS symptoms in both populations was CSVD, which was significantly more common than unilateral or bilateral vestibular deficits (Z-test for a comparison of the column proportion with *p*-values adjusted using the Bonferroni method, considering patients suffering from cerebral small vessel disease as the first group, and patients with unilateral or bilateral vestibular deficit as the second group and patients with both as the third group $p < 0.05$). CSVD is diagnosed using magnetic resonance imaging. It is often found in the imaging of asymptomatic subjects and affects almost everyone over the age of 90 (30). Because of this, not all cases of CSVD can be assumed to be the cause of vestibular symptoms and it should only be linked to some cases of gait disturbance or recurrent strokes (30), and in the case of strokes this is only when they affect the central nervous system areas of the vestibular system. Although CSVD was the most common cause of CVS, this does not reduce the importance of the tests that study the function of the peripheral vestibular system, especially considering that vestibular deficits were the second most common cause of symptoms in this group, but it does indicate that it is necessary to evaluate the vestibular system as a whole and not as a sum of separate organs. Sometimes, as we found in both populations, CVS is not the result of just one lesion, but rather the sum of many.

Antihypertensive Drugs and Vestibular Symptoms

When this study was planned, orthostatic hypotension and the side effects of medication were considered as separate conditions. However, after analyzing the results, we discovered that antihypertensive drugs played an important role in both diseases and thus they were grouped together. Orthostatic hypotension and the side effects of medication were statistically more common in the general population group. We attribute this to the fact that these are entities that are easy to recognize; sometimes, the association between drugs and symptoms is reported by the patient themselves. In our study, PCPs were able to differentiate between orthostatic symptoms and BPPV easily, but this fact could be independent from the received training. This may explain they may be less common in the specialized center population.

The Rise of Persistent Positional-Perceptual Dizziness

Finally, we can apply the same reasoning that was used for the ischemic vestibular syndromes to PPPD: if the specialized center serves a population that is 60 times greater than the sample of the general population, a larger absolute number of subjects with this diagnosis should have been found. PPPD should be the second most common diagnosis in specialized centers (31) and this diagnosis is not easy as it is necessary to systematically rule out other diseases whose symptoms are quite similar. PPPD is a term that has been recently described, and because of this professionals need to take this diagnostic option into account more often. However, the diagnosis of PPPD can become a double-edged sword: although it is incredibly useful for the diagnosis of patients whose symptoms could not be explained by reference to other older and better-known diseases, we are afraid that in many patients in which PPPD is a part of the path to recovery this label could complicate the diagnostic process.

CONCLUSION

The proportion of diagnoses in patients with vestibular symptoms is different in the general population and in the specialized center population, and therefore the two contexts have very different diagnostic and therapeutic needs and require specialized training for their staff. BPPV is the most common cause of vestibular symptoms in both contexts, but while Primary Care professionals would benefit from training on maneuvers for repositioning otoliths, those working in specialized centers need strategies for dealing with cases associated to other vestibular diseases and refractory cases. Also, further research and training is required into the treatment of headache and cardiovascular risk factors and the identification of orthostatic hypotension and

the side effects of the most commonly used drugs in patients with vestibular symptoms in Primary Care; whereas specialized centers should focus on the development of new diagnostic tools for the diagnosis of undifferentiated episodic vestibular syndrome and new therapeutic options for Ménière's disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the University Hospitals Virgen Macarena and Virgen del Rocío, Sevilla, Spain. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ED-D and IM-S contributed in the diagnoses of the patients of the series. CM-d-J and LP-S-d-P recorded and analyzed the database for the study. SS-G contributed in the diagnoses of the patients of the series and directed the project research. All authors contributed to the article and approved the submitted version.

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Co-morbidities to Vestibular Impairments—Some Concomitant Disorders in Young and Older Adults

Eva-Maj Malmström[1,2]*, *Eva Ekvall Hansson*[3], *Anna Hafström*[1], *Måns Magnusson*[1] and *Per-Anders Fransson*[1]

[1] Department of Clinical Sciences, Section of Otorhinolaryngology, Lund University, Lund, Sweden, [2] Department of Neurosurgery and Pain Rehabilitation, Skåne University Hospital, Lund, Sweden, [3] Department of Health Sciences, Lund University, Lund, Sweden

Background: Dizziness and pain are common complaints that often appear concomitantly, with or without a causal relationship. However, these symptoms might maintain and exacerbate each other and other co-morbidities. Therefore, adequate rehabilitation may have to include an expanded focus on other deficits and preconditions, especially in older adults and in patients.

Objective: To understand how frequently vestibular dysfunction coincided with medical conditions and aging, we studied two categories: Study 1: patients referred to a vestibular unit and Study 2: senior members in a fitness association.

Method: Study 1: 49 patients [34 females/15 males; mean age 52 years (SEM 2.0)] seeking health care for balance disorders and vestibular deficits were asked in questionnaires about their perception of dizziness and pain, and emotional and functional strains. Study 2: 101 senior members in a fitness association [91 females/10 males; mean age 75 years (SEM 0.6)], were assessed for vestibular and balance deficits and for any co-morbidities. The participants were monitored for falls for 12 months after the initial assessments.

Result: Study 1: Co-morbidity often existed between dizziness and pain (65%). The patients reported high emotional and functional strain related to their dizziness and pain. Patients older than 60 years reported longer durations of pain ($p \leq 0.028$) but less emotional strain ($p = 0.036$), compared to younger patients. Study 2: 84% of the participants had a vestibular impairment, often without noticing any symptoms. Furthermore, 40% reported cardiovascular illnesses, 12% musculoskeletal disorders, and 63% reported other medical conditions. Forty-two percent experienced falls within 1 year after the initial assessments (thereof 42% in the group with vestibular deficits and 38% in the group without vestibular deficits).

Conclusion: To enhance and preserve postural control, both in patients with vestibular deficits and in older adults, we suggest an expanded clinical perspective. Hence, we recommend detailed examinations of the vestibular system but simultaneously probing for possible co-morbidities. Since aging often entails deterioration of multimodal

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Lucus Augusti University Hospital, Spain

***Correspondence:**
Eva-Maj Malmström
eva-maj.malmstrom@med.lu.se

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processes related to maintained mobility and postural stability, our results add focus on the importance of addressing balance disorders together with additional medical conditions.

Keywords: dizziness, musculoskeletal pain, postural control, emotional strain, age

INTRODUCTION

Remaining in equilibrium with adequate adjustments during locomotion and movement requires a constantly ongoing feedforward and feedback postural control system (1). This system uses a redundancy of sensory information from the vestibular organs, from proprioceptors, mechanoreceptors, and vision. These sensory inputs induce executive and adjusting motor responses throughout the entire body, based on the ability to adjust the individual segmental parts and the total body, and thus, maintain stability. The adjustments depend on both the biomechanical prerequisites as well as on well-functioning sensorimotor systems that produce controlled voluntary and automated movements through the body. Joint mobility, muscle strength and postural alignment together with cognitive perception and central processing merge into a function that needs to be able to address predictable and unpredictable requirements for maintaining balance and well-controlled movement (2–4).

The peripheral vestibular sensors can be considered as part of a multifaceted sensorimotor control system with several interacting and interfering entities. However, the normal aging processes cause over time increasingly larger multisensory deficits and multimodal deterioration within the postural control system, which impair the ability to produce accurate postural adjustments (5, 6). Some deficits can be compensated for, while others may remain to exacerbate and enhance dizziness or reduce physical function, and thereby cause loss of balance and in the end, falls (7–13). Moreover, recent reports has highlighted that pain may often be a co-morbidity to vestibular dysfunctions and in patients experiencing dizziness (14, 15).

A fall is an obvious and severe outcome of dizziness or loss of balance (16). The close dependence between musculoskeletal abilities and balance functions are essential in order to allow an active lifestyle with a preserved quality of life (16–18). As a consequence, balance deficits, dizziness, and pain often lead to a decrease in activities of daily living. Reduced activity relate to a frailty syndrome, which might lead to further loss of strength, mobility and physical agility (19). The co-existence of two or more chronic diseases has become more common with increasing age of the population, and hence, the need to further explore co-morbidities to design interventions (20).

These studies aimed to identify occurrences of some co-morbidities related to vestibular impairments and dizziness. More specifically, we wanted to investigate the co-existence of dizziness with other commonly occurring ailments, and especially with musculoskeletal pain. We also wanted to analyze how common vestibular dysfunction and impaired postural control (i.e., fall incidence) is in a population of still physically active older people.

MATERIALS AND METHODS

Ethics Statement

The study conforms to the standards set by Declaration of Helsinki, 2004 and was approved by Regional Ethics Review Board (Study 1: Dnr LU178-07; Study 2: Dnr LU-2016/585), Lund University, Lund, Sweden. All subjects participated voluntary and provided written informed consent before taking part in the study. The data collection used in study 1 is an extraction from an already published article from our research group, the extracted data here categorized and analyzed to determine co-morbidities (15).

Study 1: Co-morbidities in Patients Referred to a Vestibular Unit

Subjects

The study included 49 patients [34 females/15 males; mean age 52.0 years (Standard Error of Mean (SEM)) 2.0], consecutively referred to a tertiary referral center for examination of vestibular deficits and balance dysfunction. To be included the patients had to be adult, conceding to be a part of the study, being able to understand and communicate, and fill out the required questionnaires. The patients thus had a variety of diagnosis with symptoms of dizziness or perceived balance impairment. Study questionnaires were sent to the patients 2 weeks before the visit and collected at the visit. Patients that subsequently failed to return the questionnaire were excluded. The population was divided into three subgroups for comparisons; (1) subjects $\geq$60 years of age ($n = 15$); (2) subjects <60 years of age ($n = 34$), and (3) the youngest 15 subjects in the population ($n = 15$). Results from the total study population has been partly reported, with allowance of extraction for the purpose of the present paper (15).

Test Procedure

The patients answered two questionnaires, the Dizziness Handicap Inventory (DHI) questionnaire and a custom-made questionnaire detailing the properties of pain, dizziness, and occurrence of trauma/accident, considered by the patient to be related to their symptoms.

The Dizziness Handicap Inventory Questionnaire

This questionnaire included 25 questions related to the dizzy symptoms, assessing perceived handicap and impact on quality of life (21–25). In the study, a validated version of the DHI questionnaire in Swedish was used (22). The DHI results can both be presented as "Total" (the sum of all question scores), with a maximum scored interference at 100 (questions answered with a "Yes" —scored 4, "Sometimes" —scored 2, and "No" —scored 0). The DHI questionnaire can also be divided into three symptom domains ("Physical," "Emotional," and "Functional") (21).

TABLE 1 | Pain and dizziness and accident questionnaire.

Question	
1[a]	How long have you experienced dizziness (months)?
2[b]	Dizziness intensity – How severe is the dizziness on average?
3[c]	Do you feel pain or tension in your body?
4	How long have you experienced pain (months)?
5[d]	Accident event – Have you been involved in any accident/trauma you consider caused the pain?
6[b]	Pain intensity – How severe is the pain on average?
7[b]	Pain severity – How severely has the pain affected your activities the last 24 h?
8	Pain distribution – Mark the locations where you experience pain Head ☐, Neck/Shoulder ☐, Back ☐, Arms ☐, Upper torso ☐, Lower torso ☐, Legs ☐, Feet ☐

[a] *Reporting Dizziness = Answering with a value >0 on question 1 or question 2.*
[b] *Graded by Numeric Rating Scale [0 … 10].*
[c] *Reporting Pain = Answering Yes on question 3.*
[d] *Reporting Accident event = Answering Yes on question 5.*

The Pain and Dizziness Questionnaire

This questionnaire comprised eight questions, that queried intensity and duration factors related to dizziness and if present, of a pain (**Table 1**) (15). For symptoms, the patients reported intensity and severity using an 11 point Numeric Rating Scale (NRS) (26, 27) (0 = no symptoms, 10 = most severe symptoms imaginable). If the patients reported pain, questions were also asked about interference on activities ("severity") and locations for their pain (head, including headache; neck/shoulders; back; arms; upper torso; lower torso; legs and feet). If the patients reported pain, they were asked for any potential history of accident/trauma they considered to be related with the experienced pain.

Study 2: Co-morbidities in Physically Active Older People With or Without Vestibular Deficits

Subjects

The participants were senior members of a Swedish fitness association who were invited to a balance workshop. The workshop was repeated at three different times to allow all volunteering subjects to attend. The inclusion criterion was that the subject should be an active participant in a fitness association, and by that indirectly, having an independent and physically active lifestyle. Subjects that failed to return the fall incident dairies during the 12-months observation period were excluded. In total, 150 persons attended the workshops. Out of these, 113 accepted to participate in the study. The 101 participants [91 females/10 males; mean age 75.0 years (SEM 0.6)] who performed all testes and returned at least one fall diary were included in this study. After performing a series of vestibular assessments the population was divided into two categories, subjects with vestibular deficits ($n = 85$) and subjects without vestibular deficits ($n = 16$). The subjects in the two categories had similar mean age (75.1 vs. 74.8 years) and both categories were dominated by females (**Table 5**).

Procedure

Baseline information about age, gender, illnesses, and medication was self-reported. Vestibular function was assessed by a specialist in neuro-otology (author MM) with the headshake test, Dix-Hallpike test (28), and head impulse test (29, 30). The Timed up and Go test (TUG), Timed up and Go test with a cognitive task (TUGcog), and Timed Up and Go test with a manual task (TUGman) (31) were used for measuring balance. The performance of the tasks was assessed by a registered physiotherapist recording the time (author EEH).

Vestibular Tests

Three screening tests of the vestibular function were performed (32). The eye movements were recorded with a Video Nystagmoscope from Synapsis™ during all vestibular tests. The responses were considered pathologic if nystagmus beats occurred (headshake, Dix-Hallpike tests) or a saccade occurred (head impulse test) (33).

Vestibular asymmetry was assessed by the headshake test, where the tested person was lying supine and the examiner shook their head from side to side for 15 s at ∼2 Hz.

Benign paroxysmal positional vertigo for the posterior and anterior canals were assessed by the Dix-Hallpike test. The tested person sat on an examination table, with their head turned about 45° to the left or right. The examiner thereafter moved the patient down into a supine position, with the patient's supported head leaning back about 30 degrees. The test was considered positive if nystagmus and vertigo occurred (28).

For assessing hypofunction in the angular vestibulo-ocular reflex, the head impulse test was used. The tested person was asked to focus the gaze on the examiner's nose, and the examiner rotated the patient's head slowly from side to side. The examiner then turned the head rapidly about 20° to one side. The test was repeated randomly to each side. The test was considered pathologic if the tested person was not able to keep their eyes focused on the target, but responded with a corrective or compensatory saccade (29, 30).

Balance Measures

Balance was assessed by the TUG, TUGcog, and TUGman. In TUG, the tested person was instructed to stand up from a chair, walk in a fast but safely pace three meters and cross a line marked on the floor, turn around, walk back to the chair, and sit down (31). In TUGman, the tested person was instructed to grab a glass filled with water when standing up, walk the three meters as in TUG and put the glass back before sitting down. In TUGcog, the tested person was instructed to count aloud numbers down from 100 with 3-number intervals during the tests (34). The performance of all TUG tasks were rated by the time required to complete the task sequences.

Falls

All participants were given a falls diary booklet at the workshop, in which the participant noted falls during a 12-months period. The participants were instructed to detail in the diary the number of times they had fallen and describe the situational characteristics of the incidents associated with the falls. Every

third month during the 12-months follow-up, a new diary was sent home by ordinary mail, together with a pre-paid envelope, which the participant used for returning the filled-in diary. The falls was categorized according to a classification system from St. Louis, Older Adult Service and Information System (OASIS) (35).

Statistical Analysis

Study 1

Our initial analysis revealed that patients referred to a vestibular unit often displayed symptoms that seemed related to their age above and below 60 years of age. Therefore, to reveal better any age effects, data from the subjects ≥60 years were compared with all subjects below 60 years of age, and a subgroup of the 15 youngest subjects in the population. Moreover, pain in various parts of the body was also very common and the patients reported that their symptoms might be associated with an accident they had experienced (**Table 1**). The roles of these three potential co-morbidities factors were evaluated individually with either Mann-Whitney *U* (two-tailed) analysis or the Pearson's Chi-square test when appropriate (36). In the analyses, p-values <0.05 were considered statistically significant (36). P-values to the level of trends ($p < 0.1$) are presented in the figures and marked with bold text in the tables.

Using the statistical package GPower 3.1™, sample size analyses of parameter categories, such as DHI revealed an effect size of 1.1, which shows that with the *p*-value set to 0.05 (two-tailed), our study would require $n = 14$ subjects to reach a power value of 0.8 for this parameter category (36). The sample size analysis of the pain distribution parameters revealed an effect size of 1.4, which shows that with the *p*-value set to 0.05 (two-tailed), our study would require $n = 10$ subjects to reach a power value of 0.8 for this parameter.

Study 2

The between-groups comparisons were made with either Mann-Whitney *U* (two-tailed) analysis or the Pearson's Chi-square test when appropriate (36).

Non-parametric statistical methods were used since distribution tests with Kolmogorov-Smirnov and Shapiro-Wilk methods revealed that some of the datasets did not have a normal distribution profile and that normal distribution could not be obtained by logarithmic transformation. In the analyses, *p*-values <0.05 were considered statistically significant (36). Bonferroni correction was applied but had no practical effect as all datasets in the statistical between-groups evaluations were included only once in a comparison. P-values to the level of trends ($p < 0.1$) are marked with bold text in the table.

In study 2, measures of step time was also performed, using a wearable device. Step time was used for calculation of power. Expecting 50% more falls among persons with a variance in step time of ≥2.0 ms, and the level of significance set at 0.05, a total of 60 participants would be necessary to reach a power of 80%.

The statistical analyses were performed with SPSS 26.0 software (SPSS Inc., Chicago, IL, USA) and the power analysis was performed with GPower 3.1™.

RESULTS

Study 1: Co-morbidities in Patients Referred to a Vestibular Unit

The patients with balance disorders showed signs of physical, emotional and functional strain, irrespectively of age. In the total DHI score, 19 of 49 subjects (39%) reported severe handicap; 9 of 49 subjects (18%) reported moderate handicap, whereas 14 of 49 subjects (29%) reported mild handicap. A large proportion of the patients, 65.3% (SEM 6.9), presented co-morbidity with pain to the levels that it influenced daily life. Another co-morbidity factor was that 24.5% (SEM 6.2) of the subjects reported that they believed some of the symptoms they experienced were related to a history of an accident.

Evaluation of Differences Between Age Populations

Females were overrepresented in all age categories, but the gender distribution was not significantly different between any of the categories (**Table 2**). The older population tended to score lower in the emotional subscale in the DHI questionnaire than the <60 years category ($p = 0.054$), and significantly lower than the youngest age category ($p = 0.036$), suggesting less emotional strain. Pain symptoms were commonly reported in all populations and this did not differ significantly between the older and younger populations. However, the older population reported significantly longer pain duration than the <60 years category ($p = 0.023$), and the youngest age category ($p = 0.028$). Finally, the dizziness duration tended to be longer in the older population than in the youngest population ($p = 0.063$).

Pain Distribution When Sorted in Different Factor Categories

The population <60 years tended to report pain more often in the upper torso region compared with the older population (**Figure 1A**), though not reaching significant levels ($p = 0.082$). Moreover, the youngest population tended to report more pain in the neck/shoulder region ($p = 0.065$), but significantly less pain in the arms ($p = 0.032$) and also a tendency of less pain in the legs ($p = 0.099$) than the older population.

Evaluation of Differences Between Patients With and Without Pain

The females suffered more often from pain than males ($p = 0.013$) (**Table 3**). The patients with reported pain scored significantly higher in the DHI questionnaire, both in Total ($p = 0.004$) and in the DHI sub-scales DHI_Emotional ($p < 0.001$) and DHI_Functional ($p = 0.011$). A significant number of the patients that reported pain had a history of an accident ($p = 0.004$).

Pain Distribution When Sorted in Different Factor Categories

In the patients suffering from any kind of pain, the most common location for pain were the head (56.3%), the neck/shoulder (87.5%), the back (65.6%), and the legs (43.8%) (**Figure 1B**).

TABLE 2 | Characteristics and questionnaire results for the patients, divided by age.

Material characteristics		Population[a]			Statistics[b]	
		Aged ≥0 years	Aged <60 years	15 youngest subjects	Aged ≥60 years vs. Aged <60 years	Aged ≥60 years vs. 15 youngest subjects
Gender		11f/4m	23f/11m	9f/6m	0.691	0.439
Age (years)		68.5 (1.7)	44.8 (1.5)	36.5 (1.3)	–	–
Dizziness duration (months)		110.1 (31)	52.6 (12.6)	35.4 (9.8)	0.121	**0.063**
Dizziness intensity[c]		5.5 (0.4)	6.3 (0.5)	6.2 (0.7)	0.276	0.315
DHI[d]	Total	37.5 (6.4)	43.9 (3.9)	43.3 (4.4)	0.356	0.383
	Physical	12.8 (1.8)	11.6 (1.3)	10.7 (1.5)	0.609	0.504
	Emotional	11.7 (2.5)	16.9 (1.5)	18.1 (2.1)	**0.054**	**0.036**
	Functional	13.0 (2.6)	15.5 (1.6)	14.5 (2.0)	0.339	0.506
Reporting pain (%)[e]		60.0 (13.1)	67.6 (8.1)	73.3 (11.8)	0.604	0.439
Pain duration (months)		175.2 (21)	68.7 (13)	41.6 (13.5)	**0.023**	**0.028**
Pain intensity[c,e]		5.8 (0.5)	5.3 (0.4)	4.1 (0.6)	0.604	0.114
Pain severity[c,e]		5.3 (0.7)	6.2 (0.5)	5.4 (0.8)	0.631	0.795
Reporting accident (%)		20.0 (10.7)	26.5 (7.7)	26.7 (11.8)	0.627	0.666

[a] *The values are presented as mean and SEM-values, the latter presented within the parenthesis.*
[b] *The notation "<0.001" means that the p-value is smaller than 0.001.*
[c] *Scaled as 0 = no symptoms and 10 = most severe symptoms imaginable.*
[d] *DHI represent the values obtained from the Dizziness Handicap Inventory questionnaire.*
[e] *Pain represent whether the patients reported pain anywhere at the eight locations detailed.*

Evaluation of Differences Between Patients With and Without a History of an Accident

Females tended to more often have experienced an accident than males ($p = 0.054$) (**Table 4**). The patients who reported a history of an accident scored not significantly different than the non-accident patients in the DHI questionnaire. However, the patients in the accident group suffered significantly more often from pain than the patients in the no accident group ($p = 0.004$).

Pain Distribution When Sorted in Different Factor Categories

The patients reporting that they had an accident in their history had significantly more often pain in the head ($p = 0.013$) and neck/shoulder ($p < 0.001$) and tended to have more often pain in the back ($p = 0.055$) compared with patients that had no history of an accident (**Figure 1C**).

Study 2: Co-morbidities in Physically Active Older People With or Without Vestibular Deficits

Clinical Findings

After excluding participants with incomplete baseline measurements and participants who did not return a complete set of fall-diaries, a total number of 101 participants were included in the statistical analysis. In the population category that had vestibular deficits ($n = 85$), most of the subjects had pathological headshake nystagmus (90.6%) and pathological head impulse responses (46.4%) (**Table 5**). A smaller group also had pathological Dix-Hallpike responses (22.4%). Note that subjects can present pathological responses in several of the vestibular tests.

Even if many of participants reported that they had no illnesses, many of them used some kind of medication. Therefore, the categorization of illnesses was based on both reported illness and reported medication. In the total population, 40% reported cardiovascular illnesses, 12% musculoskeletal disorders, and 63% reported other types of illnesses that affected daily life. The frequencies of illnesses were very similar in both categories of vestibular deficits and without vestibular deficits. Cardiovascular illnesses were common in both categories, 38.8 vs. 43.8%. Severe musculoskeletal disorders and other illnesses were also common in both categories (10.6 vs. 18.8%) resp. (28.2 vs. 12.5%). Some subjects in both categories reported more than one illness (14.1 vs. 18.8%).

The mean values and standard error of mean for age, the TUG, TUGman, and TUGcog tests and the results for health status and vestibular tests are shown in **Table 5**. The age, gender, health status, and stability as reflected by results in the functional tests were not significantly different between subjects with vestibular deficits and subjects without deficits. During the 12-months follow up, 42 participants reported one or more falls, and a total number of 58 falls. The majority of falls was categorized as extrinsic falls (77%), caused by perturbed stance, perturbed gait, or external cause of loss of balance. Almost one fifth of the falls was categorized as intrinsic falls (19%), caused by vertigo, legs giving away, or loss of postural control. A few falls was not classifiable (4%). There was no difference in age, cardiovascular illnesses, musculoskeletal disorders, or vestibular status between fallers and non-fallers.

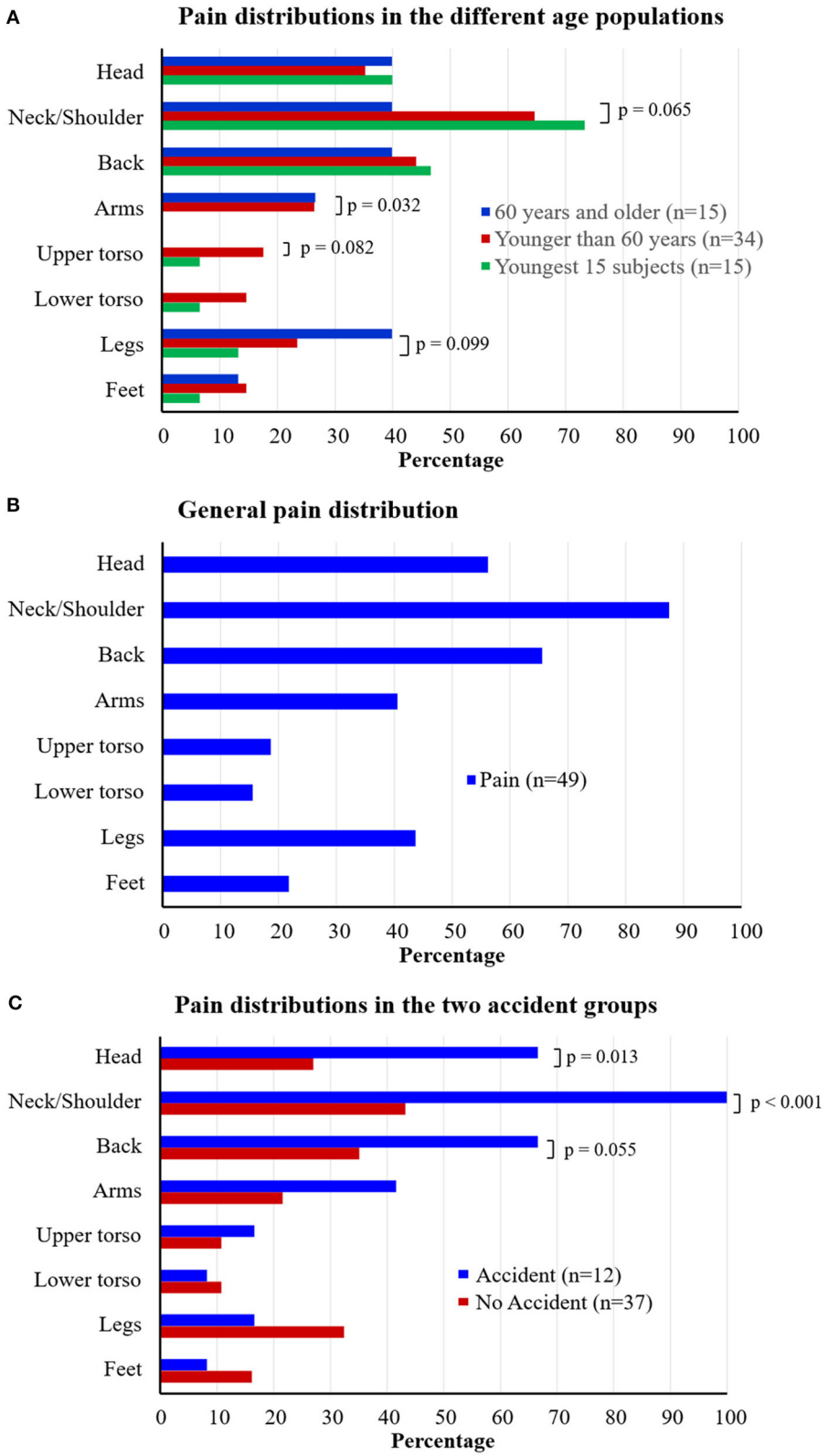

FIGURE 1 | Pain distribution patterns for: **(A)** each age population; **(B)** among all patients reporting pain; **(C)** the patients reporting a history of an accident and not having a history of an accident. The values on the x-axis denotes the percentage of the patients reporting pain with the pain localization on the y-axis. Note that a patient can report pain in more than one location. *P*-values to the level of trend <0.1 are detailed.

TABLE 3 | Characteristics and questionnaire results for the patients, divided by pain symptoms.

Material characteristics		Symptoms[a]		Statistics
		Pain[b]	No pain[b]	*p*-Values[c]
Gender		26f/6m	8f/9m	**0.013**
Age (years)		50.7 (2.5)	54.5 (3.2)	0.488
Dizziness duration (months)		86.3 (19)	34.3 (9)	0.146
Dizziness intensity[d]		6.3 (0.4)	5.5 (0.7)	0.280
DHI[e]	Total	48.7 (3.9)	29.3 (5.0)	**0.004**
	Physical	12.9 (1.3)	10.1 (1.5)	0.192
	Emotional	18.5 (1.5)	9.2 (1.9)	**<0.001**
	Functional	17.2 (1.6)	10.0 (2.2)	**0.011**
Pain duration (months)		95.4 (16)		
Pain intensity[b,d]		5.4 (0.4)	–	–
Pain severity[b,d]		5.9 (0.5)	–	–
Reporting accident (%)		37.5 (8.7)	0.0 (0.0)	**0.004**

[a] *The values are presented as mean and SEM-values, the latter presented within the parenthesis.*
[b] *Pain represent whether the patients reported pain anywhere at the eight locations detailed.*
[c] *The notation "<0.001" means that the p-value is smaller than 0.001.*
[d] *Scaled as 0 = no symptoms and 10 = most severe symptoms imaginable.*
[e] *DHI represent the values obtained from the Dizziness Handicap Inventory questionnaire.*

TABLE 4 | Characteristics and questionnaire results for the two accident groups.

Material characteristics		Symptoms[a]		Statistics
		Accident	No accident	*p*-Values[b]
Gender		11f/1m	23f/14m	**0.054**
Age (years)		50.1 (3.6)	52.6 (2.4)	0.658
Dizziness duration (months)		83.3 (23)	65.1 (16)	0.179
Dizziness intensity[c]		5.8 (0.8)	6.1 (0.4)	0.736
DHI[d]	Total	48.8 (6.6)	39.7 (3.8)	0.285
	Physical	12.2 (2.4)	11.9 (1.1)	0.916
	Emotional	18.3 (2.5)	14.3 (1.6)	0.217
	Functional	18.4 (2.6)	13.5 (1.6)	0.124
Reporting pain (%)[e]		100.0 (0.0)	54.1 (8.3)	**0.004**
Pain duration (months)		110.1 (27)	83.3 (14)	0.447
Pain intensity[c,e]		5.3 (0.6)	5.5 (0.4)	0.823
Pain severity[c,e]		6.0 (0.8)	5.9 (0.4)	0.951

[a] *The values are presented as mean and SEM-values, the latter presented within the parenthesis.*
[b] *The notation "<0.001" means that the p-value is smaller than 0.001.*
[c] *Scaled as 0 = no symptoms and 10 = most severe symptoms imaginable.*
[d] *DHI represent the values obtained from the Dizziness Handicap Inventory questionnaire.*
[e] *Pain represent whether the patients reported pain anywhere at the eight locations detailed.*

DISCUSSION

We found that patients with dizziness also commonly report musculoskeletal pain and that co-morbidities between dizziness and pain add psychological distress. In addition, older subjects, healthy enough to participate in physical activities, frequently had asymptomatic vestibular deficits. Almost half of these physically active older people experienced one or more fall incidence during the following year.

Co-morbidity Between Dizziness and Pain

Although both dizziness and pain are common in a general population, the concurrent appearance of these two symptoms might suggest an interrelationship as well as an additional burden for the individual. The patients with pain demonstrated significantly higher levels of perceived handicap of their dizziness, and thus, that this had consequences on their perceived quality of life compared to patients that did not report pain. Older patients stated significantly longer pain duration and they reported more pain in their lower extremities compared to the younger population, who had more pain in the neck/shoulder and torso regions (**Figure 1**).

In patients that reported pain, 38% also reported a history of an accident the patient themselves thought was the origin of the pain. These patients had significantly more often issues with pain in the head and neck/shoulder regions and reported more often back pain (**Figure 1C**). Pain in these segments has been shown to affect the sensorimotor control (37). Moreover, especially after neck trauma, pain in these specific segments are known to be associated with dizziness (38). The intersegmental coordination between different parts of the body changes with age and disturbances of the cervical proprioceptive information especially seems to disturb and change postural strategies (6). This emphasizes the importance of reducing pain and disability from the different body regions in order to make intersegmental coordination as optimal as possible, also in dizzy patients.

The high frequency of reported pain conditions in lower extremities, the neck and the shoulder regions in this study group of dizzy patients, highlight the importance of asking patients about any pain conditions and history of trauma.

Age-Related Multimodal Decline in Sensorimotor Systems and Comorbidities

Since aging often entails deterioration of multisensory and multimodal processes related to maintained mobility and postural stability, our results add focus on the importance of paying attention and taking direct actions when balance disorders occur together with pain and other medical conditions. Especially when dizziness occurs together with pain in the neck/shoulder regions and in the lower extremities, the symptoms might interact and maintain or even enhance each other. To some extent, the balance deficits may be compensated for, but the adjustment options might be fewer due to pain related impairments. Thus, the functional loss may become more prominent, with reduced physical function and thereby also a loss of postural stability (7–13).

Pain in the lower extremities is often related to reduced muscle strength, which *per se* is regarded as one important factor for preserved postural stability (7). In addition, mobility

TABLE 5 | Characteristics for the two vestibular groups.

Material characteristics		Symptoms[a]		Statistics
		Vestibular pathologies	No vestibular pathologies	*p*-Values
Gender		78f/7m	13f/3m	0.196
Age (years)		75.1 (0.6)	74.8 (1.6)	0.841
Medical conditions (%)		67.1 (5.1)	62.5 (12.5)	0.723
Cardiovascular illness (%)		38.8 (5.3)	43.8 (12.8)	0.712
Musculoskeletal disorder (%)		10.6 (3.4)	18.8 (10.l)	0.355
Other illnesses (%)		28.2 (4.9)	12.5 (8.5)	0.187
More than one illness (%)		14.1 (4.2)	18.8 (10.1)	0.687
Detection methods	Headshake[b] (%)	90.6 (3.2)	–	–
	Dix-Hallpike[b] (%)	22.4 (4.5)	–	–
	Head impulse[b] (%)	46.4 (5.4)	–	–
Balance	TUG[c] (s)	8.5 (0.2)	8.3 (0.3)	0.696
	TUGman[d] (s)	9.4 (0.2)	9.2 (0.4)	0.900
	TUGcog[e] (s)	10.2 (0.2)	10.2 (0.3)	0.820
	Difference TUGman-TUG (s)	1.0 (0.2)	0.9 (0.3)	0.659
	Difference TUGcog-TUG (s)	1.8 (0.1)	1.9 (0.3)	0.602
Reporting falls (%)		42.4 (5.4)	37.5 (12.5)	0.718
Number of falls classified as extrinsic/intrinsic		30/9	15/2	0.230

[a] *The values are presented as mean and SEM-values, the latter presented within the parenthesis.*
[b] *The subjects can present pathological responses simultaneously in several of the vestibular tests made.*
[c] *TUG, Timed up and Go test.*
[d] *TUGman, Timed up and Go test with a manual task.*
[e] *TUGcog, Timed up and Go test with a cognitive task.*

and proprioception that also might be affected by pain are of uttermost importance to retain postural stability during upright stance and locomotion (9, 33, 39). Here, the ankle mobility, the mechanoreceptors of the feet, and also the proprioceptors of the intertarsal muscles is of special interest, keeping us stable with adequate reactive response relative to the surface (9). Because proprioceptive information often is also decreased in older people without pain, a reduction of these sensory inputs might become even clearer in situations that demand postural stability (40). A rigid strategy caused by either pain or dizziness, or both simultaneously, might also affect intersegmental mobility and coordination. Here, older people already seem to have a different coordination strategy (6). Older people also seem to have a different postural strategy compared to younger, including less effective adaptive processes to address stability issues (10). Especially in the frontal plane, older people seem to adapt to postural disturbances in a less appropriate way than younger subjects (41), a factor that can be negatively additive to an already present pain condition in the lower extremities. With the clear importance of adequate proprioceptive input, muscular strength and coordination and good mobility, for appropriate postural responses and thereby maintained postural stability, postural control is considerably and indisputably multisensory, multifactorial and multimodal. Therefore, pain conditions and biomechanical prerequisites should be considered and examined in parallel with proper vestibular examinations when balance problems are being addressed and treated, irrespectively of age.

Psychological Distress of Co-morbidity Between Dizziness and Pain

The patients reporting both dizziness and pain showed significantly more psychological distress, demonstrated by higher scoring values on questions associated with emotional status in the DHI questionnaire (24). While high psychological distress was reported for the patients with pain, compared to younger subjects the older subjects reported somewhat less impact on the emotional domain (**Table 2**). This is consistent with results showing a negative correlation for emotional distress with age suggesting possibilities for adaptation of daily activities and thereby an increased sense of control in older, retired people (42). Still, older subjects reported high scores in the DHI questionnaire, suggesting a considerable emotional load anyway and interference of daily activities. Hence, the emotional factor is a substantial handicap to consider also among older adults (23, 43, 44).

Physically Active Older Adults With Vestibular Deficits and Fall Incidents

The majority (84%) of the older subjects active in a fitness association demonstrated vestibular asymmetry or other balance function disturbances. Most of them were not aware of such dysfunctions. That said, the study participants all had responded to an invitation to take part in a workshop about balance, which might suggest that at least some of them had a specific

interest in such a topic. Thus, one may speculate that this interest might be instigated by them having experienced instability and balance disturbance. If that was the case, that would explain the proportionally very high amounts of vestibular asymmetry in the studied group compared to other populations (5, 8, 45, 46). On the other hand, the participants considered themselves healthy, were active in a fitness association, and performed well in the TUG tests. This speculative view stands in contrast to that almost half of the whole group had sustained falls at the 1-year follow-up. However, a large majority of these falls (77%) were by the participants regarded to be caused by extrinsic factors. Furthermore, the subjects with identified vestibular pathologies did not fall more often compared to those without such disorders.

More than half of the group reported impairment related to other medical conditions, which highlight the importance to evaluate the person's entire state of health regarding their ability to maintain postural control. Vestibular dysfunctions are common in the older population and together with physiological changes and multi-morbidity it implies a risk of hazardous events, such as falls (12). Kristinsdottir et al. have demonstrated that vestibular asymmetries could contribute to falls and thereby even fractures in older people (47). Our results demonstrate that falls among older subjects are so common that specific balance training programs could be suitable for all, especially since such programs have shown to affect vestibular asymmetry positively (46). In addition, more than half the group had other declared illnesses, such as diabetes and high blood pressure, which also may be suspected to affect balance control and requiring specific attention of the primary health care. Low blood pressure is also a common issue among older people, but none of the participants in our studies reported taking any medications that could be related to treatment of low blood pressure. Hence, addressing balance problems to older persons visiting primary health care and providing specific balance programs, including vestibular rehabilitation, might decrease the risk of falls and should be offered not only to persons with severe balance problems.

Limitations

Both sub-studies were explorative, carried out independently and performed in two different contexts. However, they both addressed co-morbidities to vestibular impairments and dizziness.

Both study 1 and study 2 included subjective self-reported data. Thus, in study 1 questionnaire answers and reports of pain might be skewed by, e.g., subjective exaggeration of symptom severity. However, a recently published study with similar approach and scope as in study 1 largely confirm our findings (14). Study 2 included physically active subjects. Even if they suffered from conditions linked to aging or chronic diseases, most did not perceive themselves as being impaired. This may reflect a mental state of habituation and thus, there is a risk that co-morbidities were under-reported in study 2. Therefore, subjects were asked about medications. If medications suggested a chronic disease, such co-morbidity was considered present even if the participant did not report it initially.

In study 2, the commonly used and simple to perform TUG test was utilized to make a primary assessment of postural control, as some studies suggests that this method can determine fallers from non-fallers (48, 49). An optimal cut-off value for TUG in predicting future falls among community-dwelling older persons has been suggested to be 12.6 s (48). However, this value is much higher than we recorded in both participants with and without vestibular deficits in our study. Thus, TUG does not seem to be optimal for predicting falls among older persons who considered themselves as healthy and that regularly are physically active, in our case as part of a membership in a fitness association. This difficulty to predict falls with tests has lately been demonstrated by Bobowik et al. (49). A possible explanation to why the falls still were so common in the population category might be that the falls often (77%) were deemed caused by extrinsic factors like tripping and slipping, i.e., when the subject is exposed to external demands for which there are not sufficient resources. The aims and scope of study 2 was limited to determining if vestibular deficits and a limited set of various co-morbidities were common in a physically active cohort of older people subjects, and if so, if these factors had a substantial effect on their postural control. One reason for this approach was to determine if specific vestibular deficits were over-represented among fallers. This turned out not to be the case. Thus, falls can be caused by a number of factors, and it would be interesting if these factors could be detailed in future studies, for example, by using more advanced methods to assess postural control and by screening the study participants for more kinds of relevant medical conditions.

Clinical Implications

The findings highlight some of the most important factors for our ability to be active and in good health at old age. A good body balance, good postural alignment, and preserved muscle strength are considered to be protective factors for maintain an active lifestyle in aging. These factors also coincide with good quality of life (17). Since most stability issues originating from vestibular and other sensorimotor deficits can be addressed, or at least ameliorated, it is critical that they are detected by the health care system (46, 50). The interventions to enhance and maintain postural control during aging should preferably be multimodal, able to detect and address co-morbidities, such as pain and mental strain, and performed in a structured but still adjustable, customized way (51). Both rehabilitation and training in general for older people have to meet the impairments that are common in dizzy patients and in older people in general.

In summary, based on the clinical implications of our results, together with previously acquired knowledge, we recommend health care practitioners to always ask for, and consider the co-morbidities that often coincide with dizziness. Pain should be considered a common co-morbidity, of importance to address both for optimal vestibular rehabilitation and for training postural control. Having a history of an accident/trauma may also be a contributing factor to dizziness, pain, and sensorimotor deficits. When performing rehabilitation, the exercises should promote a holistic approach of maintaining healthy muscular and biomechanical function in all intersegmental levels, as well as to facilitate optimum use of integrated sensory information from all sensory sources, i.e., vision, vestibular, mechanoreceptors,

and proprioceptors. Older adults may be more prone to suffer from vestibular impairment, and should be diagnosed and rehabilitated accordingly.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Ethics Review Board (Study 1: Dnr LU178-07; Study 2: Dnr LU-2016/585), Lund University, Lund, Sweden. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

E-MM, EE, MM, and P-AF conceived the study design. E-MM, EE, and MM executed the data collection procedures. E-MM, EE, MM, AH, and P-AF analyzed and interpreted the results and contributed to the manuscript.

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REVIEW
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Genetics and the Individualized Therapy of Vestibular Disorders

*Christine Mei[1], Hongsong Dong[1,2], Eric Nisenbaum[1], Torin Thielhelm[1], Aida Nourbakhsh[1], Denise Yan[1], Molly Smeal[1], Yesha Lundberg[3], Michael E. Hoffer[1], Simon Angeli[1], Fred Telischi[1], Guohui Nie[2], Susan H. Blanton[1] and Xuezhong Liu[1]**

[1] Department of Otolaryngology, University of Miami, Coral Gables, FL, United States, [2] Shenzhen Second People's Hospital, Shenzhen, China, [3] Department of Otolaryngology, Boys Town National Research Hospital, Omaha, NE, United States

Background: Vestibular disorders (VDs) are a clinically divergent group of conditions that stem from pathology at the level of the inner ear, vestibulocochlear nerve, or central vestibular pathway. No etiology can be identified in the majority of patients with VDs. Relatively few families have been reported with VD, and so far, no causative genes have been identified despite the fact that more than 100 genes have been identified for inherited hearing loss. Inherited VDs, similar to deafness, are genetically heterogeneous and follow Mendelian inheritance patterns with all modes of transmission, as well as multifactorial inheritance. With advances in genetic sequencing, evidence of familial clustering in VD has begun to highlight the genetic causes of these disorders, potentially opening up new avenues of treatment, particularly in Meniere's disease and disorders with comorbid hearing loss, such as Usher syndrome. In this review, we aim to present recent findings on the genetics of VDs, review the role of genetic sequencing tools, and explore the potential for individualized medicine in the treatment of these disorders.

Methods: A search of the PubMed database was performed for English language studies relevant to the genetic basis of and therapies for vestibular disorders, using search terms including but not limited to: "genetics," "genomics," "vestibular disorders," "hearing loss with vestibular dysfunction," "individualized medicine," "genome-wide association studies," "precision medicine," and "Meniere's syndrome."

Results: Increasing numbers of studies on vestibular disorder genetics have been published in recent years. Next-generation sequencing and new genetic tools are being utilized to unearth the significance of the genomic findings in terms of understanding disease etiology and clinical utility, with growing research interest being shown for individualized gene therapy for some disorders.

Conclusions: The genetic knowledge base for vestibular disorders is still in its infancy. Identifying the genetic causes of balance problems is imperative in our understanding of the biology of normal function of the vestibule and the disease etiology and process. There is an increasing effort to use new and efficient genetic sequencing tools to discover the genetic causes for these diseases, leading to the hope for precise and personalized treatment for these patients.

Keywords: genetics, vestibular disorder, individualized therapy, genomics, Meniere disease, usher, BPV, next generation sequencing

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Maurizio Barbara,
Sapienza University of Rome, Italy

***Correspondence:**
Xuezhong Liu
x.liu1@med.miami.edu

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INTRODUCTION

Vestibular disorders (VDs) are a heterogeneous group of conditions that stem from dysfunction of the inner ear, vestibulocochlear nerve, or central vestibular pathways. Patients with VDs typically present with vertigo, although other symptoms, including dizziness, unsteadiness, and oscillopsia, are often present as well. Benign inner ear conditions, including benign positional vertigo, Meniere's disease, and vestibular neuritis, are the common causes of vertigo. Other causes of isolated vestibular dysfunction include benign paroxysmal vertigo of childhood, bilateral vestibulopathy, and motion sickness. Due to similar developmental mechanisms between vestibular structures and the human hearing apparatus, some genetic hearing loss syndromes, such as Usher syndrome, also present with vestibular symptoms. Structural malformations of the inner ear, including enlarged vestibular aqueduct syndrome and superior canal dehiscence syndrome, are also causes of hearing and vestibular deficits (1). Vestibular migraine (VM), vestibular dysfunction occurring within the context of migraine headaches, is now also a well-established cause of vertigo (2).

There is growing evidence that genetics play a role in at least a subset of VDs (3). Multiple familial clusters with a range of vestibular symptoms have been described in the literature, and epidemiological studies have shown familial aggregation and higher prevalence of vestibular syndromes in some ethnic groups (4). However, the specifics of how genetic variations and familial clustering or aggregation may contribute to the development of specific VDs are largely unknown. This is due in part to limitations in clinical phenotyping, similarities in presentation of different VDs, and variable penetrance resulting in clinical heterogeneity (5).

Recently, advances in the field of genetic sequencing have increased our understanding about the pathophysiology of VDs. Genetic tools that have been used in genome-wide association studies in VDs include whole exome sequencing (WES) and whole genome sequencing (WGS). By using WES and WGS in combination with bioinformatic tools, novel VD gene variants have been identified, allowing for finer genetic differentiation between phenotypically similar conditions. As research into this area increases, it is important to realize the goals of this knowledge. As exemplified in the ever-evolving field of treatment for sensorineural hearing loss, genetic knowledge generated with these tools can lead to exciting and effective individualized gene therapies. In this review, we will examine new evidence of genetic contributions of VDs, use of genetic tools in the discovery of causal genes and individualized treatments, and future directions in VD treatment with a particular focus on the development of precision medicine (PM) platforms.

FAMILIAL MENIERE'S DISEASE

Meniere's disease (MD) is a complex chronic inner ear disease; symptoms include episodic vertigo, fluctuating low-to-middle frequency SNHL, tinnitus, and aural fullness. The clinical course of MD is variable, but hearing loss is typically progressive. In 25–40% cases, hearing loss occurs bilaterally, though there is disagreement as to whether this represents a subtype of MD or a separate disorder (6). Per AAO-HNS criteria, a diagnosis of "definite MD" requires "2 or more episodes of vertigo lasting 20 min to 12 h, low-to-middle frequency hearing loss in one ear, fluctuating aural symptoms, and no other VD that better explains the symptoms" (7). More recently, [Lopez-Escamez et al. (6)] further divided MD into 5 clinical subtypes to better capture its clinical heterogeneity. Type 3, representing 13% of cases, is Familial MD (FMD), defined as patients meeting MD criteria with 2 or more 1st or 2nd degree relatives who also meet criteria (6).

FMD inheritance patterns have been widely studied, with most studies finding an autosomal dominant inheritance pattern; however, recessive and mitochondrial inheritance has been observed as well (8). This may indicate the existence of several different underlying genotypes that result in phenotypes meeting the clinical criteria of FMD. Several studies have published information on new genes associated with MD. Pathogenic variants in FAM136 and DTNA—both of which were found to code for proteins expressed in the vestibular neuroepithelium in adult rat models—were detected via WES in a single family with MD (9). In particular, a-Dystrobrevin—the protein product of DTNA—is associated with the dystrophin complex of proteins that is thought to play a role in cytoskeleton structure for cochlear hair cells, providing a potential mechanism for the SNHL seen in this family (9). Another family with multigenerational MD was found to have mutations in the PRKCB gene, which codes for a protein kinase and is expressed in both the adult human cochlea and semicircular canals (10). Notably, expression was greater in the tectal cells of the apical turn of the cochlea compared to the middle or basal turn, which may explain why SNHL tends to occur in lower frequencies in MD patients. Another study found pathogenic changes in DPT in one MD family, and in SEMA3D in another. Respectively coding for the extracellular matrix protein dermatopontin and the axonal guiding protein semaphoring-3D, both genes were found to be strongly expressed in the cochlea and the semicircular canals (11). Additionally, a family with a mutation in COCH, a gene related to the hearing loss disorder DFNA9, was found to have an MD-like phenotype with asymmetric SNHL, unilateral aural symptoms, and episodic vertigo (12). Recently, a rare variant analysis of FMD cases revealed the presence of missense variants in the *OTOG* gene (rs552304627) in ∼1/3 of included families. This finding indicates that *OTOG* may represent a key gene in future genetic testing for FMD (13).

While the majority of MD cases are not familial, there is growing evidence of specific genotype-phenotype relationships underpinning the clinical presentation of sporadic MD. Mutations in MICA, TLR10, and NFKB1, all of which are associated with the immune system, have been associated with differences in the occurrence and rate of progression of SNHL in sporadic MD patients (14–16). Another recent study found that sporadic MD patients had enrichment of missense mutations in a variety of genes associated with SNHL including GJB2 (connexin 26 deafness), SLC26A4 (Pendred syndrome), and USH1G (Usher 1C) compared to controls, which the authors postulate may have an additive effect on the MD phenotype (17, 18).

Vestibular Migraine

VM is a headache disorder with a prevalence of 1–3% that is characterized by episodes of migraine with vertigo (19). There is some evidence to suggest a heritable component to VM. A recent systematic review of VM prevalence studies indicated a moderate familial aggregation, with siblings of affected individuals at a four to 10 times greater risk than the general population (20). Symptoms of MD may also overlap with those of VM, making it difficult in some cases to distinguish between them clinically. Flook et al. (21) studied peripheral blood samples of patients with diagnosed MD and VM using WGS and cytokine panels and showed significant differences in gene expression and cytokine/chemokine profiles between the two patient populations. Further, two subgroups of MD were found, one with high IL-1β (MDH) and one with low IL-1β (MDL), supporting the theory that MD is a complex disorder with multiple endophenotypes. The authors proposed that a small cytokine assay of IL-1β, CCL3, CCL22, and CXCL1 levels would differentiate between VM and MD patients and may be used when a clinical diagnosis is unable to be made (21). MD as currently defined is a purely clinical diagnosis, yet is clinically heterogeneous, overlaps with other VDs, and has poorly understood pathophysiology. This state of affairs presents great challenges for both diagnosis and the development of effective treatments. The genetic discoveries summarized above can serve as a roadmap to more accurate, genotype-based classification of MD, and subsequently to better patient counseling and the development of new PM-based treatment approaches (22–24).

HEREDITARY SENSORINEURAL HEARING LOSS WITH VESTIBULAR DYSFUNCTION

In addition to the isolated vestibulopathies, dizziness, and episodic vertigo is found in combination with moderate or severe hearing loss in a number of cochleovestibular disorders. Frequently, these disorders result from mutations in genes related to development of the otic capsule and temporal bone (5). These hereditary hearing disorders can be grouped by mode of inheritance into autosomal dominant, autosomal recessive, X-linked, or mitochondrial. Monogenic sensorineural hearing loss (SNHL) disorders that also present with vestibular dysfunction include DFNA9, DFNA11, DFNA15, and familial Meniere's disease. The mutations underlying these disorders are of interest to both hearing loss and vestibular disorder researchers and may benefit from precision medicine initiatives in both fields.

DFNA

DFNA9 is an uncommon, delayed-onset disorder caused by heterozygous mutations in the COCH (coagulation factor C homology) gene (25). This disorder is primarily characterized by progressive high-frequency SNHL and can include vestibular dysfunction (gait imbalance, oscillopsia), ranging from bilateral vestibular loss to acute attacks resembling MD (12). Researchers have discovered 14 mutations in the COCH gene by studying unrelated families with DFNA9 (26). DFNA11 patients show a delayed-onset low-to-middle frequency hearing loss with a range of possible vestibular dysfunction. Seven mutations in the MYO7A gene have characterized as causes of DFNA11 (27). Myosin VIIA is a protein linked to hair bundle formation and mechanotransduction, and mutations in MYO7A are also related to Usher syndrome and DFNB2. Patients with DFNA15 display early-onset progressive high-frequency SNHL and vestibular dysfunction. DFNA15 is associated with missense mutations in the POU4F3 gene, which codes for a number of POU-domain transcription factors. Great variability in the type and severity of vestibular symptoms suggests that epigenetic factors or additional genes may be involved in defining the vestibular phenotype (28).

USHER SYNDROMES

The Usher syndromes (USH) are a clinically and genetically diverse group of autosomal recessive disorders that result in dual hearing and visual loss along with vestibular dysfunction (29). Clinically, the Usher syndromes are divided into 3 groups (USH1, USH2, USH3) based on clinical presentation. Genetically, USH has been associated with 16 loci (9 USH1, 3 USH2, 2 USH3, 2 not specified), from which 13 individual genes have been identified (6 USH1, 3 USH2, 2 USH3, 1 modifier gene, 1 atypical USH gene) (30).

Patients with USH1—most commonly resulting from mutations in MYO7A (USH1B) which codes for the actin motor protein myosin VIIa—present with severe to profound hearing loss at birth as well as vestibular areflexia. Children with USH1 frequently are unable to walk until after 18 months of age and, as they grow, have difficulty with tasks requiring balance or coordination (31, 32).

Patients with USH2—most commonly resulting from mutations in USH2A (USH2A) which codes for the transmembrane protein usherin—are generally considered to have clinically normal vestibular function (33). However, in a study of 7 patients with genetically confirmed USH2 and without history of persistent imbalance or vestibular disorders, [Magliulo et al. (34)] found that the majority had pathologic responses on vestibular testing, pointing toward possible subclinical vestibular dysfunction in this patient population (34).

Patients with USH3—most commonly resulting from mutations in CLRN1 (USH3A) which codes for the transmembrane protein clarin-1—present with progressive vision and hearing loss occurring later in life than in patients with USH1 (35). Similarly, USH3 patients develop vestibular dysfunction less frequently and later in life. In a study of 22 USH3 patients with a median age of 30.5 years, Sadeghi et al. (36) found that 45% (10/22) had vestibular hypofunction or areflexia on caloric testing. Furthermore, 8 of the 10 affected patients had a normal walking age (<16 months), implying that their vestibular function was normal at that time and subsequently worsened over time (36).

While USH has been classically grouped into three USH types, in addition to atypical USH, recent genotype-phenotype correlation testing between USH1, USH2, and USH3 revealed

that ∼1/3 (29/90) of included patients displayed vestibular findings that were not consistent with their respective USH genotype (37). Wafa et al.'s findings suggest the absence of a reliable genotype-phenotype correlation in USH with respect to vestibular symptoms.

GENETICS OF HEREDITARY VESTIBULAR DISORDERS WITHOUT HEARING LOSS

Genetic etiologies have been identified for several isolated vestibular disorders, such as benign paroxysmal vertigo of childhood and bilateral vestibulopathy. These disorders are often mistaken for vestibular migraine, with overlapping clinical presentation and concurrent diagnoses. With very few, if any, targeted interventions for these disorders, better understanding of the genetic underpinnings of these diseases may allow for both more accurate diagnoses and the development of more effective treatment modalities.

BENIGN PAROXYSMAL VERTIGO

Initially described as "benign recurrent vertigo" in a 1979 study of a group of patients with recurrent episodes of vertigo, benign paroxysmal vertigo (BPV) presents with recurrent vertiginous episodes lasting minutes to hours which first begin in childhood or early adulthood (38). Most patients with BPV eventually also meet International Headache Society (IHS) criteria for the diagnosis of migraine, which also displays a strong familial component. Furthermore, greater than 1/3 of first-degree relatives of BPV patients also suffer from BPV, and ½ of those relatives meet HIS criteria for migraine (39, 40). The largest genome wide study of BPV patients to date (20 families) showed evidence of linkage to chromosome 22q12 but also revealed significant genetic heterogeneity (24). Two other regions (5p15 and 3q24) also had NPL scores suggestive of linkage. Despite the strong association with migraine, linkage analysis of a broader phenotype of BPV or migraine headaches weakened the linkage signals compared to BPV alone. Therefore, there is no current evidence that isolated migraine is allelic with BPV (24). A more recent study of a three generation family with BPV linked the condition to chromosome 15, and found an autosomal dominant inheritance pattern (41).

FAMILIAL BILATERAL VESTIBULOPATHY

Like benign paroxysmal vertigo of childhood, bilateral vestibulopathy (BVP) is characterized by recurrent vertigo attack. A significant portion of BVP patients suffer from migraine headaches as well (42); however the attacks are briefer in duration (ranging from seconds to minutes). Diagnostic criteria for BVP were proposed by the Bárány Society in 2017, requiring significant impairment of bilateral function of the vestibulo-ocular reflex (VOR)—seen in patients with "chronic unsteadiness when walking or standing—which worsen in darkness and/or on uneven ground, or during head motion." For diagnosis, "horizontal angular VOR gain bilaterally should be <0.6 (angular velocity 150–300 degree/second), and/or the sum of the maximal peak velocities of the slow phase caloric-induced nystagmus for stimulation with warm and cold water <6 degree/second, and/or the horizontal angular VOR gain <0.1 upon sinusoidal stimulation on a rotatory chair and/or a phase lead >68 degree 9 time constant of <5 seconds)." Probable BVP is diagnosed with unsteadiness symptoms and bilateral pathologic bedside head impulse test (43). A small number of multiplex BVP families have been described, possibly because quantitative vestibular function testing is only available at major medical centers, making it challenging to identify families with bilateral vestibulopathy (44, 45). Unlike in familial deafness, where new genes have continued to be identified, no specific genes have been associated with BVP. One study was able to link BVP in four families to an area on chromosome 6q (45). However, a fifth family whose phenotype did not include migraines was not linked to 6q, suggesting that there may be multiple heterogeneous genotypes that meet the clinical diagnostic criteria. Given the relative rarity of the disease, large-scale efforts to identify and recruit patients with familial vestibulopathy are the next step to identify genes important to vestibular function that may underlie this disease (46).

HEREDITARY ATAXIAS

Hereditary ataxias are a diverse group of inherited neurological disorders related to dysfunction of the cerebellum and brainstem and the associated afferent or efferent pathways. These disorders are monogenic and can be classified by pattern of inheritance as autosomal dominant, autosomal recessive, X-linked, or mitochondrial, with mutations having been identified in genes which code for components of ion channels. A subset of these disorders are associated with attacks of vertigo, likely due in part to vestibular connections within the cerebellum (47).

Autosomal Dominant Ataxias

The autosomal dominant ataxias (ADAs) are a group of rare disorders with great genetic heterogeneity characterized by progressive ataxia, myoclonic epilepsy, dementia, and choreoathetosis. A number of ADAs are the result of point mutations, deletions or duplications associated with ion-channel dysfunction—spinocerebellar ataxias (SCA) 6, 13, 19/2, and episodic ataxias 1, 2, 5—or in signal transduction molecules (47). SCAs are a genetically diverse group characterized by slowly progressive ataxia. The clinical picture often overlaps between variations, making it difficult to diagnose from phenotype alone. However, genetic diagnosis has identified causative mutations in several SCA subtypes—which are numbered in chronological order of their causative locus or gene discovery– potentially opening the door to targeted treatment modalities (48–50). The most common ADA is SCA3 (Machado–Joseph disease), followed by SCA2, SCA1, and SCA6 (51). Episodic ataxias (EA), a group of classical monogenic recurrent vertigo syndromes, are early-onset, autosomal dominant neurologic disorders. Patients experience recurrent episodes of incoordination, slurred speech, and truncal ataxia. The EA subtypes are categorized by interictal findings and genetic mutations. Episodic ataxia type 1 (EA1)

is characterized by short episodes of ataxia with interictal myokymia. EA1 is caused by mutations in *KCNA1* located on chromosome 12 (52), which encodes Kv1.1, a human homolog of the Shaker voltage-gated potassium channel in Drosophila (53). *KCNA1* is widely expressed in the cerebellum as well as along motor axons (54). The mutations causing EA1 result in compromised potassium channel activity, potentially leading to increased neuronal excitability (55, 56). EA2 patients suffer from episodes of vertigo with interictal nystagmus and progressive ataxia. Half of patients with EA2 have migraine headaches; EA2 shares several clinical symptoms with familial hemiplegic migraine type 1 (FHM1), basilar migraine, and progressive ataxia (57, 58). Both EA2 and FHM1 are caused by mutations in the *CACNA1A* gene, which codes for the a1A subunit of the P/Q-type voltage-gated calcium channel (59–61). Recently, exome sequencing has identified both novel mutations in *CACNA1C* and other genes that may be associated with EAs, such as *PRRT2* (62, 63). EA3, which has been documented in a single large Canadian family, is associated with episodic vertigo, nausea, tinnitus, ataxia, and migraine (64). While the disease locus has been mapped to chromosome 1q42, the causative gene is still unknown (65). EA4, also called familial periodic vestibulocerebellar ataxia, is characterized by episodic vertigo and ataxia beginning between ages 30 and 60 (66, 67). A whole genome scan failed to map the EA4 locus to a specific chromosomal location, making the designation of EA4 as a unique syndrome controversial. The causative gene is still undetermined. EA5 is characterized by episodic vertigo attacks beginning in the third decade of life, with episodes of vertigo and ataxia lasting several hours to days. EA5 was identified when several families with EA were screened and found to have mutations in the calcium channel b4 subunit CACNB4, on chromosome 2q. The associated gene encodes the b4 subunit of voltage-gated calcium channels and is the "most highly expressed β subunit in the cerebellum" (68). Interestingly, the EA5 mutation was discovered in a German family displaying generalized epilepsy without ataxia, raising questions about the variable expressivity of the mutation. EA6 was described in a child with episodic, progressive ataxia and migraine attacks, seizures, and prolonged alternating hemiplegia; MRI revealed edema in the corresponding hemisphere. A *de-novo* heterozygous mutation was detected from screening of the *SLC1A3* gene, a solute carrier gene that codes for excitatory amino acid transporter type 1 (EAAT1), a glial glutamate transporter in the cerebellum (69). EA7 was found in a single family with episodes of vertigo, weakness, dysarthria and ataxia for hours to days, with onset before age 20 (70). There are no interictal findings or tinnitus (which distinguishes it from EA3). Genome scanning mapped the locus of EA7 to chromosome 19q13 (70). However, the responsible gene has also not been definitively identified.

Autosomal Recessive Ataxias

Autosomal recessive ataxias (ARAs) typically begin in childhood and are characterized by peripheral sensorimotor neuropathy. Friedreich ataxia and ataxia-telangiectasia are the most common ARAs (71). Next generation sequencing techniques (NGS) has defined new mutations in *GBA2* (72), ANO10 (73, 74), and *SYT14* genes (75); linkage analysis has identified new genes such as the gene *KIAA0226* (76). Both methods allow for a more precise diagnosis and prognosis, facilitating genetic counseling. Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS), in contrast to the other autosomal recessive ataxias, is an adult-onset neurodegenerative disorder characterized by a spectrum of disease that can include sensory neuropathy, progressive unsteadiness, dizziness, and falls beginning around age 60. Cortese et al. recently published that biallelic intronic AAGGG repeat expansions in replication factor complex subunit 1 (*RFC1*) are the causative mutations in CANVAS. *RFC1* encodes a large subunit of the replication factor complex, which is a DNA-dependent ATPase that is essential for DNA replication and repair (77, 78).

X-Linked Ataxias and Mitochondrial Ataxias

X-linked ataxias—occurring in males most commonly after age 50—are very rare, the most common being the fragile X-associated tremor/ataxia syndrome. The causative mutation is a 55–220 CGG repeat expansion in the fragile X mental retardation (PMR1) gene on chromosome Xq27.3, with expression profiling also showing deregulation in 14 microRNAs (79). Exome-sequencing has also identified a missense mutation in a kindred with X-linked with SCA (80). Very little is known of the genetics of mitochondrial ataxias, which are seen in diseases associated with cerebellar ataxia, such as Kearns-Sayre syndrome, myoclonic epilepsy with ragged-red fibers (MERRF), ataxia and retinitis pigmentosa (NARP), lactic acidosis and stroke-like episodes (MELAS), mitochondrial myopathy, neuropathy, and encephalopathy (80, 81).

DISCUSSION

Current State of Personalized Treatment in VDs

Current treatment for vestibular disorders ranges from lifestyle modifications to surgical intervention. Initial treatment is directed toward identifying and reducing triggers for vestibular symptoms, which may vary even among family members. Medications used to treat vestibular symptoms include meclizine, dimenhydrinate, and acetazolamide, though these do not target underlying disease pathophysiology and can depress central compensatory mechanisms, leading to long-term worsening of symptoms. Meniere's disease can be treated with intratympanic injections of steroids or antibiotics, which have both been shown to be beneficial in symptom relief (82, 83). Ablative or destructive surgical procedures for Meniere's disease can lead to vertigo control but are associated with high risk of hearing loss (84). Given the lack of definitive treatment options, it is crucial to better understand the pathophysiology of VDs in order to develop more effective, individualized treatment approaches.

Precision medicine (PM) is a clinical approach that aims to prevent and treat disease on an individual basis, with consideration for individual variation in genetics, environments, and experiences. Success of PM rests on effective use of genetic tools, such as next generation sequencing (NGS) screening

panels, targeted sequencing, and WGS and WES, both on an individual basis and also for the development of a genetic knowledge base. The potential inherent in a PM approach to disease can be seen in its use in the related field of hearing loss (HL), another condition with many heterogeneous genetic etiologies. Common HL-causing mutations are included on available NGS-based gene panels, which can be used for high-risk newborns and for large-scale population studies. WES has been used to find novel HL-associated genes and has aided homozygosity mapping in HL. Research approaches for personalized therapy for HL include gene therapy, stem cell therapy, and pre-implantation genetic diagnosis (85). The trajectory of PM in HL can serve as a road map for the application of PM to VDs.

While a comprehensive genetic knowledge base in VDs is in its relative infancy (**Table 1**), efforts to implement use of personalized medicine clinically in VDs are already under way, particularly in the diagnosis and treatment of MD. Genome England and the Meniere's Disease Consortium have created gene panels for sporadic (18) and familial MD (6); panelapp.com, which can potentially be used in large-scale genomic studies of MD. Further, [Lopez-Escamez et al. (6)] highlighted the multifactorial etiology of MD and proposed all individuals with MD symptoms obtain testing for rs4947296, a marker for a potentially treatable NF-kB-mediated inflammatory response and outlined an algorithm to determine candidacy for gene therapy (87). As more is discovered about the genetic bases of other VDs, similar algorithms can be generated and put into clinical practice.

TABLE 1 | A summary of potential genes involved in vestibular disorders.

Disorder	Gene or locus	References
Hereditary vestibular disorders without hearing loss		
Benign Paroxysmal Vertigo of Childhood	22q12, 5p15, 3q24, chromosome 15	(24, 41)
Familial bilateral vestibulopathy	6q	(45)
Hereditary Ataxias		
CANVAS	*RFC1*	(77, 78)
Episodic ataxias	*KCNA1, CACNA1A,* 1q42, *CACNB4, SLC1A3,* 19q13	(52, 60, 65, 68–70)
Friedreich Ataxia	*FXN*	(71)
Spinocerebellar ataxias	*ATXN1, ATXN2, BEAN*	(48–51)
Hereditary SNHL with vestibular dysfunction		
DFNA9	*COCH*	(26)
DFNA11	*MYO7A*	(27)
DFNA15	*POU4F3*	(28)
Enlarged vestibular aqueduct syndrome	*SLC26A4, FOXI1, KCNJ10*	(1, 17, 86)
Familial Meniere Disease	*DTNA*, *FAM136, PRKCB, DPT, SEMA3D, COCH, OTOG*	(9–13)
Usher Syndrome	*MYO7A, USH2A, CLRN1*	(32–35)
Episodic vestibular syndromes		
Vestibular Migraine	5q35, 11q, 22q12	(22–24)

Gene Expression Profiling, Gene Therapy, and Stem Cell Therapy Research in the Inner Ear

Multiple next-generation sequencing methods of gene expression profiling have been created, including microarray technology, serial analysis of gene expression (SAGE), and RNA-seq, and have led to the ability to study gene expression levels within the inner ear. Using microarray analysis, [Cristobal et al. (88)] assessed gene expression in vestibular epithelial cell types, eliciting more than 400 genes with differential expression between hair cells and supporting cells (88). Further, target genes showing specific expression in vestibular hair cells before and after maturation of mechano-sensitivity have also been defined, which could potentially serve in the future as gene therapy targets (89).

Many animal model studies of gene therapy in HL syndromes have included vestibular dysfunction studies. Recently, a number of studies have shown promise using gene therapy to restore auditory and vestibular function in mouse models of Usher syndrome (90–92). One study showed that the delivery of USH1c into the inner ear with an adeno-associated viral vector (Anc80L65) restored vestibular function—measured by rotarod performance and open field behavior—in USH1c mice nearly back to wild-type levels (92). Yet, to date, there have not been dedicated gene therapy studies targeting isolated vestibular symptoms in VDs. Similar to gene therapy, many stem cell therapy efforts for hearing loss syndromes include vestibular rescue as well. In addition, Taura et al. described a regenerative therapy for vestibular disorders utilizing human induced pluripotent stem cells (iPSCs) (93). Human neural stem cells (hNSCs) derived from iPSCs and injected into mouse utricle tissues produced elongated axon-like structures that contacted the vestibular hair cells. While the hNSCs showed signs of morphological maturation, they showed only partial physiological maturation, and further work must be done to investigate the potential of the therapy (93).

CONCLUSIONS

Vestibular disorders are complex diseases, with heterogeneous presentations and overlapping symptoms making purely clinical diagnoses extremely challenging. Deep phenotyping with a complete familial medical history combined with NGS will allow for the identification of rare variants and genes related to familial vestibular disorders, allowing for more accurate classification of disease processes. In turn, furthering knowledge of genetic etiologies and pathophysiology of VDs will allow for the development and implementation of precision medicine approaches and individualized treatment to VDs, including gene and stem cell therapies.

AUTHOR CONTRIBUTIONS

CM, HD, EN, and TT: literature review, analysis of data, manuscript preparation, manuscript review,

and manuscript submission. DY, AN, MS, YL, MH, SA, FT, GN, SB, and XL: manuscript preparation, manuscript review, and manuscript submission. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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Identification of Potential Meniere's Disease Targets in the Adult Stria Vascularis

Shoujun Gu[1], *Rafal Olszewski*[1], *Lacey Nelson*[2], *Alvaro Gallego-Martinez*[3], *Jose Antonio Lopez-Escamez*[3,4,5] *and Michael Hoa*[1,2]*

[1] Auditory Development and Restoration Program, National Institute on Deafness and Other Communication Disorders, National Institutes of Health (NIH), Bethesda, MD, United States, [2] Department of Otolaryngology-Head and Neck Surgery, Georgetown University School of Medicine, Washington, DC, United States, [3] Otology and Neurotology Group CTS495, Department of Genomic Medicine, Centre for Genomics and Oncological Research, Pfizer/Universidad de Granada/Junta de Andalucía (GENYO), Granada, Spain, [4] Department of Otolaryngology, Instituto de Investigación Biosanitaria ibs.GRANADA, Hospital Universitario Virgen de las Nieves, Granada, Spain, [5] Division of Otolaryngology, Department of Surgery, University of Granada, Granada, Spain

The stria vascularis generates the endocochlear potential and is involved in processes that underlie ionic homeostasis in the cochlear endolymph, both which play essential roles in hearing. The histological hallmark of Meniere's disease (MD) is endolymphatic hydrops, which refers to the bulging or expansion of the scala media, which is the endolymph-containing compartment of the cochlea. This histologic hallmark suggests that processes that disrupt ion homeostasis or potentially endocochlear potential may underlie MD. While treatments exist for vestibular symptoms related to MD, effective therapies for hearing fluctuation and hearing loss seen in MD remain elusive. Understanding the potential cell types involved in MD may inform the creation of disease mouse models and provide insight into underlying mechanisms and potential therapeutic targets. For these reasons, we compare published datasets related to MD in humans with our previously published adult mouse stria vascularis single-cell and single-nucleus RNA-Seq datasets to implicate potentially involved stria vascularis (SV) cell types in MD. Finally, we provide support for these implicated cell types by demonstrating co-expression of select candidate genes for MD within SV cell types.

Keywords: stria vascularis, single-cell, nucleus, RNA-seq, Meniere's, ion homeostasis

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***Correspondence:**
Michael Hoa
michael.hoa@nih.gov

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INTRODUCTION

The cochlea is composed of 3 fluid-filled chambers, including the scala vestibuli, scala media, and scala tympani. Two of these chambers, the scala vestibuli and tympani, contain perilymph, which is characterized by a high sodium concentration and low potassium concentration similar to plasma in the blood. The remaining chamber, the scala media, contains endolymph, which is a special fluid characterized by a high potassium and low sodium concentration. The stria vascularis (SV) is a specialized non-sensory epithelial tissue which resides in the lateral wall of the cochlea facing the endolymph-containing scala media. The SV is composed of three main layers of cells (**Figure 1**), consisting predominantly of marginal, intermediate and basal cells, respectively, that function together to regulate cochlear ionic homeostasis, including potassium

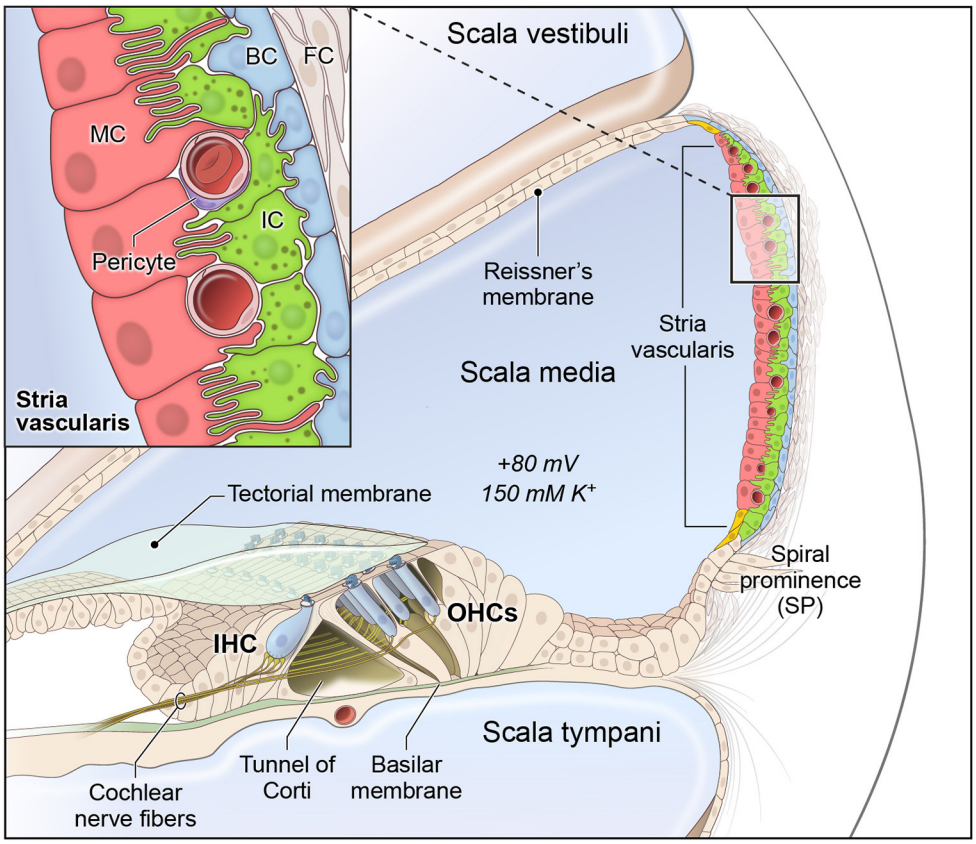

FIGURE 1 | Stria vascularis cellular heterogeneity and organization. Schematic of the stria vascularis (SV) and its relationship to structures in the cochlea. The SV is composed of three layers of cells and is responsible for generating the +80 mV endocochlear potential (EP) and the high potassium concentration in the endolymph-containing scala media. The relationship between the marginal, intermediate and basal cells are demonstrated with the marginal extending basolateral projections to interdigitate with intermediate cells, which have bidirectional cellular projections that interdigitate with both marginal and basal cells. In addition to these cell types, other cell types, including spindle cells (yellow), endothelial cells, pericytes, and macrophages (not shown) are present in the SV. Used with permission from Korrapati and colleagues.

concentration in the endolymph, and generate the endocochlear potential (EP), which enables hair cell mechanotransduction and hearing (1–6). While the mechanisms by which both EP and cochlear ionic homeostasis are maintained remain incompletely characterized, we have recently characterized the transcriptional profiles of SV cell types and identified homeostatic gene regulatory networks using both single-cell and single-nucleus RNA-sequencing in the adult mammalian stria vascularis (7).

The histopathological hallmark of Meniere's disease (MD) is endolymphatic hydrops (EH), which refers to the expansion and bulging of the endolymph-containing scala media into the scala vestibuli as seen in processed human temporal bones (8, 9). As the stria vascularis plays a role in cochlear ionic homeostasis, the finding of EH suggests that dysfunctional ion homeostasis possibly involving the stria vascularis may result in EH and be involved in MD (10–14). Studies in human temporal bones suggest that structural changes in the stria vascularis in Meniere's disease patients exceed those in age-matched controls with presbycusis and that these structural changes correlate with observations of EH (12, 14). Despite these findings, the mechanisms underlying MD remain poorly understood and the observation of EH is thought to be an epiphenomenon or by-product of the underlying disease rather than a causal factor (8, 15). While a variety of histopathological changes in both cochlear and vestibular inner ear structures in humans have been observed, including, but not limited to, hair cells, supporting cells, the tectorial membrane, the SV and lateral wall, spiral ganglion neurons, saccule, utricle, and endolymphatic sac (9, 12–14, 16, 17), none of these findings have been definitively correlated with premortem auditory and vestibular findings (9). Furthermore, the cell types and tissues involved in the initiating pathophysiology underlying Meniere's disease remain undefined.

Despite these gaps in knowledge, a number of studies have examined genomic and transcriptomic changes in patients with MD (10, 18–32). The recent availability of single cell and single nucleus RNA-sequencing datasets from the adult mammalian stria vascularis (7) provide an opportunity to localize genes implicated in MD to relevant cell types. With this in mind, we compare the existing genomic and transcriptomic studies of MD to adult mouse SV single cell and single nucleus transcriptional

profiles to identify cell types in the adult SV that express genes implicated in MD.

MATERIALS AND METHODS

Literature Review of Meniere's Disease Implicated Genes

To provide an expanded view of the genes and variants associated with MD, we performed a systematic literature review. Facilitated by a web tool, we classified, curated, and annotated most of the genes and PubMed abstracts related to MD. Each abstract was systematically annotated for gene names and symbols. The abstracts were computationally organized by genes to be manually reviewed. After the review, the genes were classified to determine which genes showed evidence of mutations or altered expression, as defined by increased or decreased compared to control patients, in MD. In parallel, a systematic review was also performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines. Results of both reviews were combined to create a master list of implicated genes in Meniere's disease. The complete literature search strategy as well as study inclusion and exclusion criteria are documented in a PRISMA flowchart (**Supplementary Figure 1**).

PubTerm Search Strategy

To obtain unbiased updated information on genes with mutations or altered expression in MD, we acquired PubMed abstracts related to reported polymorphisms or altered expression in MD in August 2020 using a web tool called PubTerm (http://bioinformatica.mty.itesm.mx:8080/Biomatec/pubterm.html) to initially organize, annotate, and curate abstracts per gene (33–35). Briefly, the PubTerm tool organizes abstracts by genes, chemicals, diseases, species, and other terms, or by the co-occurrences of genes and diseases that facilitate classification, annotation, curation, and tracking of genes of interest. We used the following query terms: ("Meniere's Disease"[TIAB] OR "Meniere disease" OR "endolymphatic hydrops"[TIAB]) AND (mutations[TIAB] OR mutation[TIAB] OR polymorphisms[TIAB] OR polymorphism[TIAB] OR variant[TIAB] OR variants[TIAB] OR RNA-sequencing[TIAB]) NOT review[Publication Type]. Addition of the term "GWAS" or "genome-wide association study" did not change the number of references identified. Subsequently, full text reviews of all identified references were performed to identify genes not mentioned in the abstract and to include gene candidates provided in datasets that accompanied references where applicable.

PRISMA Search Strategy

The following databases and gray literature sources were searched from inception through August 1, 2020: PubMed-NCBI, MEDLINE, Embase, CINAHL, Cochrane Library, ClinicalTrials.gov, OpenGrey, GreyNet, GreyLiterature Report, and European Union Clinical Trials Registry. No language restriction was employed. Previously described search terms were utilized. Article titles and abstracts were screened for eligibility before full-text articles were obtained and assessed for possible inclusion. Additional relevant articles were identified through the reference lists of included studies. References identified by PubTerm were also incorporated into the PRISMA strategy and screened as described (36). The final reference list was reviewed independently by two reviewers. Data from included studies was extracted and compiled in a standardized electronic data collection sheet. The primary outcome of interest was the gene(s) or genetic polymorphism(s) of interest being investigated.

Animals

Inbred CBA/J males and females were purchased from JAX (Stock No. 000656). Breeding pairs were set up to obtain P30 mice for immunohistochemistry and single molecule RNA FISH.

Single Molecule Fluorescent *in situ* Hybridization (smFISH) and Immunohistochemistry

smFISH Using RNAscope Probes

Briefly, *in situ* hybridizations were performed as previously described (7). The following RNAscope probes were utilized: *Kcne1* (Cat# 541301), *Atp1b2* (Cat# 417131), *Esrrb* (Cat# 565951-C3), *Met* (Cat# 405301), *Ednrb* (Cat# 473801-C3), and *Tmem176a* (Cat# 432641-C2). RNAscope probes were obtained from Advanced Cell Diagnostics (Newark, CA) and used with sections of cochleae from CBA/J wild type mice at P30. Adult cochleae were dissected from the head and fixed overnight at 44°C in 4% PFA in 1x PBS. Fixed adult mouse inner ears were decalcified in 150 mM EDTA for 5–7 days, transferred to 30% sucrose, and then embedded and frozen in SCEM tissue embedding medium (Section-Lab Co, Ltd.). Adhesive film (Section-Lab Co, Ltd.; Hiroshima, Japan) was fastened to the cut surface of the sample in order to support the section and cut slowly with a blade to obtain thin midmodiolar sections. The adhesive film with section attached was submerged in 100% EtOH for 60 s, then transferred to distilled water. The adhesive film consists of a thin plastic film and an adhesive and it prevents specimen shrinkage and detachment. This methodology allows for high quality anatomic preservation of the specimen. Frozen tissues were sectioned (10 μm thickness) with a CM3050S cryostat microtome (Leica, Vienna, Austria). Sections were mounted with SCMM mounting media (Section-Lab Co, Ltd., Hiroshima, Japan) and imaged using a 1.4 N.A. objective. Labeling with 4,6-diamidino-2-phenylindole (DAPI, 1:10,000, Life Technologies) was included to detect cell nuclei.

Bioinformatics

Data and Software Availability

Previously published single cell and single nucleus RNA-Seq datasets of postnatal day 30 (P30) mouse stria vascularis (7) were utilized (GEO Accession ID: GSE136196) which can be found at the following link (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE136196) and are available through the gene Expression Analysis Resource (gEAR), a website for visualization and comparative analysis of multi-omic data, with an emphasis

on hearing research (https://umgear.org//index.html?layout_id=b50cae7a) (37).

Data Visualization

P30 SV scRNA-Seq & snRNA-Seq

Previously published P30 SV scRNA-Seq and snRNA-Seq data were preprocessed by Scanpy (v1.5.1) with criteria as previously described (7). Briefly, low-quality and outlier cells were computationally removed if: (1) gene number per cell or nuclei was less than the 5th percentile or more than 95th percentile; (2) total counts per cell or nuclei was less than the 5th percentile or more than 95th percentile; (3) >20% mitochondria genes (snRNA-Seq only). Predicted doublets by Scrublet (v0.2.1) with default settings were also filtered.

Preprocessed data were normalized by total with parameter *exclude_highly_expressed* set as "True" and scaled by the function *pp.log1p()*. Cell clustering and annotation was performed using modularity-based clustering with Leiden algorithm implemented in Scanpy (v1.5.1). Heatmap were plotted by Seaborn (v0.10.1). Dotplots were plotted by the Scanpy function *pl.dotplot()*.

Gene Ontology and Gene Set Enrichment Analysis

Gene ontology analyses and gene set enrichment analyses were performed using Enrichr (http://amp.pharm.mssm.edu/Enrichr/) as previously described (7, 38–41). The combined score approach where enrichment score is calculated from the combination of the *p*-value computed using the Fisher exact test and the z-score was utilized. Top gene ontology (GO) terms were chosen by utilizing the combined score approach as described. Genes were further functionally classified using the Protein Analysis Through Evolutional Relationships (PANTHER, pantherdb.org) database (42).

RESULTS

Systematic Literature Reviews Curate Genes Implicated in Meniere's Disease

In order to curate a comprehensive list of genes implicated in MD, we utilized parallel methodologies for systematic reviews including the use of a webtool, PubTerm, to perform an unbiased search for implicated genes using the previously noted search terms in PubMed and a manual search of public databases and gray literature using PRISMA guidelines. In total, 389 unique abstracts were identified. Abstracts unrelated to Meniere's disease ($n = 196$) were excluded resulting in 193 abstracts related to Meniere's disease. Exclusion criteria including non-English language ($n = 22$), animal studies ($n = 21$), non-gene outcome defined as an absence of genes studied in relation to Meniere's disease ($n = 45$), and unrelated to Meniere's disease ($n = 28$) were applied to a full-text review of these references resulting in 77 references being included for systematic review (**Supplementary Figure 1**). Full-text reviews of identified reference articles as well as review of attached datasets were utilized to ensure that a comprehensive list of genes was defined from these references. Based on the described search strategy, a total of 832 genes were investigated in relation to MD, and 122 of the genes were reported in more than one study. A table of the identified genes and their references is included (**Supplementary Table 1**). We have provided descriptive tables summarizing the studies implicating genes in MD (**Supplementary Datas 1**, **2**).

Gene ontology (GO) biological process analysis of these genes implicated in MD identified significant enrichment for cellular metal ion homeostasis (GO:0006875), positive regulation of calcium ion transport into cytosol (GO:0010524), positive regulation of calcium ion transmembrane transport (GO:1904427), equilibrioception (GO:0050957), sensory perception of mechanical stimulus (GO:0050954), and sensory perception of sound (GO:0007605). GO molecular function analysis identified significant enrichment for cytokine activity (GO:0005125), water transmembrane transporter activity (GO:0005372), water channel activity (GO:0015250), chemokine activity (GO:0008009), chemokine receptor binding (GO:0042379), and sodium ion transmembrane transporter activity (GO:0015081). GO cellular component analysis identified significant enrichment for integral component of plasma membrane (GO:0005887), MHC protein complex (GO:0042611), and junctional sarcoplasmic reticulum membrane (GO:0014701). The PANTHER classification identified 20 functional groups and revealed that genes implicated in MD encoded metabolite interconversion enzymes (15.1%, including *Mif* and *Sod2*), transporters (14.3% including *Atp1b2* and *Trpv4*), intercellular signaling proteins (12.6%, including *Tgfb2*), protein modification proteins (10.5%, including *Wnk2*, *Wnk4*, and *Sgk1*), transmembrane signaling receptors (10.1%, including *Adrb2*), and others (**Figure 2**).

Single-Cell and Single-Nucleus RNA-Sequencing of the Adult Mouse Stria Vascularis Reveal Expression of Genes Associated With MD in Major SV Cell Types

Heatmaps demonstrating genes reported in MD with cell type specificity in both the single-cell and single-nucleus RNA-sequencing datasets are shown in **Figures 3A,B** (single-cell RNA-Seq) and **Figures 4A,B** (single-nucleus RNA-Seq), respectively. In the single-cell RNA-seq dataset, fibrocytes were detected and the number of spindle and root cells captured did not enable their transcriptional profiles to be distinguished. In the single-nucleus RNA-seq dataset, spindle and root cell transcriptional profiles were distinguishable, Reissner's membrane cells were detected, and fibrocytes were not detected. Stria vascularis from adult mice were collected, attempting to remove as much of the spiral ligament from the strial tissue. Fibrocytes that were detected represent contaminating cells from the spiral ligament. In both datasets, marginal cells, intermediate cells, basal cells, macrophages, B cells and neutrophils were detected. Details related to these datasets have been described previously (7). Heatmaps demonstrating expression of genes without cell type-specific expression are shown in the supplement for single-cell RNA-seq (**Supplementary Figures 2–4**) and single-nucleus RNA-Seq (**Supplementary Figures 5–9**), respectively.

Genes with preferential expression in SV marginal cells include *Kcne1*, *Atp1b2*, *Esrrb*, *Add2*, *Sgk1*, *Atp13a5*, *Hmx2*,

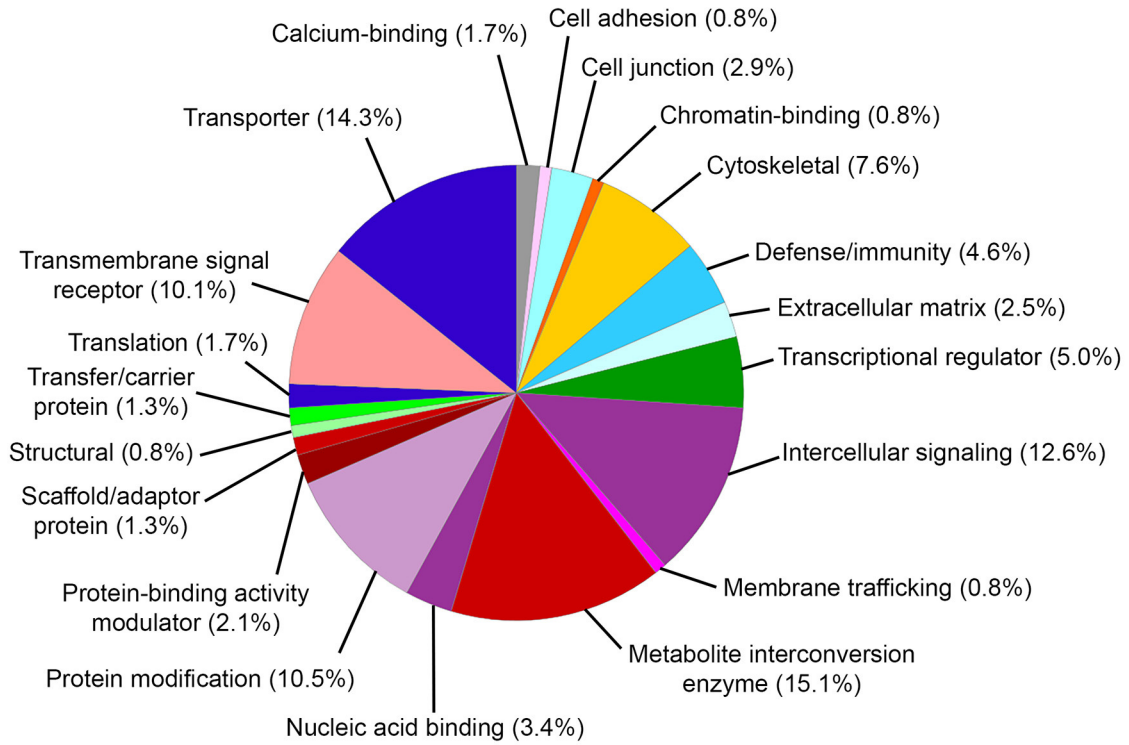

FIGURE 2 | PANTHER protein classification of genes implicated in Meniere's disease. Pie chart depicts protein classes found in gene list. Percentages reflect number of gene hits against total number of process hits. The top protein classes include metabolite interconversion enzymes, transporters, intercellular signaling proteins, protein modification proteins, and transmembrane signaling receptors.

Cacna2d1, *Car12*, *Eya4*, *Tnfrsf12a*, *Shroom3*, *Wnk2*, and *Dtna* (**Figures 3A**, **4A**). Dot plots demonstrate differential expression amongst the major strial cell types including marginal, intermediate and basal cells, as well as some rarer cell types, including SV spindle and root cells, and SV macrophages (**Figures 5A,B**). In each dot plot, the more orange the dot, the greater the expression level of a given gene and the larger the dot, the greater the proportion of cells that express the given gene. Genes with previously published expression in marginal cells, include *Kcne1* (2, 43), *Atp1b2* (2, 44–47), *Esrrb* (7, 48), and *Atp13a5* (7). Examination of SGK1 protein expression in the rat and guinea pig cochlea demonstrates expression in the stria vascularis in addition to the spiral ganglion neurons, spiral limbus, organ of corti, and spiral ligament (49, 50). Examination of *in situ* hybridization of *Sgk1* in the E15.5 mouse in the Allen Brain Atlas suggests localization to the organ of Corti and the roof of the cochlear duct where future marginal cells reside (**Supplementary Figure 10A**). *Hmx2* has been previously localized to the developing mouse stria vascularis (51). *Cacna2d1* has been previously localized to spiral ganglion neurons (52) and examination of *in situ* hybridization in the E15.5 mouse in the Allen Brain Atlas suggests possible localization to the roof of the cochlear duct where future marginal cells reside (**Supplementary Figure 10B**). Carbonic anhydrases have been previously shown to be expressed in the stria vascularis (53–55) and *Car12* expression has been previously shown to be expressed in root cells with reduced expression in marginal cells (56). *Eya4* has not been previously localized to the mouse stria vascularis but examination of *in situ* hybridization in the E15.5 mouse in the Allen Brain Atlas suggests widespread expression in the cochlear duct including the region of the future stria vascularis (**Supplementary Figure 10C**). *Add2*, *Tnfrsf12a*, *Shroom3*, *Wnk2*, and *Dtna* have not been previously localized to structures in the cochlea.

Genes with preferential expression in SV intermediate cells include *Met*, *Cdc14a*, *Ednrb*, *Tmem176a*, *Tmem176b*, and *Gsta1* (**Figures 3A**, **4A**). Dot plots demonstrate differential expression amongst marginal, intermediate, and basal cells in the SV (**Figures 5A,B**). *Met* has been previously shown to be expressed in intermediate cells in the developing and adult mouse SV (7, 57). *Cdc14a* has been previously shown to be expressed in hair cell stereocilia and hair cells, supporting cells, the osseous spiral lamina, and spiral ganglion neurons (58). *Ednrb* has been previously localized to the SV but the specific cell type expressing *Ednrb* could not be defined (59). *Tmem176a*, *Tmem176b*, and *Gsta1* have not been previously localized to structures in the cochlea.

Genes with preferential expression in basal cells within the SV include *Wnk4*, *Col11a2*, and *Slc44a2* (**Figures 3B**, **4B**). Dot plots demonstrate differential expression amongst marginal,

intermediate, and basal cells in the SV (**Figures 5A,B**). These genes are co-expressed by SV basal cells which are identified by *Cldn11* expression that has been previously demonstrated in both adult mouse and humans (7, 60, 61). *Col11a2* has been previously shown to be expressed in the spiral ligament (SL) and has been associated with a variety of syndromic as well as non-syndromic sensorineural hearing loss including both autosomal-dominant (DFNA13) and autosomal-recessive

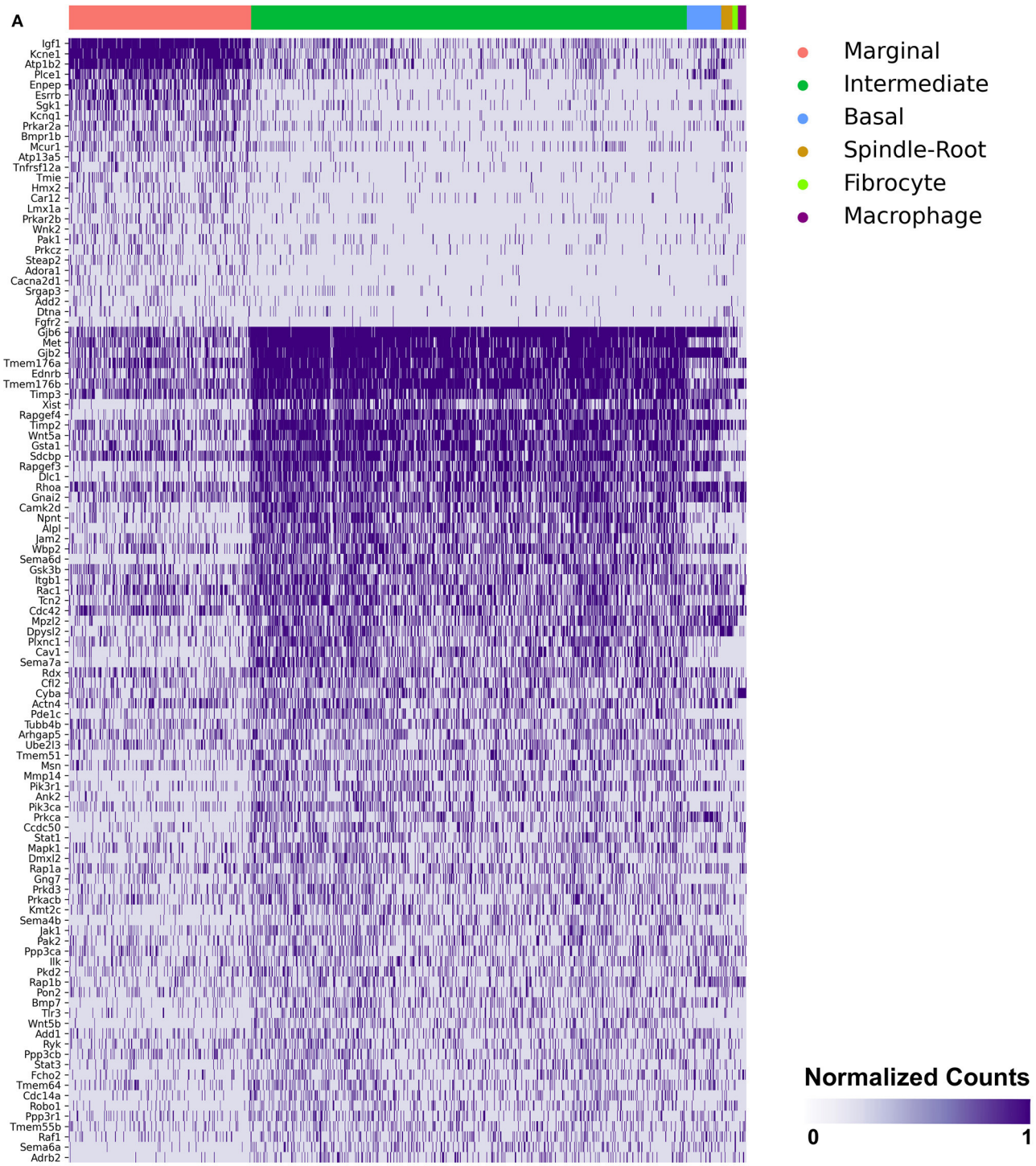

FIGURE 3 | Continued

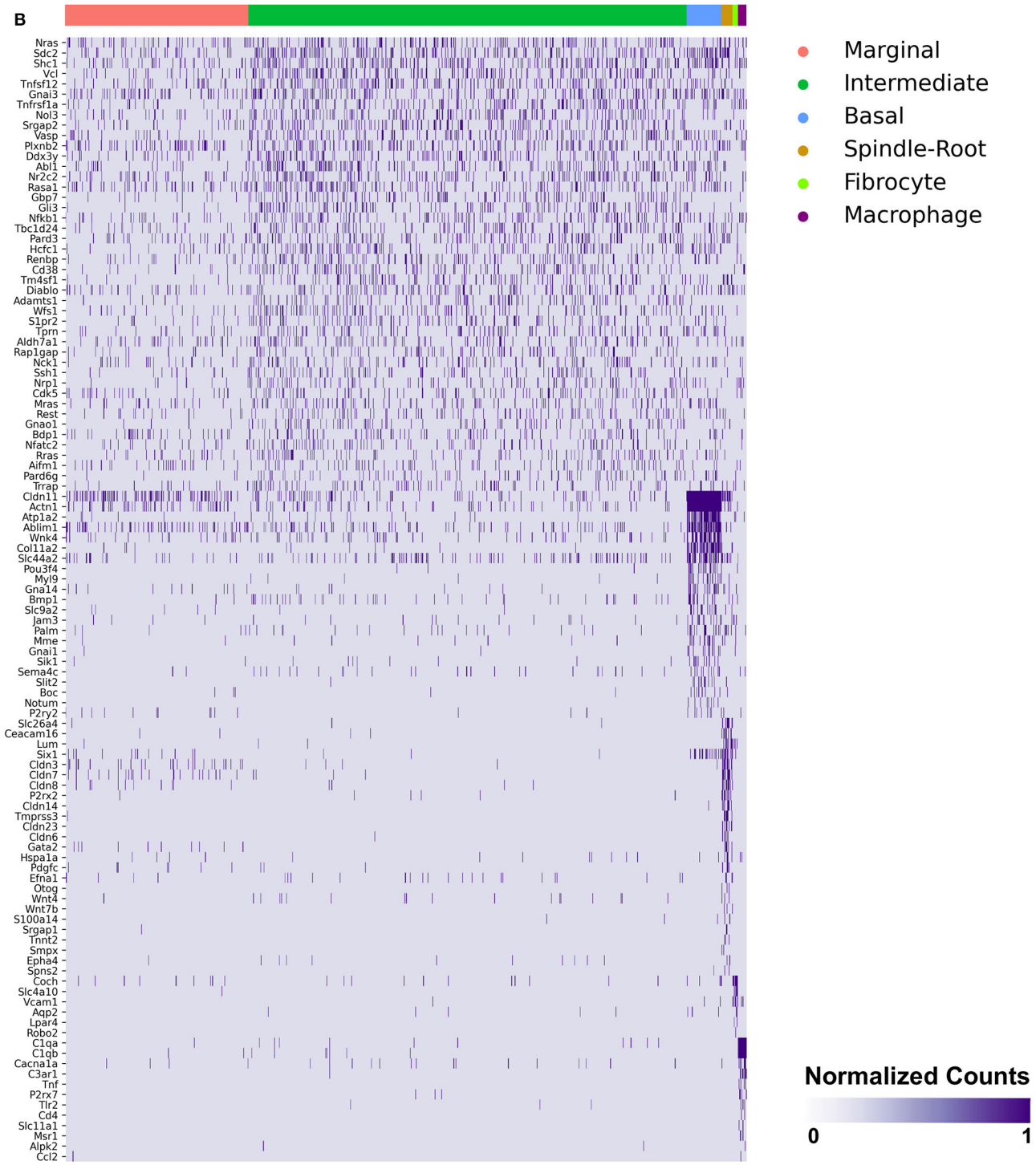

FIGURE 3 | (A) SV cell type-specific expression of genes implicated in Meniere's disease as demonstrated by single-cell RNA-seq: marginal & intermediate cells. Heatmap displays genes with cell type-specificity predominantly to marginal and intermediate cells, respectively. Heatmap displays cell types along the horizontal axis and genes along vertical axis. Gene expression is displayed in normalized counts. Cell types displayed include marginal cells, intermediate cells, basal cells, spindle-root cells, fibrocytes, and macrophages. **(B)** SV cell type-specific expression of genes implicated in Meniere's disease as demonstrated by single-cell RNA-seq: intermediate cells, basal cells, spindle-root cells, fibrocytes, and macrophages. Heatmap displays genes with cell type-specificity predominantly to intermediate, basal cells, spindle-root cells, fibrocytes, and macrophages, respectively. Heatmap displays cell types along the horizontal axis and genes along vertical axis. Gene expression is displayed in normalized counts. Cell types displayed include marginal cells, intermediate cells, basal cells, spindle-root cells, fibrocytes, and macrophages.

(DFNB53) forms (62–64). Slc44a2, formerly known as choline transporter-like protein 2 (CTL-2), is a multi-transmembrane protein originally discovered as a target of antibody-induced hearing loss (65) and more recently implicated in Meniere's disease (28). Others have suggested that because of its prominent expression in cells facing the scala media, SLC44A2 may play a role in cochlear ionic homeostasis (65). *Wnk4* is a known regulator of claudins and paracellular chloride ion

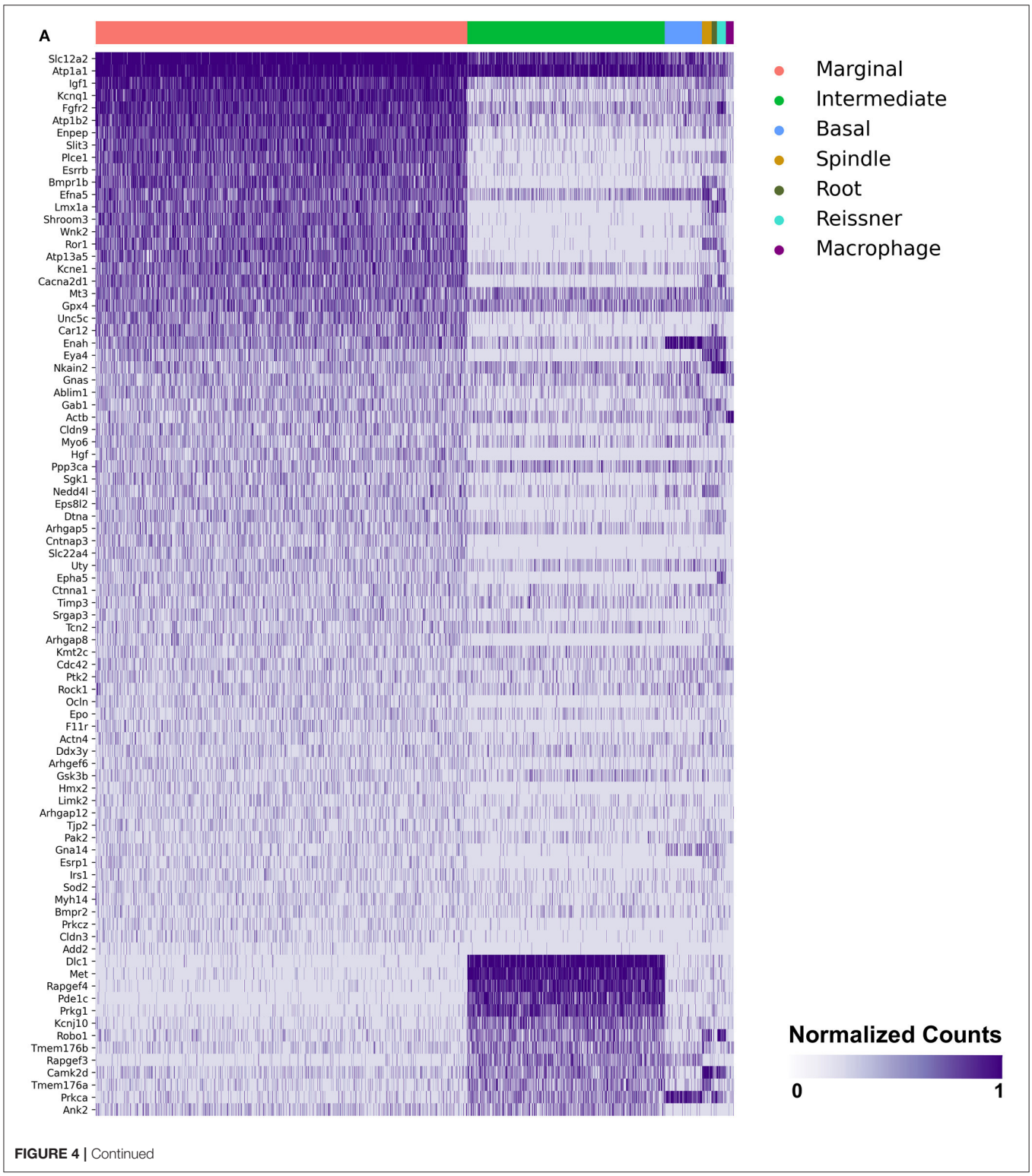

FIGURE 4 | Continued

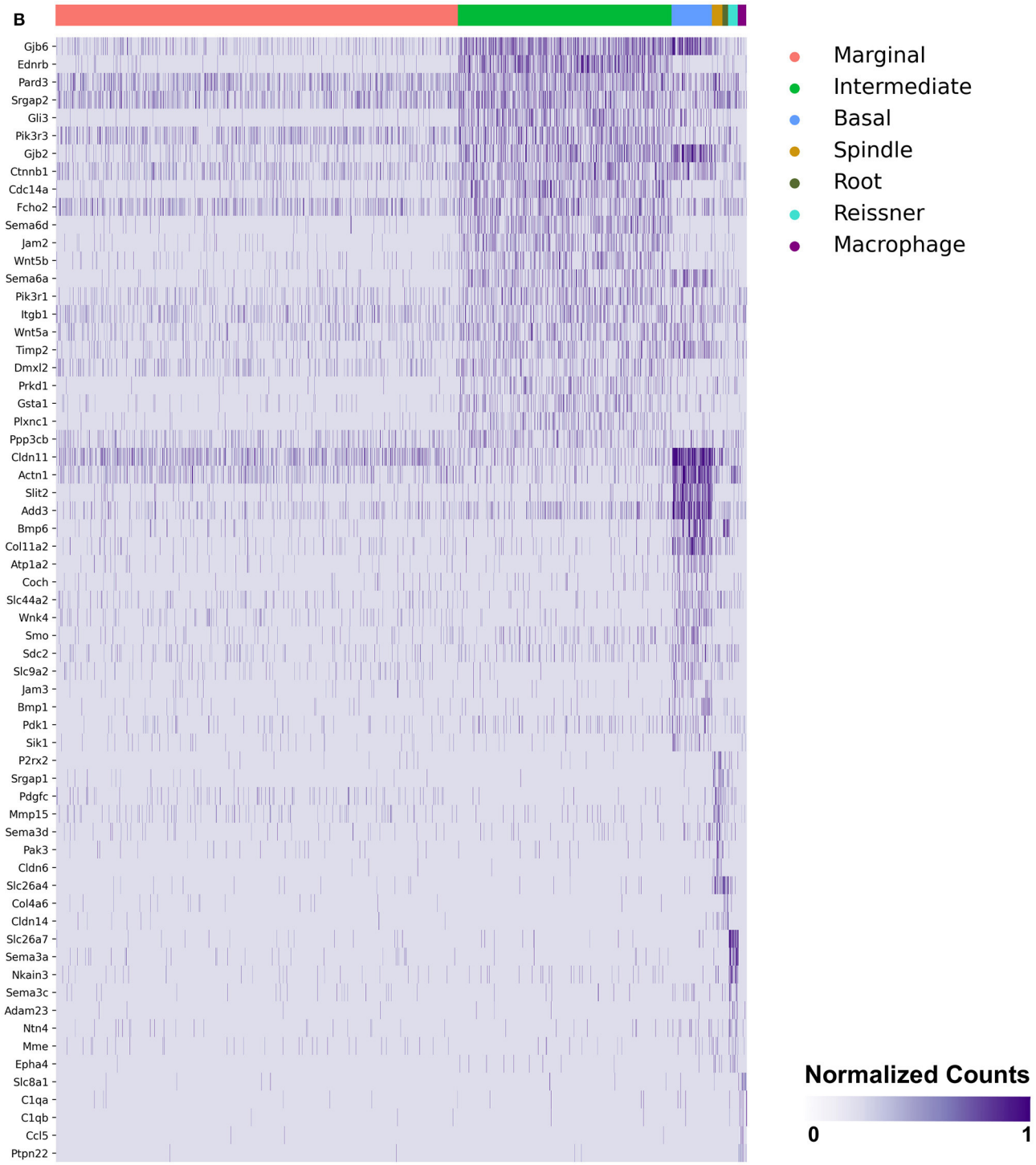

FIGURE 4 | (A) SV cell type-specific expression of genes implicated in Meniere's disease as demonstrated by single-nucleus RNA-seq: marginal & intermediate cells. Heatmap displays genes with cell type-specificity predominantly to marginal and intermediate cells, respectively. Heatmap displays cell types along the horizontal axis and genes along vertical axis. Gene expression is displayed in normalized counts. Cell types displayed include marginal cells, intermediate cells, basal cells, spindle cells, root cells, Reissner's membrane cells, and macrophages. **(B)** SV cell type-specific expression of genes implicated in Meniere's disease as demonstrated by single-nucleus RNA-seq: intermediate cells, basal cells, spindle cells, root cells, Reissner's membrane cells, and macrophages. Heatmap displays genes with cell type-specificity predominantly to intermediate cells, basal cells, spindle cells, root cells, Reissner's membrane cells, and macrophages, respectively. Heatmap displays cell types along the horizontal axis and genes along vertical axis. Gene expression is displayed in normalized counts. Cell types displayed include marginal cells, intermediate cells, basal cells, spindle cells, root cells, Reissner's membrane cells, and macrophages.

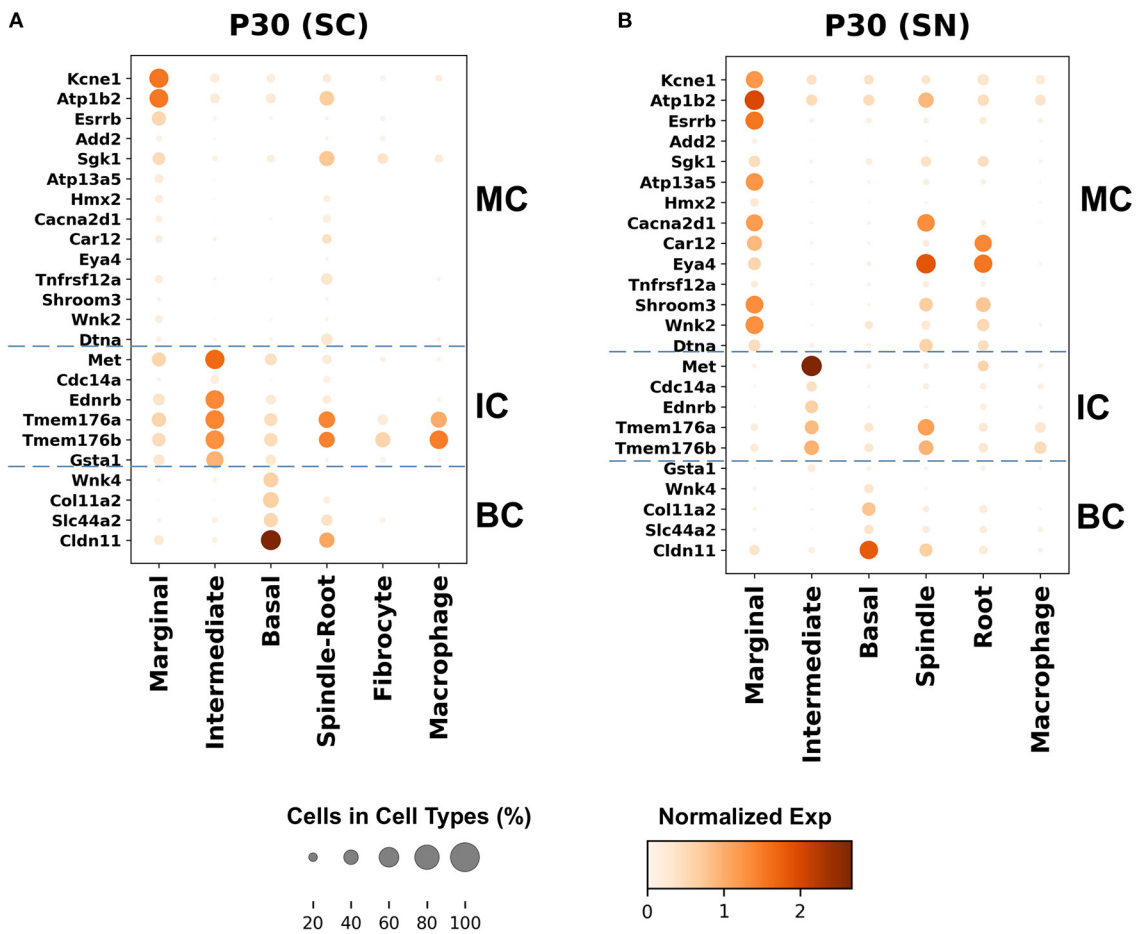

FIGURE 5 | Top differentially expressed Meniere's disease genes in the adult SV. **(A)** Top differentially expressed genes in the single-cell RNA-seq dataset. **(B)** Top differentially expressed genes in the single-nucleus RNA-seq dataset. Cell types are displayed along the horizontal axis and genes are displayed along the vertical axis. The size of the dot corresponds to the proportion of cells expressing the given gene (see legend). The darker the color of the dot, the greater the level of expression of the given gene (see scale bar).

permeability (66–68). *Wnk4* has not been previously localized to SV basal cells. In summary, these data demonstrate RNA expression of Meniere's candidate genes in adult mouse SV cell types.

Single Molecule Fluorescent *in situ* Hybridization (smFISH) Localizes Genes Implicated in Meniere's Disease to the Stria Vascularis

To localize genes implicated in Meniere's disease to the stria vascularis, we performed smFISH. Having previously validated expression of *Esrrb* RNA in adult SV marginal cells (7), we demonstrate co-expression of this Meniere's disease-implicated gene with *Atp1b2* (**Figures 6A,A′**) and *Kcne1* (**Figures 6B,B′**) in adult mouse SV marginal cells. The RNA of both *Atp1b2* (**Figure 6A**) and *Kcne1* (**Figure 6B**) localizes predominantly to the marginal cell nuclei. Images without DAPI labeling are shown to emphasize the co-localization of *Esrrb* with *Atp1b2* (**Figure 6A′**) and *Kcne1* (**Figure 6B′**), respectively. Missense mutations in *ESRRB* have been identified in patients with Meniere's disease (27) as well as an autosomal recessive non-syndromic sensorineural hearing loss DFNB35 (48). *ATP1B2* is upregulated in peripheral blood mononuclear cells (PBMCs) obtained from patients with Meniere's disease compared to patients without Meniere's disease (23). Single nucleotide polymorphisms (SNPs) in KCNE1 have been identified in patients with Meniere's disease (29, 69–72). However, Campbell and colleagues, in comparing two larger cohorts of Meniere's disease and control patients in the Caucasian population, failed to find a significant association between several KCNE1 SNPs and Meniere's disease (73). In intermediate cells, we co-localize expression of *Met*, *Ednrb*, and *Tmem176a* RNA (**Figures 6C–E**). *Met* (in turquoise), *Ednrb* (in red), and *Tmem176a* (in green) RNA are localized to the intermediate cell layer of the stria vascularis (**Figure 6C**).

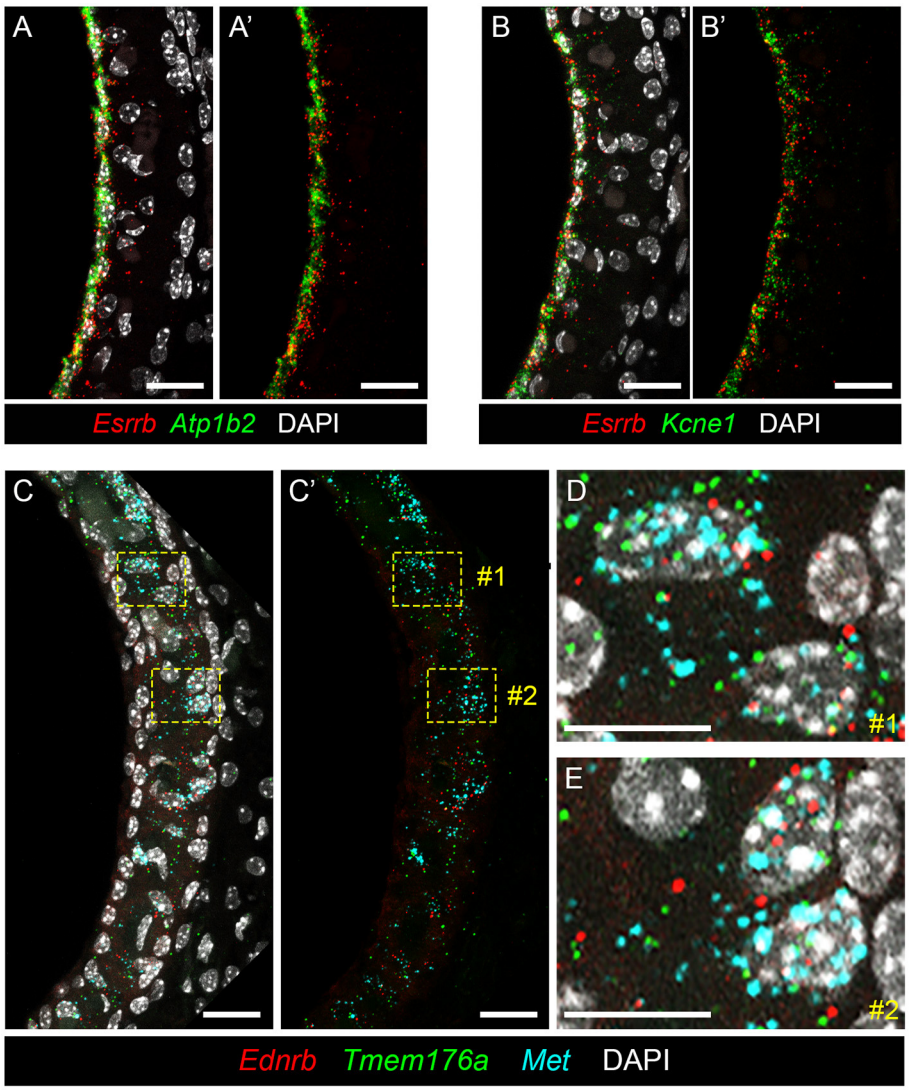

FIGURE 6 | Validating expression of genes implicated in Meniere's disease in the adult mouse SV. **(A,A')** RNA probes demonstrate co-expression of *Esrrb* (red) and *Atp1b2* (green) RNA in marginal cells of the adult mouse SV (P30 mouse) with DAPI (white) labeling of cell nuclei **(A)** and without DAPI labeling of cell nuclei **(A')**. Scale bars are 20 microns. **(B,B')** RNA probes demonstrate co-expression of *Esrrb* (red) and *Kcne1* (green) RNA in marginal cells of the adult mouse SV (P90 mouse) with DAPI (white) labeling of cell nuclei **(B)** and without DAPI labeling of cell nuclei **(B')**. Scale bars are 20 microns. **(C,C')** RNA probes demonstrate co-expression of *Met* (turquoise), *Ednrb* (red) and *Tmem176a* (green) RNA in the intermediate cell layer of the adult mouse SV (P90 mouse) with DAPI labeling of cell nuclei **(C)** and without DAPI labeling of cell nuclei **(C')**. Scale bars are 20 microns. Yellow dashed line boxes outline representative cells where the RNA signal of all 3 probes are colocalized to intermediate cell nuclei. **(D,E)** Closeup images of representative cells #1 **(D)** and #2 **(E)** from **(C,C')** where RNA expression of *Met* (turquoise), *Ednrb* (red), and *Tmem176a* (green) are co-expressed in intermediate cell nuclei. Scalebars are 10 microns.

Image without DAPI labeling (**Figure 6C'**) is shown to emphasize co-expression of *Ednrb*, *Tmem176a*, and *Met* in the intermediate cell layer. Yellow dashed line boxes delineate two representative regions (region #1 and region #2) that are enlarged to serve as representative examples of *Ednrb*, *Tmem176a*, and *Met* RNA co-localizing to intermediate cell nuclei within the SV (**Figures 6D,E**, respectively). While SNPs of uncertain significance for *MET* were identified in patients with familial Meniere's disease (22), PBMCs from Meniere's patients demonstrate increased expression of both *EDNRB* and *TMEM176A* compared to control patients (23). These examples validate the ability of these datasets to localize genes implicated in Meniere's disease to specific cell types in the adult stria vascularis.

DISCUSSION

Meniere's disease may represent multiple disease entities with a common set of presenting symptoms. The identification of

a heterogeneous group of mutations in human genes with variable expressivity within familial MD cohorts (74) and an increased burden of rare missense variants in several SNHL genes (27) and axonal guidance signaling genes (24) in sporadic MD in the Spanish population serve as examples of evidence that support this contention. Given this suspected heterogeneity, single-cell and single-nucleus transcriptional datasets offer the opportunity to localize genes identified in poorly understood diseases like MD to involved cell types and tissue structures. Gene ontology analysis of systematically curated genes implicated in MD suggests that these genes may play a role in ion homeostasis and immune function and functional analysis suggests that these genes are involved in metabolism, transport, intercellular and transmembrane cell signaling, and protein processing. While possibly not surprising given that ion homeostatic dysfunction and immune dysfunction (10, 25, 30, 75) have been postulated mechanisms underlying MD likely motivating at least some of the reviewed studies, this review utilizes an integrative approach to derive a top-down perspective on genes implicated in MD. More importantly, we utilize these systematically curated genes implicated in MD and single-cell transcriptional profiles from the adult mouse SV to localize these genes to the adult stria vascularis and for the first time, implicate SV cell types in the underlying pathophysiology of MD. Based on these results, we suggest that these data have several implications.

Localization of Genes Implicated in Meniere's Disease to Major SV Cell Types Suggests That Dysfunction in These Cell Types May Contribute to Mechanisms Underlying Meniere's Disease

First, the larger number of genes expressed by marginal and intermediate cells in the SV suggests that these cells may play a more prominent role in MD. The global localization of these implicated genes to major SV cell types is supported by both our previous work in localizing *Kcne1*, *Atp13a5,* and *Esrrb* RNA to marginal cells, *Met* RNA to intermediate cells, and *Slc26a4* and *P2rx2* RNA to spindle cells (7) as well as newly localized gene candidates to major adult SV cell types, including *Ednrb* and *Tmem176a* (**Figure 5**). Connexin 26 (GJB2) and Connexin 30 (GJB6) protein have been previously localized to SV intermediate and basal cells in humans (76, 77) and in mouse (78). While localization of expression does not necessarily imply functional significance, the localization of many genes to specific cell types in the SV may suggest that understanding the roles of these cell types may be critical for insight into the underlying pathophysiology of MD.

Implications for the Development of Mouse Models of Meniere's Disease

In localizing Meniere's disease genes to major SV cell types, we suggest that this points to the need to better understand the role that dysfunction in each of these cell types plays in hearing instability and loss. Work by Gallego-Martinez and colleagues suggests the possibility that the accumulation of missense variants in some which include genes expressed by SV cell types may contribute to the development of MD (27). Meniere's disease has an onset of disease in adulthood (79–81) and this suggests a need for inducible mouse models of gene dysfunction which would distinguish the effects of gene dysfunction on hearing in the mature cochlea from those related to dysfunction during development. More generally, mouse models that replicate hearing fluctuation seen in human patients with MD could serve as useful pre-clinical models to examine the mechanism and efficacy of repurposed and novel therapeutics for MD.

Implications for Mechanisms Underlying Meniere's Disease

Furthermore, investigating dysfunctional calcium homeostasis may be important to understanding mechanisms underlying hearing loss in MD. Gene ontology analysis of genes implicated in MD identified mechanisms involving calcium ion transport as being enriched. Of these genes, single-cell and single-nucleus RNA-Seq datasets demonstrate expression of *Atp13a5*, *Cacna2d1*, and *Trpv4* in SV marginal cells, *Ank2*, *Cav1*, and *Wfs1* in SV intermediate cells, and *P2rx2* in SV spindle cells. Of these genes, evidence of decreased expression in MD has been noted for *Atp13a5*, *Cacna2d1*, and *Ank2* (23) while *Cav1* expression has been shown to be increased in patients with MD (23, 31). While it has been suggested that *TRPV4* expression may be decreased in human endolymphatic sac tissue from MD patients (82), a subsequent case-control replication study examining *TRPV4* expression failed to demonstrate an association between *TRPV4* expression and MD (83). Expression of *TRPV4* in the cochlea in patients with MD has not been compared to unaffected human patients. Missense variants of uncertain significance for *WFS1* were identified in patients with MD but a significant excess of these variants was not seen when compared to control populations (27). In contrast, an excess of *P2RX2* missense variants was noted in patients with MD (27). In an experimental model for endolymphatic hydrops, Salt and DeMott have previously demonstrated that elevations in calcium concentration in the endolymph, at a time when endolymph volume and the EP are no longer changing, correlates with elevated auditory thresholds (84). These authors suggest that the gradual dysregulation of calcium concentration in the endolymph while associated with endolyphatic hydrops, may be the underlying mechanism of hearing loss in these settings. Providing further evidence to support this theory, Wangemann and colleagues have shown that the loss of *Slc26a4* expression in a mouse model for Pendred syndrome results in acidification of the endolymph, a failure of calcium reabsorption from the endolymph, and hearing loss (85). Thus, these data suggest that dysfunctional calcium homeostasis within the endolymph may potentially contribute to mechanisms resulting in the development of MD.

Limitations

Despite these observations, several caveats apply to the data presented. A large proportion of gene expression changes for genes implicated in MD were determined from expression changes in the peripheral blood mononuclear cells, which may not reflect changes in the inner ear. Furthermore, genomic features, including single nucleotide variants, copy number variants, and other structural variants have not been systematically and uniformly examined in relation to these investigated genes, potentially contributing to bias in the interpretation of the genomic underpinnings of Meniere's disease. Nonetheless, we localize these genes implicated in MD to the SV providing a context for beginning to understand these observed changes and identifying potentially relevant inner ear gene targets. Furthermore, identifiable expression does not necessarily equate to functional importance. Finally, while we acknowledge the limited ability to connect functional attribution of broad expression changes in the blood to mechanisms underlying MD, identifying meaningful gene and cellular targets in the SV establishes a basis for testing hypotheses related to the underlying pathophysiology of MD.

In conclusion, utilizing single-cell and single-nucleus transcriptional profiles, we localize genes implicated in MD to adult stria vascularis cell types. We identify trends in potentially involved SV cell types based on this top-down approach and in doing so, provide justification for the development of inducible cell type-specific models of SV dysfunction as a means of investigating the underlying pathophysiology of MD. Finally, we provide evidence of the reliable ability of our published transcriptional profiles to localize several candidate gene targets to specific SV cell types, establishing a justification for testing the role of these candidate genes in MD.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found at: Previously published single cell and single nucleus RNA-Seq datasets of postnatal day 30 (P30) mouse stria vascularis (7) were utilized (GEO Accession ID: GSE136196) which can be found at the following link (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE136196) and are available through the gene Expression Analysis Resource (gEAR), a website for visualization and comparative analysis of multi-omic data, with an emphasis on hearing research (https://umgear.org//index.html?layout_id=b50cae7a) (37).

AUTHOR CONTRIBUTIONS

SG and MH contributed to bioinformatic analysis of previously published scRNA-Seq and snRNA-Seq datasets. RO and MH were responsible for smFISH and immunohistochemistry. LN performed systematic review of Meniere's disease-implicated genes. SG, RO, LN and MH contributed to primary draft of manuscript. SG, LN, AG-M, JL-E, and MH contributed to critical revising and editing the manuscript. All authors read and approved final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.630561/full#supplementary-material

Supplementary Figure 1 | Flow chart depicting systematic review of genes implicated in Meniere's disease. Results of PRISMA search strategy and PubTerm search were combined and PRISMA systematic review criteria were applied to all identified references. In total, 389 unique abstracts were identified. Abstracts unrelated to Meniere's disease ($n = 196$) were excluded. The following exclusion criteria including non-English language ($n = 22$), animal studies ($n = 21$), non-gene outcome defined as an absence of genes studied in relation to Meniere's disease ($n = 45$), and unrelated to Meniere's disease ($n = 28$) were applied to a full-text review of these references resulting in 77 references being included for systematic review.

Supplementary Figures 2–4 | Expression of Meniere's disease implicated genes without cell type-specific expression in the adult mouse SV as demonstrated by single-cell RNA-Seq. Heatmap displays cell types along the horizontal axis and genes along vertical axis. Gene expression is displayed in normalized counts. Cell types displayed include marginal cells, intermediate cells, basal cells, spindle-root cells, fibrocytes, and macrophages.

Supplementary Figures 5–9 | Expression of Meniere's disease implicated genes without cell type-specific expression in the adult mouse SV as demonstrated by single-nucleus RNA-Seq. Heatmap displays cell types along the horizontal axis and genes along vertical axis. Gene expression is displayed in normalized counts. Cell types displayed include marginal cells, intermediate cells, basal cells, spindle cells, root cells, Reissner's membrane cells, and macrophages.

Supplementary Figure 10 | Expression of Meniere's disease implicated genes in the developing mouse cochlea as see in the Allen Brain Atlas. **(A)** In the E15.5 mouse, *Sgk1* is expressed in the organ of Cort and the roof of the cochlear duct where future marginal cells reside. **(B)** In the E15.5 mouse, Cacna2d1 is localized to the roof of the cochlear duct where future marginal cells reside. **(C)** Eya4 is widely expressed in the cochlear duct including the region of the future stria vascularis at E15.5. Scale bars are 200 microns.

Supplementary Data 1 | Descriptive tables summarizing studies in humans implicating genes in Meniere's disease.

Supplementary Data 2 | Accompanying lists of genes for human studies that examined larger groups of genes as referred to in **Supplementary Data 1**.

Supplementary Table 1 | Genes investigated in relation to Meniere's disease.

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OPINION
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Ten Vestibular Tools for Primary Care

Otto R. Maarsingh and Vincent A. van Vugt*

Department of General Practice, Amsterdam University Medical Center (UMC), Vrije Universiteit Amsterdam, Amsterdam Public Health, Amsterdam, Netherlands

Keywords: vestibular symptoms, vertigo, dizziness, primary care, general practice, diagnosis, treatment, prognosis

INTRODUCTION

Although primary care physicians (PCPs) regularly encounter patients with dizziness or vestibular symptoms, they often consider these patients as difficult, challenging or even heartsink (1, 2). Given the current scientific evidence and available "vestibular tools," this is unnecessary. We will provide ten vestibular tools that should not be missed, following definition, diagnosis, treatment, and prognosis, respectively (**Figure 1**).

DEFINITION

When approaching a potentially complex problem, the use of a uniform nomenclature is crucial. To date, most primary care guidelines use the typology of Drachman and Hart (3). This typology distinguishes four dizziness subtypes, i.e., vertigo (rotational dizziness), presyncope (lightheadedness), disequilibrium (unsteadiness when walking), and non-specific dizziness. The Drachman-Hart typology is primarily based on how patients describe the nature of their symptoms, assuming that this will provide etiological insight, and therefore, diagnostic guidance (4, 5). However, both doctors and patients use the term "vertigo" differently (6–8), patients are inconsistent when describing their symptoms (7), the identified subtype does not reliably match the suggested etiology (5, 9), and regularly patients have more than one dizziness subtype (10). Therefore, it is time to leave the Drachman-Hart typology and to adopt a more accurate and uniform way to describe vestibular symptoms. The Bárány society, the leading international organization for clinicians and researchers involved in vestibular medicine, previously realized such a nomenclature: the International Classification of Vestibular Disorders (ICVD) (11, 12). The ICVD identifies four main vestibular symptoms, i.e., dizziness ("the sensation of disturbed or impaired spatial orientation without a false or distorted sense of motion"); vertigo ("the sensation of self-motion when no motion is present or the sensation of distorted self-motion during normal head movement"); vestibulovisual symptoms ("visual symptoms that result from vestibular pathology or visual-vestibular interaction"); postural symptoms ("balance symptoms related to maintenance of postural stability, occurring only while upright—seated, standing, or walking") (13). These vestibular symptoms are not specific in terms of etiology, not overlapping, and not hierarchical (a single patient can experience multiple symptoms) (13). When assessing a patient with vestibular symptoms, the Bárány society recommends to focus on timing (onset, duration, and evolution of symptom) and triggers (actions, movements, or situations that provoke onset of symptoms) (11, 12). Combining the mentioned vestibular symptoms with timing and triggers results in three vestibular syndromes, i.e., acute vestibular syndrome (AVS), episodic vestibular syndrome (EVS), and chronic vestibular syndrome (CVS). AVS is defined as acute-onset, continuous vertigo/dizziness, lasting days to weeks, generally including symptoms that suggest new dysfunction of the vestibular system (like vomiting, nystagmus, and severe postural instability). Disorders presenting with AVS include vestibular neuritis, labyrinthitis, stroke affecting vestibular structures, and traumatic vestibulopathy. EVS is defined as transient vertigo/dizziness

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Edited by:
Eva Grill,
Ludwig Maximilian University of Munich, Germany

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Filipp Filippopulos,
LMU Munich University Hospital, Germany

****Correspondence:***
Otto R. Maarsingh
o.maarsingh@amsterdamumc.nl

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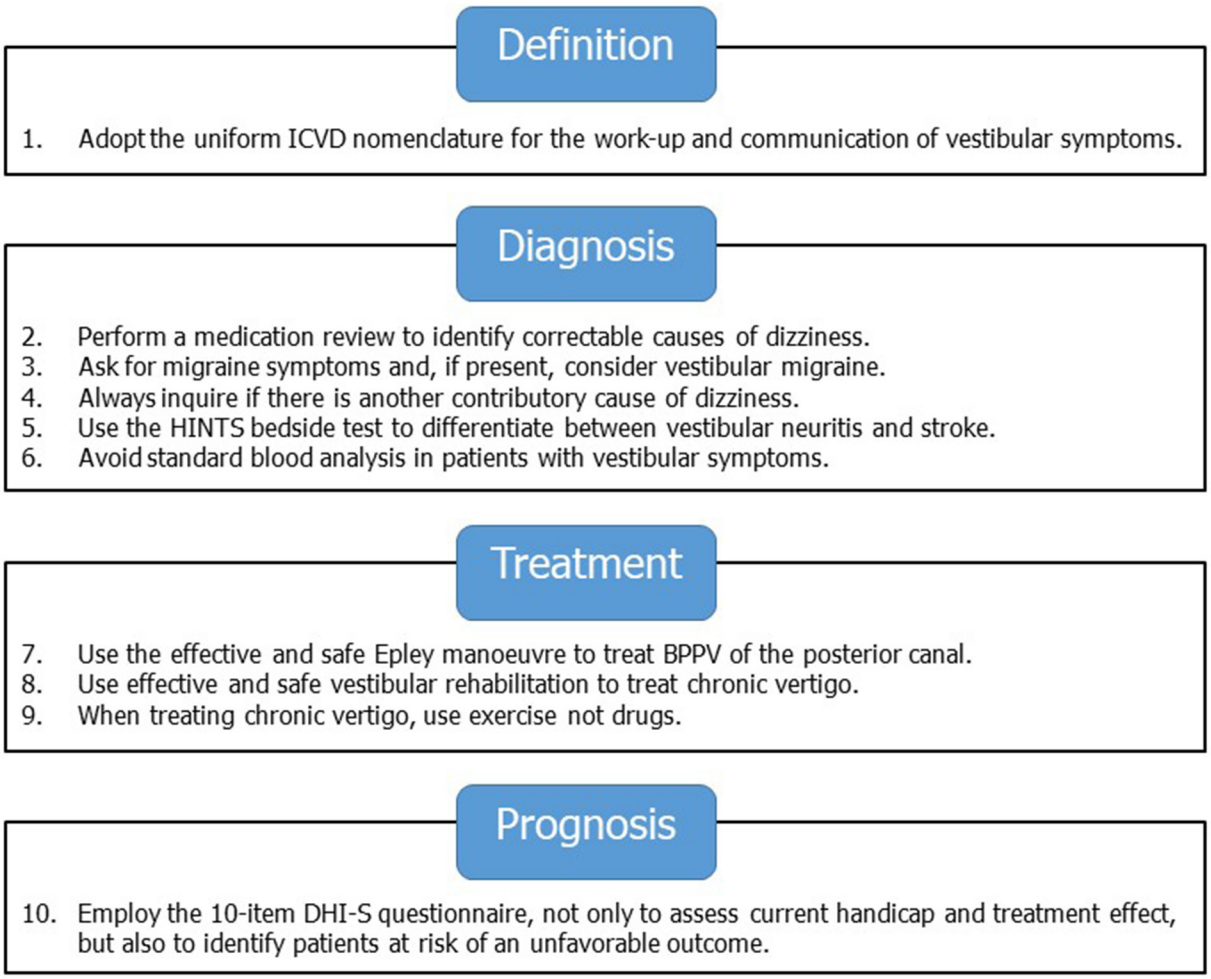

FIGURE 1 | Ten vestibular tools for primary care.

lasting seconds to hours, generally including symptoms that suggest temporary dysfunction of the vestibular system (like nausea, nystagmus, and sudden falls). Disorders presenting with EVS include vestibular migraine, benign paroxysmal positional vertigo, Menière's disease, and panic attacks. CVS is defined as chronic vertigo/dizziness lasting months to years, generally including symptoms that suggest persistent dysfunction of the vestibular system (like oscillopsia, nystagmus, and gait unsteadiness). Disorders presenting with CVS include poorly compensated vestibulopathy, bilateral vestibulopathy, and persistent postural perceptual dizziness (13). In short, the ICVD nomenclature provides an essential tool for the work-up and communication of vestibular symptoms in primary care (tool #1).

DIAGNOSIS

Up to 40% of patients presenting with vestibular symptoms in primary care remain undiagnosed (14, 15). Although this is not unusual for comparable reasons for encounter (like tiredness), we firmly believe it is possible and necessary to reduce the number of undiagnosed dizzy patients in primary care. An accurate diagnosis starts with thorough history taking, focusing on symptom characteristics, timing, and triggers according to the ICVD.

During history taking, the importance of a medication review is apparent. Although an adverse drug effect is a rare cause of vertigo/dizziness in younger patients, it is much more prevalent and regularly missed in older patients. Previous research studies showed that Dutch PCPs scarcely reported adverse drug effect as a cause of dizziness in older patients (1–3%) (14, 15), whereas a diagnostic panel study among the same population found a much higher proportion (25%) (10). Drug-induced vertigo can be caused by aminoglycosides, azithromycin, pregabalin, mefloquine, and α-blockers, whereas drug-induced dizziness can be caused by anticonvulsants, antidepressants, anti-psychotics, β-blockers, Calcium channel blockers, antiarrhythmics, diuretics, vasodilators, anxiolytics, and antispasmodics (16–18). As a medication review costs little time and may provide much insight

(i.e., clues for intervention), it should not be missed in the diagnostic phase (*tool #2*). During such a review, a practical guide may help to rapidly identify potential adverse drug effects (19).

A more common cause of episodic vertigo is vestibular migraine (VM). This is a migraine variant with vestibular symptoms and poorly understood pathophysiology. Despite its prevalence and high impact on healthcare cost and utilization, VM remains clinically underdiagnosed (20). The diagnostic criteria for VM include the presence or history of migraine, at least five episodes with vestibular symptoms of moderate or severe intensity and at least 50% of episodes associated with migraine features (i.e., headache, motion sensitivity, photo- or phonophobia, or visual aura) (21). We recommend physicians to structurally ask for migraine symptoms (*tool #3)*, and, if present, consider vestibular migraine (VM).

Another small but effective tool, especially regarding older patients, is to incorporate the following question in your diagnostic work-up: Is there another contributory cause of dizziness? (*tool #4*) According to a diagnostic panel study among 417 older dizzy patients in Dutch primary care, 62% had two or more contributory causes of dizziness (10). Among a consecutive cohort of 621 patients in tertiary care (average age 56 years, range 11–90 years), 30% of dizzy patients had more than one diagnosis (22).

If the history reveals red flags (e.g., neurological symptoms, new headache, or acute deafness) (23), it is important to minimize the probability of a central cause of dizziness. However, when sharpening one's diagnostic tools, population awareness is crucial: the prior probability of a central cause of dizziness in a primary care population is very low compared to secondary/tertiary care. In a study cohort that consisted of patients hospitalized with isolated vertigo, the risk for stroke during 4-year follow-up was 3.01-times higher compared to the general population; vertigo patients with three or more risk factors (including age >55 years, male gender, hypertension, diabetes, coronary artery disease, and hyperlipidemia) even had a 5.51-fold higher for stroke (24, 25). However, in a surveillance study among patient presenting with dizziness symptoms to the emergency department, only 0.7% with isolated dizziness symptoms had a stroke/TIA (26). According to the ecology of medical care (27), this proportion will be even lower for patients presenting with the same symptoms in primary care. In case of an acute vestibular syndrome (i.e., rapid onset of vertigo, nausea/vomiting, and gait unsteadiness in association with head-motion intolerance and nystagmus) another promising tool comes in: the three-step Head Impulse–Nystagmus–Test of Skew (HINTS) exam (HINTS; *tool #5*). The HINTS exam is a simple bedside test that is relatively easy to learn (https://medicinetoday.com.au/vertigovideos). The HINTS exam can help differentiate between vestibular neuritis and stroke, because the presence of any of three oculomotor signs (normal horizontal head impulse; gaze-direction nystagmus; or skew deviation) indicates a central cause of acute vestibular syndrome. A recent systematic review revealed that the HINTS exam has a pooled sensitivity of 96% and specificity of 71% to detect stroke (28), which indicates an even higher diagnostic accuracy than early MRI (29).

When revising one's diagnostic tools, it is important to reconsider overrated tools. According to an observational study (n = 2,812), Dutch PCPs performed blood analyses in 22% of older patients presenting with dizziness (15). Until present, there is no scientific evidence that standardized blood analysis has additional value during the work-up of vestibular symptoms. Among 4,538 patients included in etiologic studies, laboratory abnormalities that explained dizziness were limited to three patients with electrolyte disturbances, 11 with glucose disorders, 11 with anemia, and one with hypothyroidism (30). In a community based study, the results of standardized blood analysis among 149 dizzy subjects and 97 controls did not differ (31). Therefore, use blood tests only on a strict medical indication and avoid standard blood analysis in patients with vestibular symptoms (*tool #6*).

TREATMENT

A very rewarding vestibular tool is the Epley maneuver (*tool #7*). This is a relatively simple, safe, and highly effective treatment for the most prevalent cause of episodic vertigo, i.e., benign paroxysmal positional vertigo of the posterior canal—a diagnosis that can be confirmed by using the Dix-Hallpike test (https://www.youtube.com/watch?v=kEM9p4EX1jk&feature=youtu.be) (32). Despite its proven effectiveness, PCPs have not yet embraced the Epley maneuver. During a survey among 426 PCPs, only 57% used the Epley maneuver. The most common reason (50%) for PCPs not to use the maneuver was that they did not know how to perform the technique (33). The second reason (30%) was not being convinced of its effectiveness. Both deserve reconsideration, as the Epley maneuver can be easily learned (https://medicinetoday.com.au/vertigovideos) and the scientific evidence is convincing [Epley vs. sham maneuver, complete resolution of vertigo: OR 4.42 (95% CI 2.62–7.44); Epley vs. sham maneuver, conversion of Dix-Hallpike: OR 9.62 (95% CI 6.0–15.42)] (32).

Another effective, safe, and neglected tool is vestibular rehabilitation (VR; *tool #8*). VR is an exercise based treatment that gradually stimulates the vestibular system and vestibular compensation (34). Chronic vertigo occurs when natural vestibular compensation fails (35). Although a clear definition is lacking (36), chronic vertigo is—based on clinical course and expert opinion—often defined as symptoms persisting more than 1 month (17, 37). In primary care, physicians can refer patients to a specialized physiotherapist for VR. Despite the scientific evidence for VR, < 10% of PCPs in the Netherlands and UK reported its use (33, 38). As this may be due to a lack of availability or access to VR (38), the University of Southampton developed a freely available online VR intervention (https://balance.lifeguidehealth.org). This online VR intervention was investigated among two different cohorts in primary care (n = 296 and n = 322, respectively), showing both reduction of dizziness and dizziness-related impairment after 3 and 6 months (39, 40). Being easily accessible, safe, effective and low cost, online VR has the potential to substantially improve the quality of life for a largely undertreated group of patients.

Although nowadays VR is the preferred treatment for chronic vertigo according to US, Dutch, and UK clinical practice guidelines (17, 41–43), anti-vertigo drugs like betahistine are still regularly prescribed. According to a large observational study, betahistine was initially prescribed to more than two thirds of vertigo patients in general practice and was still being used after 6 months (44). This enthusiastic prescribing contradicts with the state of the science, though, as a recent Cochrane review showed only weak evidence for the effectiveness of betahistine to treat chronic vertigo (45). Also, long term prophylactic treatment with betahistine does not change the time course of vertigo episodes related to Meniere's disease compared with placebo (46). When using GRADE methodology to compare VR (4 RCTs, $n = 565$ adults with different causes of chronic vertigo) with betahistine (11 RCTs, $n = 606$ adults with different causes of chronic vertigo), there is a difference in effectiveness and quality of evidence: vertigo patients receiving VR reported higher improvement compared to sham/no treatment [odds ratio 2.67 (95% CI 1.85–3.86); moderate quality of evidence], whereas vertigo patients treated with betahistine reported limited improvement compared to placebo [risk ratio 1.30 (95% CI 1.05–1.60); low quality of evidence]. In contrast to VR, none of the betahistine trials was conducted in primary care, which limits the generalizability (47). In short, when treating chronic vertigo, use exercise not drugs (*tool #9*).

PROGNOSIS

Until present, many risk factors of handicapping dizziness and/or vertigo have been identified, like chronic dizziness, daily dizziness, activity limitation or avoidance due to dizziness, anxiety or depression, polypharmacy, and impaired functional mobility (48–51). One of the most powerful predictors of an unfavorable course of dizziness, though, is significant impairment at baseline as measured with the Dizziness Handicap Inventory (DHI) (49, 52). The DHI is a 25-item self-report questionnaire, developed to measure impairment due to vestibular symptoms (53). Nowadays, it has been translated in at least 17 languages and considered to be the most used vestibular PROM (54). However, the length of the DHI limits its use in daily clinical practice. Therefore, the abbreviated 10-item DHI-S questionnaire (fill-in time ± 2 min) was developed in 1998 (55). During a psychometric evaluation in primary care, the DHI-S showed excellent criterion validity, test-retest reliability, and responsiveness (56). Recently, a prediction study with external validation in primary care showed that the ability of the DHI-S to identify patients at risk of an unfavorable course of dizziness improved when combined with the predictors age, history of arrhythmia, and looking up as a provoking factor (area under the curve after external validation = 0.78) (52). Given the fact that—in addition to its prognostic qualities—the DHI-S provides information on current handicap and can be used to monitor treatment effect, we believe that this questionnaire should not be missed in the vestibular toolkit of the PCP (*tool #10*).

CONCLUSIONS

In this article, we present ten vestibular tools for primary care. PCPs can use these tools to improve diagnosis, treatment, and prognosis of vestibular symptoms. All tools are readily available and do not require intensive training. By simplifying proper management of vestibular symptoms, we hope that PCPs will embrace dizziness as an exciting symptom.

AUTHOR CONTRIBUTIONS

OM and VV wrote and approved the manuscript. All authors contributed to the article and approved the submitted version.

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Association Between Circular RNAs and Intracranial Aneurysm Rupture Under the Synergistic Effect of Individual Environmental Factors

Qing Huang[1†], Yi Sun[2†], Qiuyu Huang[1†], Yile Zeng[1†], Shaowei Lin[2], Shuna Huang[2], Yingying Cai[2], Xingyan Xu[2], Dezhi Kang[3], Huangyuan Li[2*] and Siying Wu[2*]*

[1] Department of Neurosurgery, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, China, [2] School of Public Health, Fujian Medical University, Fuzhou, China, [3] Department of Neurosurgery, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China

Introduction: To study the association between specific circular RNAs and rupture of intracranial aneurysm. To explore its clinical diagnostic significance and synergistic effects with individual environmental influencing factors.

Methods: Three hundred and forty seven cases and controls were included in this study. Multivariate analysis was used to explore the main individual environmental factors. Intracranial aneurysm rupture related circular RNAs screened based on sequencing was verified in peripheral blood by PCR. ROC curve, logistic regression model and fork analysis were used to study the association, diagnostic values, and synergistic effects of circular RNA with intracranial aneurysms and individual environmental factors.

Results: Smoking, hair dyeing, sitting time ≥6 h/day, single animal oil intake and hypertension are the main risk factors for intracranial aneurysm rupture; People with higher education, sleeping time ≥7 h/day, tea drinking, diabetes, higher levels of (hemoglobin, low density lipoprotein, serum calcium, and apolipoprotein-A1) have a low risk of intracranial aneurysm rupture. Hsa_circ_0008433 and hsa_circ_0001946 are closely related to intracranial aneurysm rupture and have certain clinical diagnostic significance (AUC = 0.726; 95% CI: 0.668∼0.784). Hsa_circ_0008433 (OR = 0.497, 95% CI: 0.338∼0.731), hsa_circ_0001946 (OR = 0.682, 95% CI: 0.509∼0.914) were independent epigenetic factors affecting intracranial aneurysm rupture, and have a multiplicative interaction with age (OR = 3.052, 95% CI: 1.006∼9.258).

Conclusions: Low expressions of hsa_circ_0008433 and hsa_circ_0001946 are risk factors for intracranial aneurysms rupture and have good clinical diagnostic value. There was a multiplicative interaction between epigenetic score and age. The older and the higher the epigenetic score was, the more likely to have intracranial aneurysm rupture.

Keywords: individual environmental factors, circular RNAs, intracranial aneurysm, high-throughput sequencing, diagnostic value

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Jian Zhang, Tianjin Medical University, China

***Correspondence:**
Siying Wu
fmuwsy@163.com
Huangyuan Li
fmulhy@163.com
Dezhi Kang
kdzy99988@163.com

[†]These authors have contributed equally to this work

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INTRODUCTION

Intracranial aneurysm (IA) is a localized abnormal bulging caused by high blood flow due to congenital weakness of the cerebral artery wall or acquired lesions (1). About 0.7 to 1.9% of IA can rupture under various inducements, leading to aneurysmal subarachnoid hemorrhage. The annual incidence of IA rupture worldwide is about 9.1 per 100,000, accounting for 3 to 5% of all acute strokes. The age of onset is between 40 and 60 years old, the mortality rate is close to 40%, and 46% of survivors can be disabled or long-term cognitive impairment due to multiple complications (2–5). Therefore, it is called the "time bomb" in the brain, which seriously threatens human life and health (6). Current research showed that the traditional risk factors for IA rupture are smoking, alcoholism, high blood pressure, gender, drugs, and blood lipid levels, etc (7–13). However, under the current social and economic development, the tremendous changes in people's life and habits will inevitably be accompanied by new individual environmental impact factors, and these new impact factors are not yet very clear.

Circular RNA is a type of novel nucleic acid molecule that does not have a characteristic 5'cap end and 3'poly (A) end, and exists in the form of a covalent loop (14). It is widely distributed, highly conservative in structure, and has specificity of temporal expression and spatial distribution. At present, circRNA has rapidly become a hot spot in the field of cerebrovascular disease research, and has been deeply explored as an emerging biological marker and therapeutic target (15). Recent studies have found that circRNA can competitively bind to specific miRNA through sponge adsorption to regulate the expression of downstream target genes. The related mechanism is called competitive endogenous RNA (ceRNA). A large number of subsequent studies have confirmed that the ceRNA mechanism involves many important pathological processes such as inflammation, SMC phenotype transformation and extracellular matrix, which play an important role in epigenetic regulation in vascular diseases (16, 17). However, there are still few studies on the relationship between circRNA and IA rupture recently and its synergistic effects with individual environmental factors are even rarer.

In this study, a questionnaire survey was conducted on 347 cases of IA rupture and 347 healthy examinees. Logistic regression analysis was used to study the individual environmental factors that affect the rupture of IAs. At the same time, based on the previous high-throughput sequencing results, the selected IA rupture-related circRNA were verified in the population peripheral blood, the potential correlation of these specific circRNA expression patterns in IA tissues and peripheral blood was further explored.

Abbreviations: IA, intracranial aneurysm; circRNA, circular RNA; ceRNA, competitive endogenous RNA; CTA, computed tomography angiography; MRA, magnetic resonance angiography; DSA, digital subtraction angiography; OR, odds ratio; 95%CI, 95% confidence interval; AUC, area under the ROC curve.

MATERIALS

Study Population

A total of 347 patients with IA rupture and 347 healthy persons who were admitted to the Neurosurgery and Physical Examination Center of the Second Affiliated Hospital of Fujian Medical University from June 2017 to September 2019 were selected as case and control groups for individual environmental factors study. At the same time, 140 subjects with matching baseline data were selected from the case and the control group, their peripheral blood was collected for PCR experiments. Case group inclusion criteria: ① Age> 18 years old, and first onset; ② IA rupture confirmed by computed tomography angiography (CTA), magnetic resonance angiography (MRA), or digital subtraction angiography (DSA); ③ There was no significant systemic disease that might affect the indexes; ④ The medical record is complete and willing to cooperate with the questionnaire. Case group exclusion criteria: ① Those with the first head CT scan or negative lumbar puncture; ② Patients with a clear history of subarachnoid hemorrhage or a family history of severe cerebrovascular disease; ③ Those with previous stroke, brain tumor, or history of craniocerebral surgery; ④ Patients with coagulopathy, fever, or pregnancy. Control group inclusion criteria: ① Immediate relatives of IA or a family history of a clear subarachnoid hemorrhage; ② Patients with previous stroke, brain tumor, or history of craniocerebral surgery; ③ Recently infected or pregnant; ④ Patients with systemic severe chronic diseases; ⑤ People with incomplete clinical data or refuse to participate in this study. All research subjects signed an informed consent form and approved by the Ethics Committee of the Second Affiliated Hospital of Fujian Medical University (2018-50).

Questionnaire

A unified structured questionnaire is adopted, and the research objects are investigated by strictly trained staff with certain epidemiological and clinical work experience. The content includes general conditions such as gender, age, occupation, education, marital status, personal medical history, and so on. Individual behaviors include smoking, drinking alcohol and tea, chemical poison exposure, taking medicine, sitting and sleeping time per day, physical exercise and labor intensity. Eating habits mainly include eating habits and edible oil types. Define smokers who smoke $\geq$1 cigarette per day for >6 consecutive months or cumulatively smoke $\geq$100 cigarettes; Define a single drinking volume of $\geq$50 g as drinking; Drinking tea $\geq$1 cup per week for more than 6 months is defined as drinking tea; Actively participate in sports activities at least once a week and last at least 20 min each time is defined as physical exercise (18). The daily sleep time of adults over 18 years old <7 h is defined as insufficient sleep, and the daily sleep time $\geq$9 h is defined as excessive sleep (19). Sedentary or semi-recumbent time >6 h was definited as sedentary (20).

RNA Extraction

Two milliliters peripheral venous blood was extracted and centrifuged. Lymphocyte separation solution was added to the blood cell layer, the suspension layer of white blood cells

was further extracted. Then absorb the leukocyte suspension layer and add Trizol to fully lyse, further extract the total RNA of the peripheral blood according to the kit instructions. The NanoDrop® ND-2000 nucleic acid quantifier was used to determine the RNA content. The specimens with OD260/280 values between 1.80 and 2.00 were considered qualified samples.

cDNA Synthesis and PCR

Strictly follow the instructions of PrimeScript™ RT reagent Kit-RR037 kit for cDNA synthesis of circular RNA. According to the instructions of the TB Green™ Premix EX Taq™-RR820 kit, the Real-Time PCR System amplifier (Light Cycler 480) was used for qRT-PCR detection, establish 20 μl system. The reaction conditions are: Pre-denaturation 95°C (30 s), 1 cycle; PCR reaction 95°C (5 s), 60°C (34 s), 45 cycles; melting reaction 95°C (15 s), 60°C (1 min), 95°C (15 s), 1 cycle. Take GAPDH as internal reference.

Statistical Analysis

Two independent sample *t*-tests were used to compare the difference between normal distribution quantitative data. Two independent sample rank sum tests (Mann-Whitney *U*) were used to analyze quantitative data that did not obey the normal distribution. Chi-square test was used to infer the overall rate or composition ratio. Multivariate logistic regression was used to analyze the odds ratio (OR) of each variable and the risk of IA rupture and its 95% confidence interval (95% CI). Real-time fluorescence quantitative PCR results are expressed by -ΔΔCT method. To study the interaction between epigenetic indicators and environmental factors by using fork analysis, and further calculate the interaction index (S), attribution ratio (AP), and excess relative risk (RERI).

RESULT

General Demographic Characteristics

A total of 347 cases and controls were included using the propensity score method. Among them, 392 were male (56.48%) and 302 were female (43.51%), with an average age of 51.91 ± 10.15 years. The general demographic characteristics of the case group and the control group are balanced and comparable ($p > 0.05$) (**Supplementary Table 1**).

Univariate Analysis of Individual Environmental Factors in IA Rupture

Correlation Between Behavioral Factors and IA Rupture

The analysis of behavioral factors showed that there were statistical differences between the case and control group in education level, exposure to chemical poisons, daily sitting and sleeping time, physical exercise, tea drinking, and smoking ($p < 0.05$) (**Supplementary Table 2**).

Correlation Between Eating Habits and IA Rupture

The analysis of the eating habits showed that the salty and light diet, edible oil types were statistically different between the case and the control group ($p < 0.05$) (**Supplementary Table 3**).

Correlation Between Specific Physiological Indicators and IA Rupture

The analysis of the physiological indicators showed that diastolic blood pressure and the pulse pressure were statistically different between the case and the control group ($p < 0.05$) (**Supplementary Table 4**).

Correlation Between Specific Biochemical Indicators and IA Rupture

The analysis of the biochemical indicators showed that hemoglobin, low density lipoprotein, triglyceride, cholesterol, serum calcium, apolipoprotein A1, and apolipoprotein B were statistically different between the case and the control group ($p < 0.05$) (**Supplementary Table 5**).

Correlation Between Disease History and IA Rupture

The analysis of the biochemical indicators showed that hypertension and stroke disease and family history are statistically different between the case group and the control group ($p < 0.05$) (**Supplementary Table 6**).

Logistic Regression Analysis of Individual Factors of IA Rupture

Taking IA rupture as the dependent variable, the single factors with statistically significant difference were further included in logistic regression model for analysis (Backward: Wald). The results showed that smoking, chemical poison exposure (hair dye), sitting time >6 h/day, single animal oil intake, hypertension, larger diastolic pressure and pulse pressure differences, higher levels of plasma globulin are the risk factors for IA rupture. Tea drinking, higher education, sleep time >7 h/day, diabetes, and higher levels of (hemoglobin, low density lipoprotein, apolipoprotein A1, and serum calcium) are the protective factors for IA rupture (**Table 1**).

Expression of IA Rupture-Related circRNAs in Peripheral Blood

Based on the previous high-throughput sequencing results, seven IA rupture-related circRNAs were selected (**Supplementary Table 7**), and the reverse splicing technology was used in circ RNA primer design (**Table 2**). Their expression in the peripheral blood of the same patient (the one who provided tissue samples for RNA-Seq) was verified ($n = 4$). The results showed that hsa_circ_0008433 and hsa_circ_0005571 were both highly expressed in IA tissues ($p < 0.05$), but the expression of them in peripheral blood was low and high, respectively (**Figures 1A,B**). Similar to hsa_circ_0008433, hsa_circ_0033144 was highly expressed in IA tissues and low in peripheral blood of the two groups (**Figure 1C**, $p < 0.05$). Hsa_circ_0040809 was highly expressed in IA tissues and low in peripheral blood, but the differences were only statistically significant in the tissue expression (**Figure 1D**, $p < 0.05$). Hsa_circ_0056285 was highly expressed in IA tissues and low in peripheral blood, but the differences were not statistically significant (**Figure 1E**, $p > 0.05$). Hsa_circ_0007142 and hsa_circ_0072309 showed low expression both in IA tissues and peripheral blood, the differences between

TABLE 1 | Logistic regression analysis of individual factors of IA rupture.

Variable	β	S.E	Wald χ^2	*p*	OR (95% CI)
Education level			16.585	<0.001	
Primary school					
Middle school	−0.208	0.232	0.804	0.370	0.812 (0.515∼1.280)
University	−1.125	0.290	15.035	<0.001	0.325 (0.184∼0.573)
Poison exposure			7.698		
No				0.053	
Organic reagents	0.558	0.256	4.757		1.747 (1.058∼2.883)
Pesticides	−0.949	0.685	1.920		0.387 (0.101∼1.482)
Chemical	−0.434	0.584	0.554	0.029	0.648 (0.206∼2.033)
				0.166	
				0.457	
Sitting time	1.218	0.503	5.868	0.015	3.382 (1.262∼9.062)
Sleep time	−0.718	0.352	4.170	0.041	0.488 (0.245∼0.972)
Tea drinking			11.261		
No				0.004	
1∼4 /week	−0.644	0.231	7.746		0.525 (0.334∼0.827)
≥5 /week	−0.899	0.313	8.273		0.407 (0.221∼0.751)
				0.005	
				0.004	
Somking			16.782	<0.001	0.783 (0.346∼1.769)
No					2.545 (1.552∼4.172)
Quit smoking	−0.245	0.416	0.347	0.556	
Smoking now	0.934	0.252	13.717	<0.001	
Drinking			6.333	0.096	
No					
1∼2 /week	−0.910	0.480	3.589	0.058	0.403 (0.157∼1.032)
3∼4 /week	−2.099	1.238	2.874	0.090	0.123 (0.011∼1.388)
≥5 /week	−0.217	0.563	0.149	0.699	0.805 (0.267∼2.424)
Salty diet	0.428	0.240	3.165	0.075	1.534 (0.957∼2.456)
Edible oil type			4.816	0.090	
Vegetable oil					
Animal oil	0.970	0.468	4.295	0.038	2.638 (1.054∼6.602)
Mixed food	0.278	0.267	1.083	0.298	1.321 (0.782∼2.231)
Diastolic pressure	0.045	0.009	26.789	<0.001	1.046 (1.028∼1.064)
Pulse pressure	0.020	0.006	9.413	0.002	1.020 (1.007∼1.033)
Hb	−0.017	0.007	6.456	0.011	0.983 (0.971∼0.996)
GLB	0.072	0.024	8.884	0.003	1.075 (1.025∼1.127)
LDL	−0.221	0.104	4.510	0.034	0.802 (0.654∼0.983)
Ca^{2+}	−1.907	0.657	8.425	0.004	0.149 (0.041∼0.538)
Apo-A1	−0.752	0.305	6.082	0.014	0.471 (0.259∼0.857)
Hypertension	0.857	0.227	14.257	<0.001	2.355 (1.510∼3.675)
Diabetes	−1.303	0.538	5.865	0.015	0.272 (0.095∼0.780)

cases and control group of the two samples were statistically significant (**Figures 1F,G**, $p < 0.05$).

To further study the potential association of circRNA expression in tissue and peripheral blood, the expression of the above-mentioned specific IA rupture-related circRNAs in the same patient's IA tissues and peripheral blood was described (**Figures 2A–G**). Pearson correlation analysis was used to study the expression patterns and correlations of these specific circRNAs in two samples of the same patient. The results showed that the expression of hsa_circ_0008433 ($r = -0.778$, 95% CI: −0.958 to −0.163) and hsa_circ_0033144 ($r = -0.749$, 95% CI: −0.951 to −0.093) in IA tissues was negatively correlated with that in peripheral blood (**Figures 2A,C**). The expression of hsa_circ_0007142 ($r = 0.926$, 95% CI: 0.635∼0.987) in IA tissues was positively correlated with that in peripheral blood (**Figure 2F**).

Detection of Peripheral Blood PCR in Small Sample Population

Screening circRNA Indicators for PCR Detection

Based on the RNA-Seq results and combined with the preliminary experiments (21), we further selected 4 of the 7 circRNAs verified above for PCR detection in the population

TABLE 2 | CircRNA and internal reference gene primer sequences.

circRNA	Primer	Sequence (5′-3′)
β-actin (H)	Forward	5′ GTGGCCGAGGACTTTGATTG 3′
	Reverse	5′ CCTGTAACAACGCATCTCATATT 3′
hsa_circ_0008433	Forward	5′ TCCAAGCATTGCTATTACAACTG 3′
	Reverse	5′CCCTCTTAGGATGTCTGTTATTCA 3′
hsa_circ_0033144	Forward	5′ TGCTCTCACCCACGAAAGG 3′
	Reverse	5′ CTCCACATGGTCAGCCTCTG 3′
hsa_circ_0005571	Forward	5′ TGCGTGTTGGATGAACTTGA 3′
	Reverse	5′ AGGTATAGATTGCCTGTTAGTGG 3′
hsa_circ_0040809	Forward	5′ GCAACAAAGTGCGATGGTGA 3′
	Reverse	5′ CAGCTCTGTACCTGGGTGGTC 3′
hsa_circ_0007142	Forward	5′ TCACAAATTCTTTCTGGAACTCTG 3′
	Reverse	5′ CCGCTCCTCTGGCATCATA 3′
hsa_circ_0072309	Forward	5′ AGTTTTTCCACACCGCTCAA 3′
	Reverse	5′ TCCAGGATGGTCGTTTCAA 3′
hsa_circ_0056285	Forward	5′ GCGTGCAGTACGTGGAGAC 3′
	Reverse	5′ GTCTTCTACAAACTCGTCATACATG 3′
hsa_circ_0001946	Forward	5′ AGTCTTCCATCAACTGGCTCA 3′
	Reverse	5′ GACACAGGTGCCATCGGA 3′
hsa_circ_0000284	Forward	5′ TATGTTGGTGGATCCTGTTCGGCA 3′
	Reverse	5′ TGGTGGGTAGACCAAGACTTGTGA 3′

peripheral blood. Among them, the expression differences of hsa_circ_0005571, hsa_circ_0033144, and hsa_circ_0007142 in case and control group of IA tissues and peripheral blood were statistically significant ($p < 0.05$); hsa_circ_0008433 had a large differential expression multiple in RNA-Seq (FC = 52.077). At the same time, hsa_circ_0001946 (circ-CDR1as) and hsa_circ_0000284 (circ-HIPK3) were selected by reviewing the literature, they are two circRNAs closely related to cerebrovascular diseases that are currently being noticed (**Table 3**) (22, 23).

qRT-PCR Detection of circRNA

Peripheral blood of 30 patients with IA rupture and 30 healthy people were collected for qRt-PCR. The results showed that the expressions of hsa_circ_0008433, hsa_circ_0005571, hsa_circ_0007142, hsa_circ_0001946 in peripheral blood of patients with IA rupture were lower than that of the control group, and the difference was statistically significant ($p < 0.05$) (**Figure 3**).

Detection of Peripheral Blood PCR in Expanded Sample Population

Study Population and General Demographic Characteristics

The peripheral blood of 140 patients with IA rupture and 140 healthy people were further collected for PCR verification in expanded sample population. The general demographic characteristics of the case and the control group were balanced and comparable ($p > 0.05$) (**Supplementary Table 8**).

qRT-PCR Detection of circRNA

Select 4 IA rupture-related circRNA indicators (hsa_circ_0008433, hsa_circ_0005571, hsa_circ_0007142, hsa_circ_0007142, hsa_circ_0001946) with significant expression differences in small sample population peripheral blood for the next verification in an expanded sample population. The results showed that compared with the control group, 75.0% (105/140), 65.71% (92/140), 55.71% (78/140), and 67.14% (94/140) of patients showed down-regulation of the four circRNAs in peripheral blood (**Figure 4**).

The results of qRT-PCR showed that compared with the control group, the expression of hsa_circ_0008433, hsa_circ_0005571, and hsa_circ_0001946 in the peripheral blood of patients with IA were down-regulated, and the difference was statistically significant (**Figures 5A–D**, $p < 0.05$).

Association of circRNA and IA Rupture

A logistic regression model was established to analyze whether these specific circRNAs were independent influencing factors for IA rupture. The results showed that after adjustment of the main individual environmental factors, has_circ_0008433 (OR = 0.497, 95% CI: 0.338∼0.731) and has_circ_0001946 (OR = 0.682, 95% CI: 0.509∼0.914) were independent influencing factors of IA rupture. High-level expression of has_circ_0008433 and has_circ_0001946 in peripheral blood may have a protective effect on IA rupture (**Table 4**).

ROC Curve

ROC curve is used to explore the significance of specific circRNA in the diagnosis of IA rupture. The results show that the area under the ROC curve (AUC) of has_circ_0008433 is 0.7028, the maximum Youden index is 0.3000, and the corresponding cutoff is −1.9051 (**Figure 6A**); The AUC of has_circ_0005571 is 0.617, the maximum Youden index is 0.1928, and the corresponding cutoff is −0.5889 (**Figure 6B**); The AUC of has_circ_0001946 is 0.664, the maximum Youden index is 0.2571, and the corresponding cutoff is −1.6451 (**Figure 6C**, **Supplementary Table 9**).

The three circRNAs were further adopted into the logistic regression equation to construct new combined diagnostic factors and draw ROC curves. The results showed that the AUC of the new combined diagnostic factor was 0.726 (95% CI: 0.668∼0.784), the sensitivity and specificity were 0.793 and 0.564, respectively. Further comparison test results of the ROC curves of these circRNA and new combined factors showed that, hsa_circ_0008433 has higher diagnostic recognition than has_circ_0005571, and when the three circRNAs were jointly predicted, the diagnostic value is better than that of circ_0005571 or circ_0001946 single index ($p < 0.05$) (**Figure 6**, **Supplementary Table 10**).

The Combined Effects of Epigenetics and Other Influencing Factors

has_circ_0008433, has_circ_0005571, and has_circ_0001946 were further adopted in logistic regression model, the case and the control group were divided into high and low risk score according to the median epigenetic risk score of the control

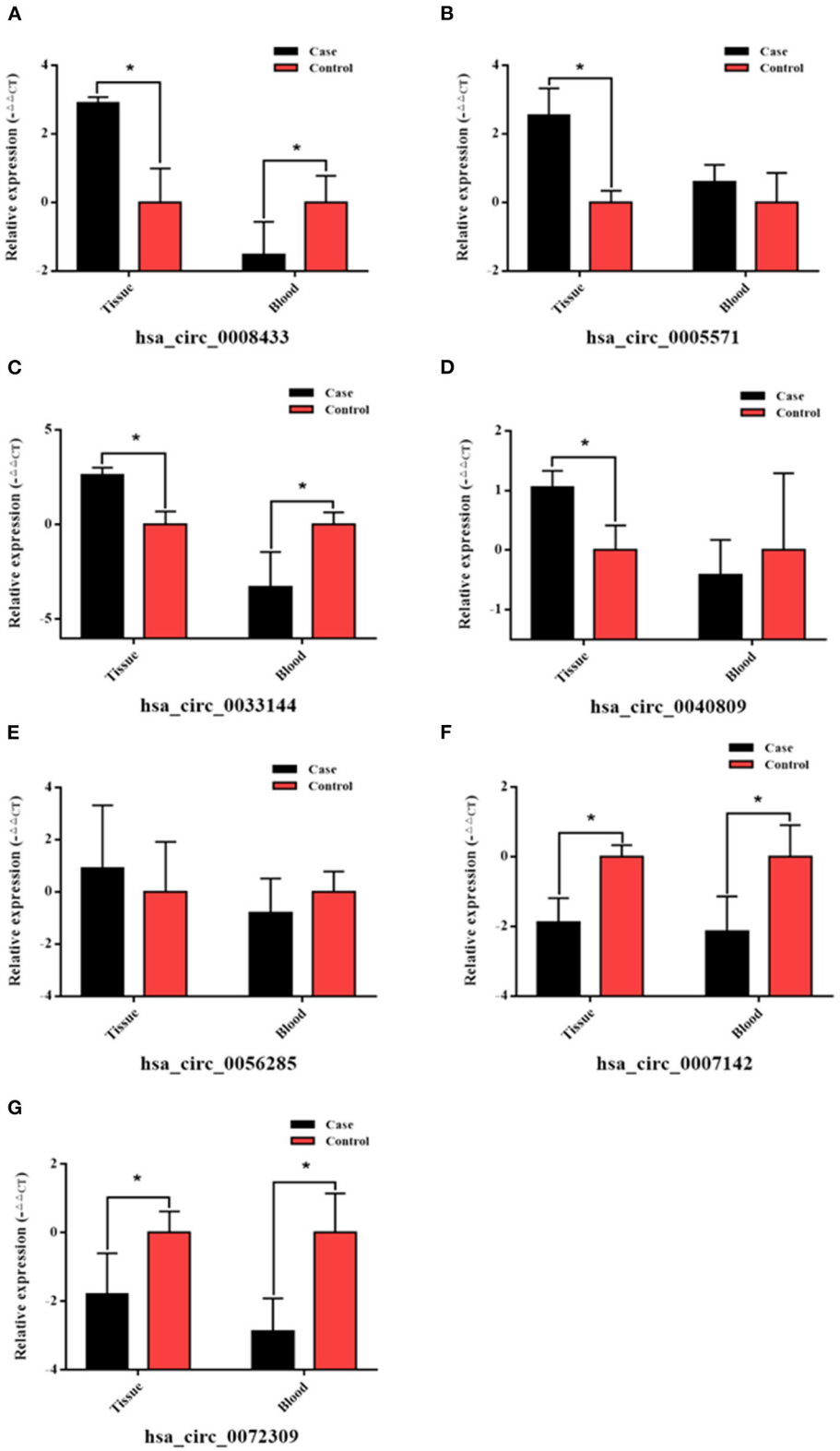

FIGURE 1 | qRT-PCR verification of specific circRNAs in IA tissue and peripheral blood of case and control group. **(A–G)** The PCR results of the 7 selected circRNAs hsa_circ_ (0008433, 0005571, 0033144, 0040809, 0056285, 0007142, 0072309) in IA tissue and peripheral blood, $^{*}P < 0.05$.

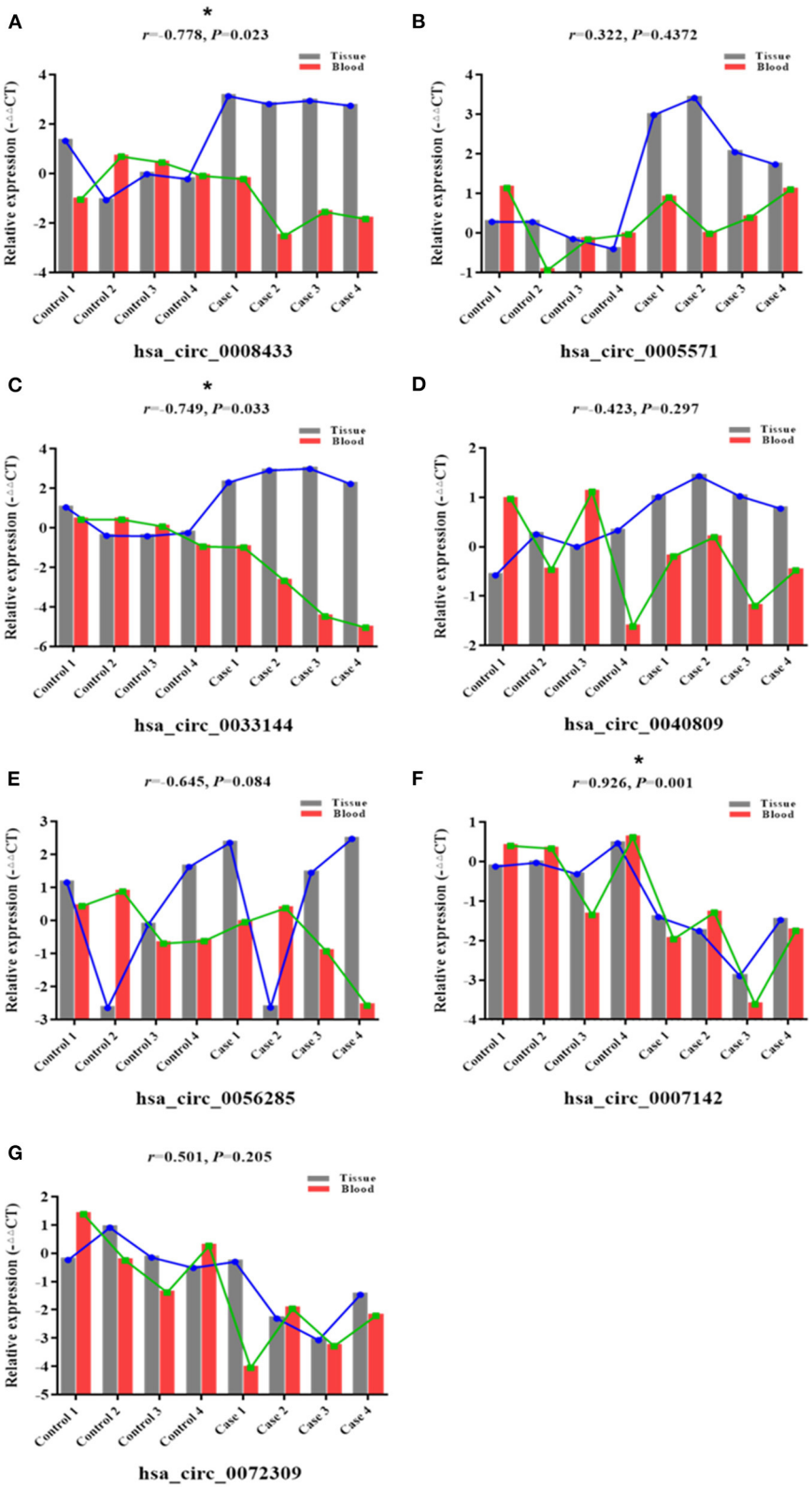

FIGURE 2 | Correlation between specific circRNAs in IA tissue and blood. **(A–G)** Correlations between 7 selected circRNAs hsa_circ_ (0008433, 0005571, 0033144, 0040809, 0056285, 0007142, 0072309) in each IA and STA sample from tissue and peripheral blood, $^{*}P < 0.05$.

TABLE 3 | CircRNAs for PCR in peripheral blood of small sample population.

circRNA ID	Host gene	Length (nt)	Log2 FC	*p*
hsa_circ_0008433	PDE4B	351	5.7026	<0.001
hsa_circ_0033144	BCL11B	369	3.8851	<0.001
hsa_circ_0005571	IFI30	658	3.6338	<0.001
hsa_circ_0007142	DOCK1	427	−4.2062	0.003
hsa_circ_0001946	CDR1	1,485		
hsa_circ_0000284	HIPK3	1,099		

group, and the epigenetic IA risk score was constructed (the higher the score, the greater the risk of IA rupture), so as to explore the joint effects of circRNA epigenetic risk and age. The results show that patients with high epigenetic risk and older (≥55 years old) have an increased risk of IA rupture ($p < 0.05$), which indicated that there is a certain multiplicative interaction between epigenetic factors and age (**Table 5**).

DISCUSSION

Current studies suggest that IA is a complex disease with multiple environmental factors and genetic regulation (24). This study indicated that people with smoking, chemical poison exposure (hair dye), sitting time >6 h/day, single animal oil intake, larger diastolic pressure and pulse pressure differences, higher levels of plasma globulin have higher risk of IA rupture. On the contrary, people with tea drinking habits, higher education, sleep time >7 h/day, diabetes and higher levels of (hemoglobin, low density lipoprotein, apolipoprotein A1, and serum calcium) have a lower risk of IA rupture. After controlling these major individual environmental factors, the low expression of circular RNA has_circ_0008433 and has_circ_0001946 in peripheral blood is the independent epigenetic risk factors for IA rupture. At the same time, the epigenetic risk score constructed based on these two circular RNAs interacts with age and has a certain clinical diagnostic values for IA repture.

It is worth mentioning that, in addition to the traditionally recognized influencing factors (smoking, drinking, biochemical indicators changes, etc.), this study also has some new findings. For example, people with higher diastolic pressure and pulse pressure differences are susceptible to IA rupture. Our data shows that for every 1 mmHg increase in diastolic blood pressure, the risk of IA rupture increases by about 4.6% (OR = 1.046, 95% CI: 1.007∼1.033). For every 1 mmHg increase in pulse pressure difference, the risk of IA rupture increases by about 2.1% (OR = 1.021, 5% CI: 1.007∼1.033). Studies have shown that patients with simple diastolic hypertension have a higher incidence of cerebrovascular disease. In Asian and European populations, for every 5 mmHg decrease in diastolic blood pressure, the risk of stroke is reduced by 44 and 27%, respectively (25). At the same time, the increase in pulse pressure difference means that the stress load on the blood vessel wall increases, which will lead to changes in arterial structure and decreased compliance, that constitutes a local weakening factor of the blood vessel wall (26, 27). Therefore, more attention should be paid to the monitoring and management of diastolic blood pressure and pulse pressure difference in the control of IA rupture, which may have more positive significance for its prevention.

Sedentary activity has been listed by the American Heart Association (AHA) as one of the independent risk factors for cerebrovascular diseases (28). According to research data, the all-cause mortality rate of people who sit for more than 11 h a day will increase by 40% (29). Sedentary reduces muscle contraction and blood circulation, promotes an increase in arterial pressure. In addition, pathological changes such as oxidative stress and endothelial dysfunction induced by continuous high perfusion become an important pathological basis for the occurrence of vascular diseases (30). In our data, the OR of people with sitting time> 6 h/day suffering from IA rupture was 3.382 (95% CI: 1.262∼9.062). Meanwhile, hair dye exposure is probably another bad lifestyle that may induce IA rupture. P-phenylenediamine (PPD) in hair dyes has a variety of target organ toxicity, which can induce TP53 gene mutation and cause DNA damage (31, 32). Although there is no direct report that the hair dye is related to the occurrence of IA, PPD as an environmental chemical poison and a new potential influencing factor of IA still deserve our attention.

Recent studies have shown that inflammation is the most important pathological mechanism in IA rupture, and circRNA may play an epigenetic regulatory role in the pathological mechanism of IA through various inflammatory pathways (33–35). including regulation of inflammatory gene expression, recruitment, chemotactic aggregation of macrophages and regulation of key signaling pathways, etc (36). In this study, based on high-throughput sequencing to analyze the circRNA expression profiles in IA tissues and their biological functions. The results showed that these abnormally expressed IA-related circRNAs are closely related to inflammation, and some important biological processes and cell signal pathways that associate with inflammation may be regulated by circular RNA epigenetic regulation.

Among them, the expressions of hsa_circ_0008433 and hsa_circ_0005571 in IA tissues and normal blood vessels are significantly different, and they still show such expression differences in the subsequent peripheral blood verification to expand the sample size. After adjusting for individual environmental factors that are important for the rupture of IA, the circular RNAs hsa_circ_0008433, hsa_circ_0005571, and hsa_circ_0001946 are independent epigenetic influencing factors

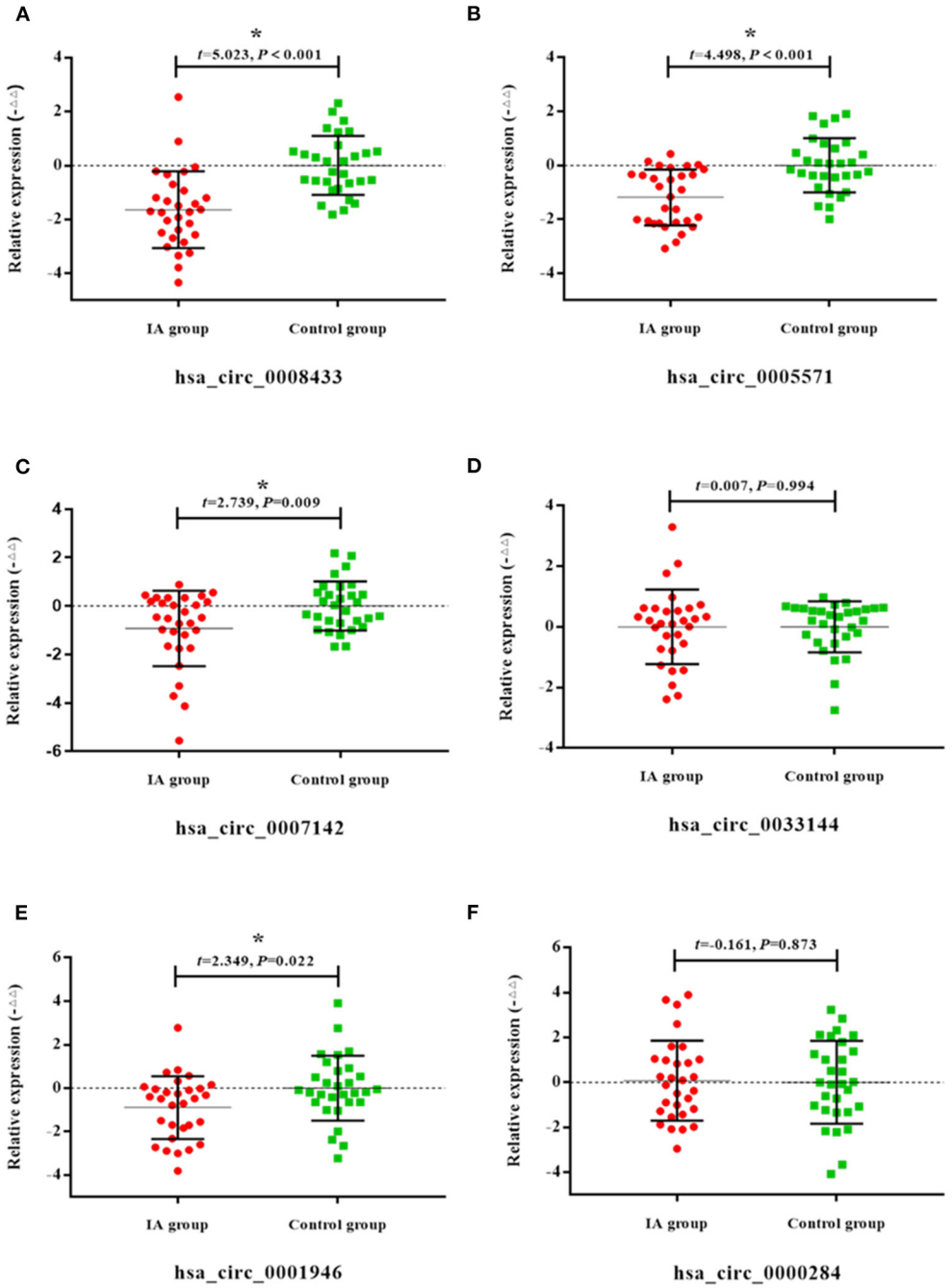

FIGURE 3 | Expression of specific circRNAs in peripheral blood. **(A–F)** Population qRT-PCR verification of hsa_circ_ (0008433, 0005571, 0007142, 0033144, 0001946, 0000284) expression in peripheral blood of IA and control group, IA group ($n = 30$) vs. control group ($n = 30$), $^*P < 0.05$.

for IA rupture, and have a certain synergistic interaction with age (>55 years). The results indicated that circRNA may participate in the pathological process of IA through various of mechanisms, which provide certain references for the molecular etiology of IA rupture (37–40).

At the same time, a "star molecule" of circular RNA circ-CDR1as (hsa_circ_0001946) which is widely studied currently was selected for peripheral blood PCR verification of IA rupture. The results showed that the expression of hsa_circ_0001946 in IA patients and healthy people was significantly different ($p < 0.05$). Studies have shown that circ-CDR1as can specifically bind miR-7-5p through ceRNA mechanism, regulate downstream target genes expression, and participate in the pathological mechanisms of various cerebrovascular diseases (22, 41). Genes such as

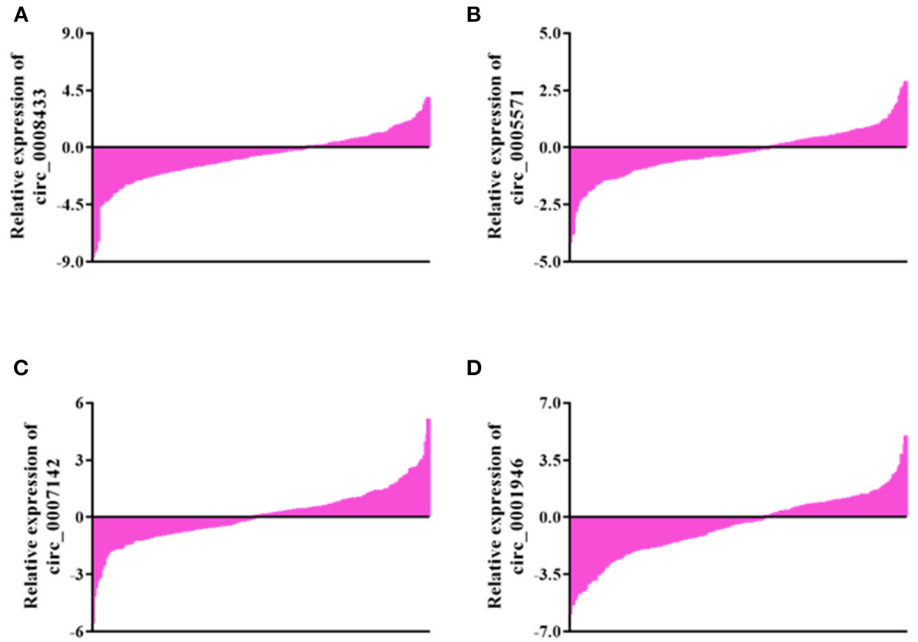

FIGURE 4 | General expression of specific circRNAs in blood of population. **(A–D)** General expression of hsa_circ_ (0008433, 0005571, 0007142, 0001946) in blood of population.

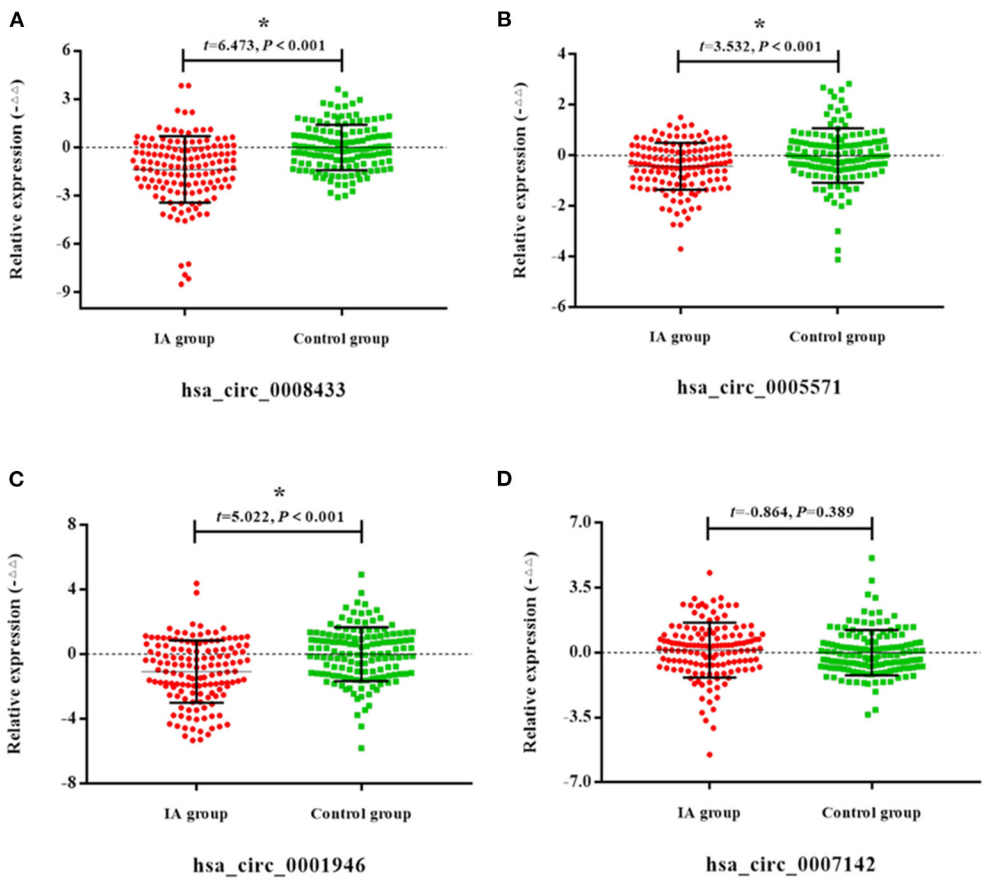

FIGURE 5 | Expression of specific circRNAs in peripheral blood. **(A–D)** Population qRT-PCR verification of hsa_circ_ (0008433, 0005571, 0001946, 0007142) expression in peripheral blood of IA and control group, IA group ($n = 140$) vs. control group ($n = 140$), $^*P < 0.05$.

PARP1 and RAF1, which are important downstream targets of miR-7-5p, are closely related to various pathophysiological mechanisms. Dysregulation expression of these genes can affect the proliferation and migration of important functional cells such as microvascular endothelial and vascular smooth muscle cells, causing damage and weakness of the vessel wall, and promoting the development of atherosclerosis (42).

TABLE 4 | Multivariate analysis of circRNA and IA rupture.

circRNA	OR	95% CI	[a]OR	[a]95% CI
has_circ_0008433	0.613	0.516∼0.727	0.497	0.338∼0.731
has_circ_0005571	0.648	0.504∼0.835	0.857	0.535∼1.374
has_circ_0001946	0.717	0.622∼0.827	0.682	0.509∼0.914

[a]Is the adjustment of sitting and sleeping time, drinking tea, smoking, edible oil, diastolic blood pressure, pulse difference, hemoglobin, globulin, LDL, serum calcium, Apo-A1, hypertension, and diabetes adjustment.

circRNA has good structural stability and can be released into extracellular space through exosomes, so it can be detected in body fluids such as saliva, milk, and plasma (43), these

TABLE 5 | Combined effects of epigenetic risk and age on IA.

Age	Genetic risk	Case/Control	[a]OR (95% CI)
<55	Low	23/50	1.000
<55	High	59/45	2.872 (1.497∼5.511)
≥55	Low	10/29	0.816 (0.333∼2.000)
≥55	High	16/48	7.981 (3.609∼17.649)
Age×Genetic point			3.052 (1.006∼9.258)
RERI			3.501 (-0.167∼7.168)
AP			1.147 (1.039∼1.255)
S			−1.418 (−2.314∼3.365)

[a]Is adjusted by gender, marital status, education level.

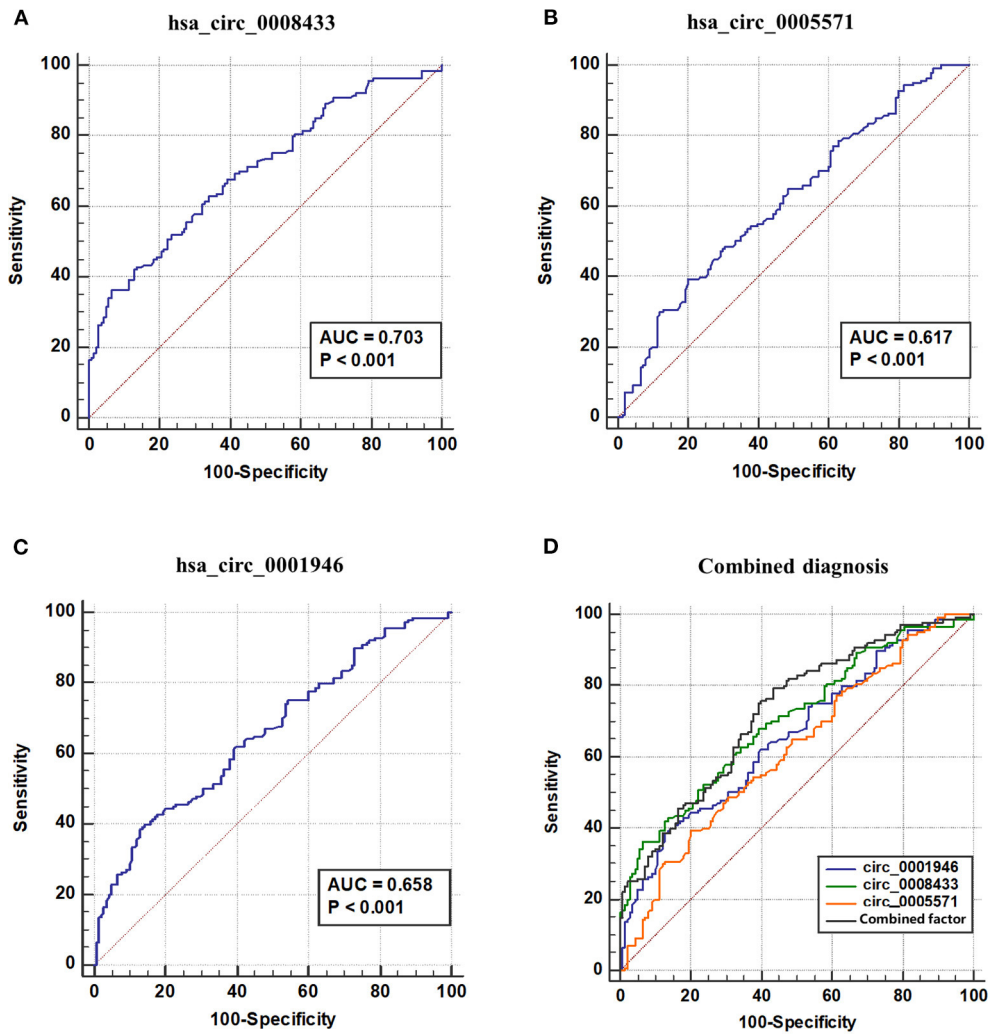

FIGURE 6 | ROC curve of IA related circRNAs and combined diagnosis. **(A–D)** ROC curve of hsa_circ_ (0008433, 0005571, 0001946) and combined factor. AUC, Area under ROC curve.

characteristics make the exploration of circRNA as a biological marker of disease widely concerned and deeply studied. In this study, we verified the results of high-throughput sequencing of circRNA simultaneously in two human specimens of cerebral artery tissue and peripheral blood. The analysis showed that certain circRNAs are differentially expressed in ruptured IA tissues and peripheral blood, there is a certain correlation between their expression patterns. For example, compared with the control group, hsa_circ_0007142 was down-regulated in IA tissues and peripheral blood, hsa_circ_0005571 and hsa_circ_0033144 were up-regulated in IA tissues, and down-regulated in IA peripheral blood. In the same patient, the expression pattern of hsa_circ_0007142 in tissue and peripheral blood were positively correlated, and the expression patterns of hsa_circ_0008433 and hsa_circ_0033144 in tissue and peripheral blood were negatively correlated. However, there was no significant difference in the expression of hsa_circ_0033144 and hsa_circ_0007142 in the follow-up population validation, which may be related to the influence factors such as the small number of tissue samples included in RNA-Seq, so the verification of further expansion of the sample size is needed.

However, these circRNAs with certain expression correlation in small samples of IA tissue and peripheral blood are still worthy of attention. This suggests that circRNA may have some correlation expression characteristics in central tissue and peripheral blood, which provides a theoretical possibility for the use of peripheral blood to diagnose IAs. In addition, we screened the three circular RNAs (has_circ_0008433, has_circ_0005571, has_circ_0001946) indicators closely related to IA rupture, and confirmed that they have certain reference significance for IA discrimination (AUC = 0.726, 95% CI: 0.668∼0.784). This also indicated that circRNA may have potential values as a biomarker, and provides a new perspective for the future research of IA non-invasive diagnosis and treatment strategies. In the study, we also found that specific circRNAs also interact with individual factors such as age, thereby further confirming that IA is a complex disease caused by the combination of environment and genetic factors.

The study also has certain limitations. First of all, we used a structured questionnaire to collect the data of the research subjects in the case-control study, and tried to reflect the clinical status of the patient as objectively as possible, but there were still some deviations inevitably. For example, the information distortion caused by the subjective will or memory bias of the respondents may have a certain impact on the research results. In the follow-up research, the sample size can be further expanded, and cross-regional cooperation can be strengthened to precisely control confounding factors and make the results more scientific. Secondly, this study does not yet have the conditions for multi-center research, so there are certain deficiencies in sample representativeness. Multi-center research needs to be carried out in follow-up studies to diversify the target population, thereby improving the accuracy and accuracy of the research. Finally, due to the limitations of case-control studies, it is not yet possible to accurately determine the causal relationship between circRNA and IA rupture, so further functional tests are needed to verify.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Fujian Medical University (2018-50). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SW, HL, and DK contributed to the study design. QinH, YS, QiuH, and YZ performed statistical analysis, interpretation, and drafted the manuscript. QiuH, YS, SL, YC, and XX contributed to data collection and laboratory test. QinH, YS, SW, and HL revised the manuscript. All authors contributed to critical revision of the final manuscript and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.594835/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A Set of Eight Key Questions Helps to Classify Common Vestibular Disorders—Results From the DizzyReg Patient Registry

Ralf Strobl[1,2*], *Michael Grözinger*[1], *Andreas Zwergal*[2,3], *Doreen Huppert*[2,3], *Filipp Filippopulos*[2,3] *and Eva Grill*[1,2,4]

[1] *Institute for Medical Information Processing, Biometrics and Epidemiology, Ludwig-Maximilians-Universität München (LMU) Munich, Munich, Germany,* [2] *German Center for Vertigo and Balance Disorders, University Hospital Munich, Ludwig-Maximilians-Universität München (LMU) Munich, Munich, Germany,* [3] *Department of Neurology, University Hospital Munich, Ludwig-Maximilians-Universität München (LMU) Munich, Munich, Germany,* [4] *Munich Centre of Health Sciences, Ludwig-Maximilians-Universität München (LMU) Munich, Munich, Germany*

Precise history taking is the key to develop a first assumption on the diagnosis of vestibular disorders. Particularly in the primary care setting, algorithms are needed, which are based on a small number of questions and variables only to guide appropriate diagnostic decisions. The aim of this study is to identify a set of such key variables that can be used for preliminary classification of the most common vestibular disorders. A four-step approach was implemented to achieve this aim: (1) we conducted an online expert survey to collect variables that are meaningful for medical history taking, (2) we used qualitative content analysis to structure these variables, (3) we identified matching variables of the patient registry of the German Center for Vertigo and Balance Disorders, and (4) we used classification trees to build a classification model based on these identified variables and to analyze if and how these variables contribute to the classification of common vestibular disorders. We included a total of 1,066 patients with seven common vestibular disorders (mean age of 51.1 years, SD = 15.3, 56% female). Functional dizziness was the most frequent diagnosis (32.5%), followed by vestibular migraine (20.2%) and Menière's disease (13.3%). Using classification trees, we identified eight key variables which can differentiate the seven vestibular disorders with an accuracy of almost 50%. The key questions comprised attack duration, rotational vertigo, hearing problems, turning in bed as a trigger, doing sport or heavy household chores as a trigger, age, having problems with walking in the dark, and vomiting. The presented algorithm showed a high-face validity and can be helpful for taking initial medical history in patients with vertigo and dizziness. Further research is required to evaluate if the identified algorithm can be applied in the primary care setting and to evaluate its external validity.

Keywords: vertigo, diagnosis, machine learning, surveys and questionnaires, clinical decision-making

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Edited by:
Sun-Young Oh,
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Reviewed by:
Sun-Uk Lee,
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Seung-Han Lee,
Chonnam National University,
South Korea

****Correspondence:***
Ralf Strobl
ralf.strobl@med.uni-muenchen.de

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INTRODUCTION

With a lifetime prevalence between 20 and 30% (1), vertigo and dizziness (VaD) belong to the most common complaints in primary care and emergency departments (2, 3). VaD have an annual prevalence of 9% in medical claim databases (4) and a high impact on daily life (5, 6).

In most cases, the diagnosis and treatment of VaD is straightforward (1, 7, 8). Inappropriate or delayed management of VaD, however, may contribute to chronic symptoms, increase disability, and cause secondary psychosomatic disorders (9–11).

While patients with VaD are mostly processed in primary care, a recent systematic review found considerable inconsistencies in the management of dizzy patients in this setting (12). Experts claim that taking a structured medical history is the key to make basic triage, namely, to decide if the patient can be managed in primary care, needs referral to a specialist, or, in the rare case of a life-threatening etiology of VaD (e.g., stroke), even needs emergency care. Concepts for a structured history taking such as the "Five Keys" (13) proposed that a limited number of symptom characteristics allows to successfully differentiate a majority of all VaD cases. Commonly recommended questions for rational history taking are whether VaD the complaints are paroxysmal or permanent, short or long lasting, spontaneous or triggered, of stereotypical presentation, direction-specific, or accompanied by neurological or otological symptoms (13, 14).

However, it has been argued that successful medical reasoning is often based on implicit experience and "gut feeling" that supplements explicit structured knowledge (15). Intuitive elements in the diagnostic process seem to play an important role in diagnostic reasoning (16). For example, the physician's feeling that "there was something wrong" turned out to predict serious infections in children (17). Personal preferences play an important role not only in taking medical history but also for assessing diagnostic thresholds and how to interpret them (18), probably as a function of experience and specialization. In this case, history taking in VaD may be less evidence-based than proposed.

In the current study, we validated a set of questions proposed by neurootological experts by a data-driven approach and hypothesized that a limited number of key characteristics will facilitate the differentiation of the most prevalent vestibular disorders with sufficient validity. To identify these key characteristics, several different statistical approaches are possible. In a previous study, we could show that machine learning methods may display good prediction accuracy but do not yield information about the causal pathways leading to good prediction (19). However, there are methods such as classification and regression trees (20) and their more recently developed methodological refinements (21) to rank characteristics according to their importance.

The aim of this study is to identify a set of key variables that are based on both expert experience and empirical knowledge, can be easily collected in clinical practice, and can be used as indicators for correct diagnosis of the most common vestibular disorders.

MATERIALS AND METHODS

To assemble an *a priori* collection of variables that are meaningful according to expert opinion, we first conducted a worldwide online survey among experts in the field about relevant themes and aspects of history taking in vestibular disease. We then used qualitative content analysis to clarify and structure these aspects. Thirdly, resulting contents were linked to variables from a specialized clinical patient registry that contained verified diagnoses according to current diagnostic guidelines as a gold standard. Fourthly, we used classification trees to analyze if these variables with apparent face validity also had statistical predictive validity.

Online Survey for Expert Opinions

Data Collection

This survey was conducted as an anonymous online survey in 2018. The participants were recruited from members of the Bárány Society, an international society for experts committed to vestibular disorders, and members of the DizzyNet, a European network initiative for vertigo and balance research (22). The online questionnaire was created with SoSci Survey (23) and made available to the participants on "www.soscisurvey.com." All experts were contacted by e-mail and provided with detailed information about the study.

Measures

The experts were asked to specify a limited number of questions that would be most salient and relevant during history taking to establish a preliminary diagnosis, e.g., to differentiate vestibular from non-vestibular or peripheral from central etiologies of VaD, along with any response options that would be indicative of a specific diagnosis. In addition to this, the respondents provided information on their institution, country, clinical specialization, and personal experience in the field.

Structuring Expert Opinions

Structured content analysis (24–26) was used to develop categories from the text passages provided by the experts. Two researchers (EG and RS) read the text and, independently from each other, identified "meaning units," i.e., distinct meaningful and manageable units. We then organized these units into a taxonomy consisting of main categories and subcategories hierarchically nested within main categories. To give an example, "associated symptoms" was defined as a main category, with "visual symptoms, oscillopsia" and "headache" being subcategories, respectively. The results of the two independent analyses were then synthesized. In case of differences, the final structure was decided on by discussion and consensus. MaxQDA® 2020 was used to support the content analysis and to assign weights to frequently used codes and priorities for interpretation (27).

Linking of Expert Opinion to Registry

Database

DizzyReg is an ongoing prospective clinical patient registry that collects information currently stored in electronic health records and medical discharge letters to create a comprehensive clinical database of patient characteristics, symptoms, diagnostic procedures, diagnosis, therapy, and outcomes in patients with VaD (28). Routinely, the patients also report quality of life and functioning in a few standardized questionnaires (2, 29–32). Adult patients are included if they presented at the German Center for Vertigo and Balance Disorders (DSGZ),

a tertiary reference unit for outpatients at the Hospital of Ludwig-Maximilians-Universität, Munich, and provided written informed consent. Recruitment into the registry commenced in December 2015. Data protection clearance and institutional review board approval has been obtained (no. 414-15).

Linking Procedure

We chose those variables from DizzyReg that would correspond most closely to the categories from our content analysis described above, e.g., the subcategory "duration of attacks" would fit to the corresponding variable "If you had vertigo, how long did it last? Less than 20 min; up to 20 min; 20 min to 1 h; several hours; more than 12 h; several days." If more than one variable could be linked, we chose the variable which was most accurately measurable and which had the smallest number of missing values.

Prediction of VaD Diagnoses

Ascertainment of Diagnoses

Diagnoses in the DizzyReg are based on a complete neurootological workup carried out by experienced neurootologists of the DSGZ and which conform to current guidelines (33–42). The neurootological examination includes a comprehensive battery of bedside tests, audiologic and vestibular function tests, and, if necessary, further imaging (e.g., cranial MRI) or consultation with other medical specialties, e.g., otorhinolaryngology, neurology, psychiatry, or ophthalmology. For this study, we included the seven most frequent diagnoses at the DSGZ (28, 43): benign paroxysmal positional vertigo (BPPV), functional dizziness (FD), Menière's disease (MD), vestibular paroxysmia (VP), unilateral vestibulopathy (UVP), bilateral vestibulopathy (BVP), and vestibular migraine (VM). There were no patients with both definite MD and VM in our data set.

Statistical Analyses

For data description, we used mean values and standard deviation for continuous variables and absolute and relative frequencies for categorical variables. In DizzyReg, missing values are routinely replaced by a neutral value, reflecting the practice that tests will not be applied if they are not needed or that symptom status are not reported if not indicated, i.e., the result is expected to be neutral.

Classification and Regression Trees

Classification and regression trees (CART) have the main advantage to yield a visually attractive tree structure that mimics human decision making and is easy to interpret (20). In brief, the CART procedure starts by splitting the entire data set with all individuals into smaller subsets that are more homogenous regarding a defined outcome in the current study "diagnosis." This splitting process is visualized by an upside-down tree structure, with each node in the tree representing a variable and each branch representing a split of the data.

For example, a first split assigns all individuals reporting headache to the left subnode and all individuals not reporting headache to the right subnode. The left subnode would then contain a higher percentage of persons with VM than the right subnode. However, the left subnode would also contain persons who reported headache but were not diagnosed with VM. Each of these subnodes is subsequently split again until each branch terminates in an end node, which is maximally homogeneous regarding diagnosis. Each end node will be assigned with the class, which occurs most often in it. A patient is allocated according to his/her individual characteristics to a certain end node and subsequently classified with the diagnosis assigned to the class of this end node.

Without any restrictions, the final tree would grow until it perfectly classifies each individual in the data set but would perform poorly to classify new individuals, i.e., it overfits. Thus, the final tree needs to be shrunk ("pruned") (20) to gain this external validity. We applied cost-complexity pruning that yields a trade-off between the complexity of the tree and its fit to the data. To get an unbiased estimate of this fit, we applied k-fold cross-validation (CV). CV assesses how a classification method will generalize to an independent data set by using out-of-sample estimates. The k-fold CV partitions the data set into k distinct subsets, trains the model on k-1 of the subsets, and estimates the test error on the remaining one. This will be repeated k times, with each subset acting once to assess the performance. The final fit is calculated as the average over the k estimates. Following common recommendations, k was set to 10 (44).

Some diagnoses were more frequent than others, resulting in imbalanced data. Imbalanced data may bias the tree toward the majority class, i.e., the final tree assigns individuals predominantly to the most frequent diagnosis. To avoid this, cases were weighted with the inverse of their class frequency (45), and class assignment of the end nodes was based on these weighted cases. Thus, cases with less frequent diagnoses were weighted higher than cases with more frequent diagnoses, for example, patients diagnosed with functional dizziness were weighted by 0.44 (1,066 divided by 7×346) and patients with unilateral vestibulopathy by 1.34 (1,066 divided by 7×114).

Estimating Variable Importance

In contrast to standard regression methods, a classification tree does not indicate which of the variables contributed most to the result, i.e., which questions will be most relevant for the diagnostic decision. To estimate variable relevance, we applied random forest classification (46), which yields estimates of variable importance values (47–49).

To assess variable importance, we applied an importance measure based on permutation (46). Here the mean decreases in accuracy, i.e., the proportion of correctly classified patients, for each variable is assessed by randomly permuting the values of this variable and measuring the decrease in accuracy due to this permutation. This permutation importance does not measure the full effect on prediction of a variable because other variables could act as surrogates. Recent developments also suggest other concrete importance parameters, among others the number of time a variable formed the root of a tree (50). In the current study, we report both the prediction accuracy based on permutation and the number of times a variable was used to split the root node.

Statistical significance was set at a two-tailed 5% level. R 3.6.1 was used for descriptive analyses (51) and the

machine learning library scikit-learn (52) for learning and pruning the tree. Variable importance was assessed with the "RandomForestExplainer" package in R (50). Visualization of the trained tree was obtained using the open-source python library dtreeviz (53).

Diagnostic Properties

Overall accuracy was estimated as the number of correctly classified patients divided by the total number of included patients. Thus, a patient was correctly classified if the assigned class of the end node that the patient belongs to matches the final diagnosis made at the DSGZ and incorrectly if otherwise. To judge the quality of the classification for each VaD syndrome, we reported sensitivity (SEN), specificity (SPEC), positive predictive value (PPV), and negative predictive value (NPV). As these measures are only defined for binary classification and not for multi-class classification, we reduced the classification problem by the "one vs. all" approach exclusively for this calculation.

RESULTS

In total, 21 experts from 16 different countries took part in the online survey. The experts worked in a total of 19 centers treating an average of 1,000 patients per year (median: 700, range: 40–4,000). The participants reported an average of 23 years of clinical experience (median: 25, range: 4–42). A total of 152 different statements were reported.

Content analysis yielded nine main categories and 39 subcategories, which are shown in **Table 1**.

A total of 98 variables contained in the DizzyReg could be linked to one of the categories. Ten categories were not represented in the registry, e.g., alcohol or pressure changes as a trigger. A complete description of the linking results can be found in the electronic appendix (**Supplementary Table 1**).

We included a total of 1,066 patients with a mean age of 51.1 years (standard deviation, SD = 15.3), 56% of whom were female. Functional dizziness was the most frequent diagnosis (32.5%), followed by vestibular migraine (20.2%) and Menière's disease (13.3%). A total of 47% of patients had vertigo or dizziness for <2 years (for more details, see **Table 2**).

Eight variables were found to be indicative for vertigo and dizziness diagnoses: attack duration, rotational vertigo, hearing problems, turning in bed as a trigger, doing sport or heavy household chores as a trigger, age, having problems with walking in the dark, and vomiting. The resulting tree is shown in **Figure 1**.

To give an example for interpretation: two paths in the tree could identify 56.7% of patients with BPPV correctly. In the first path, a short attack duration (<2 min) and turning in bed as a trigger lead to a correct classification of 55 patients with BPPV. In the second path, a longer attack duration, no hearing problems, age >60, and turning in bed as a trigger lead to 21 patients being correctly classified. In summary, of the 134 patients with BPPV, 76 (56.7%) were assigned to the correct classification of BPPV. The overall accuracy of the algorithm for all diagnoses as a multi-class problem was 42.2%. Further details of the diagnostic properties can be found in **Table 3**.

TABLE 1 | Main and subcategories identified from the expert survey.

Main category	Subcategory
Description of attacks/episodes	Duration of attacks
	Episodic/continuous
	Strength of attacks
	Evolution of attacks
	Frequency of attacks
	Type of vertigo
Associated symptoms	Aural symptoms
	Headache
	Visual symptoms, oscillopsia
	Photophobia, phonophobia
	Gait/balance unsteadiness
	Psychological symptoms
	Nausea/vomiting
	Neurological symptoms
	Autonomic symptoms
	Cervical tension/pain
Medication	
Effect on daily life	
Comorbidities	Musculoskeletal
	Diabetes
	Autoimmune disease
	Psychiatric (anxiety, depression)
	Neurological
	Cardiovascular disease
Trigger	Alcohol
	Noise
	Movement
	Pressure change (air pressure, valsalva)
	Specific situation
	Trauma
	Stress/lack of sleep
	Current herpes viral infection
	Changing body position
Family history	Comorbidities
	Vertigo
	Hearing loss
	Migraine
Duration of disease (first–last episode)	Last episode
	Age of onset
Mitigating factors	

The variable importance of the 20 variables that contributed most to the classification was determined by random forest analysis and is shown in **Table 4**. The variable with the most influence on the performance of the algorithm was vomiting, followed by age and hearing problems.

DISCUSSION

This study was able to identify a set of eight key questions that can help to differentiate seven common vestibular diagnoses. The key questions comprised attack duration, rotational vertigo,

TABLE 2 | Description of the study sample for the seven different diagnoses.

Variable	Levels	All	FD	VM	MD	BPPV	UVP	BVP	VP
Sample size	–	1,066	346	215	142	134	114	66	49
Gender	Female	602 (56%)	178 (51%)	145 (67%)	78 (55%)	88 (66%)	66 (58%)	27 (41%)	20 (41%)
Age	–	51.06 (SD = 15.29)	47.19 (SD = 14.51)	44.48 (SD = 13.95)	53.42 (SD = 13.3)	57.04 (SD = 12.06)	56.95 (SD = 15.01)	64.97 (SD = 16.96)	51.59 (SD = 14.16)
Falls last 12 months	Yes	288 (27%)	74 (21%)	53 (25%)	38 (27%)	41 (31%)	36 (32%)	26 (39%)	20 (41%)
Time since first onset	<3 months	191 (18%)	69 (20%)	48 (22%)	20 (14%)	18 (13%)	25 (22%)	8 (12%)	3 (6%)
	3 months to 2 years	314 (29%)	103 (30%)	47 (22%)	39 (27%)	45 (34%)	48 (42%)	22 (33%)	10 (20%)
	2–5 years	264 (25%)	88 (25%)	54 (25%)	32 (23%)	31 (23%)	25 (22%)	15 (23%)	19 (39%)
	5–10 years	160 (15%)	49 (14%)	33 (15%)	23 (16%)	23 (17%)	11 (10%)	11 (17%)	10 (20%)
	More than 10 years	137 (13%)	37 (11%)	33 (15%)	28 (20%)	17 (13%)	5 (4%)	10 (15%)	7 (14%)
Vertigo	Yes	574 (54%)	121 (35%)	131 (61%)	113 (80%)	102 (76%)	60 (53%)	20 (30%)	27 (55%)
Postural imbalance	Yes	609 (57%)	216 (62%)	113 (53%)	74 (52%)	64 (48%)	65 (57%)	44 (67%)	33 (67%)
Dizziness	Yes	555 (52%)	223 (64%)	110 (51%)	66 (46%)	58 (43%)	55 (48%)	22 (33%)	21 (43%)

FD, functional dizziness; VM, vestibular migraine; MD, Menière's disease; BPPV, benign paroxysmal positional vertigo; UVP, unilateral vestibulopathy; BVP, bilateral vestibulopathy; VP, vestibular paroxysmia.

hearing problems, turning in bed as a trigger, doing sport or heavy household chores as a trigger, age, having problems with walking in the dark, and vomiting.

The negative predictive values were higher than the positive predictive values, indicating that it was mostly easier to exclude a diagnosis than to confirm it.

Using expert opinion and a statistical classification approach, we arrived at combinations of symptoms with high face validity. Positive predictive value in our study was highest for functional dizziness. This is not surprising because FD is characterized by the combination of typical symptoms and the absence of others (38). Thus, the sequence of longer attack duration, no hearing problems, younger age, no vegetative symptoms, and a presentation as dizziness rather than rotational vertigo indicated FD, which is in line with the approach presented by Dieterich et al. (54, 55). On the other hand, FD was also frequently present in other nodes (between 13 and 29%). This finding may be explained by the relatively high prevalence of FD in this sample. Furthermore, patients with FD report a multitude of uncharacteristic symptoms fluctuating in time and intensity, triggered by various situations (55). Furthermore, FD often manifests as a comorbidity to or consequence of different organic vestibular disorders, most commonly VM, BPPV, and MD (56).

BPPV was likewise characterized in our study by a short duration of attacks and the movement of body and head in the horizontal plane, which is in line with the typical clinical presentation (43, 57). These two questions identified more than half of BPPV patients. Head movement while turning in bed was also indicative for an older group of patients with BPPV who had longer attacks. This finding also aligns with literature (58).

In addition, the characterization of MD, BVP, and UVP in our study is in accordance with the clinical key features described previously (39, 59, 60). In our study, MD patients had a longer attack duration and hearing loss. BVP was characterized by symptoms aggravating in darkness, permanent dizziness, and higher age. Increase of symptoms in darkness and older age are highly characteristic for BVP (61). UVP characteristics were a longer duration of symptoms, lack of hearing loss, younger age (<60 years), vomiting, and aggravation by sports or heavy household chores. Although UVP can occur in all age groups and is thus not considered to be typical for younger adults, a peak between the ages of 30 and 50 was suggested (62).

Vestibular migraine was difficult to classify in our study. The clearest differentiation was age, which is confirmed by the finding that VM typically manifests in younger adults (63) without typical triggers (34). Interestingly, we were not able to confirm headache as a typical feature of VM. It has been shown that about 30% of patients with VM do not report headache associated to vertigo attacks (64), and <50% of patients report the simultaneous presence of headache and vertigo during attacks (65).

A specific objective of the present study was to train a machine learning model for classification that is transparent, easy to use in daily clinical practice, and easy to understand. In the past, several different approaches have been used to help classify the underlying pathologies of vertigo and dizziness. A complex method like deep neural networks (DNN), which was applied to vestibular disorders with promising results (19, 66), is difficult to directly transfer to a real-world clinical setting since DNN does not provide transparency on how the classification decision was made. Similarly, applying support vector machine (SVM) is accompanied by a lack of procedural transparency but comes with a high power regarding accuracy. A study on classifying unilateral vestibulopathy using SVM yielded an accuracy of 76% (67). Another study was able to differentiate the peripheral and central causes of acute vestibular disorders with a high

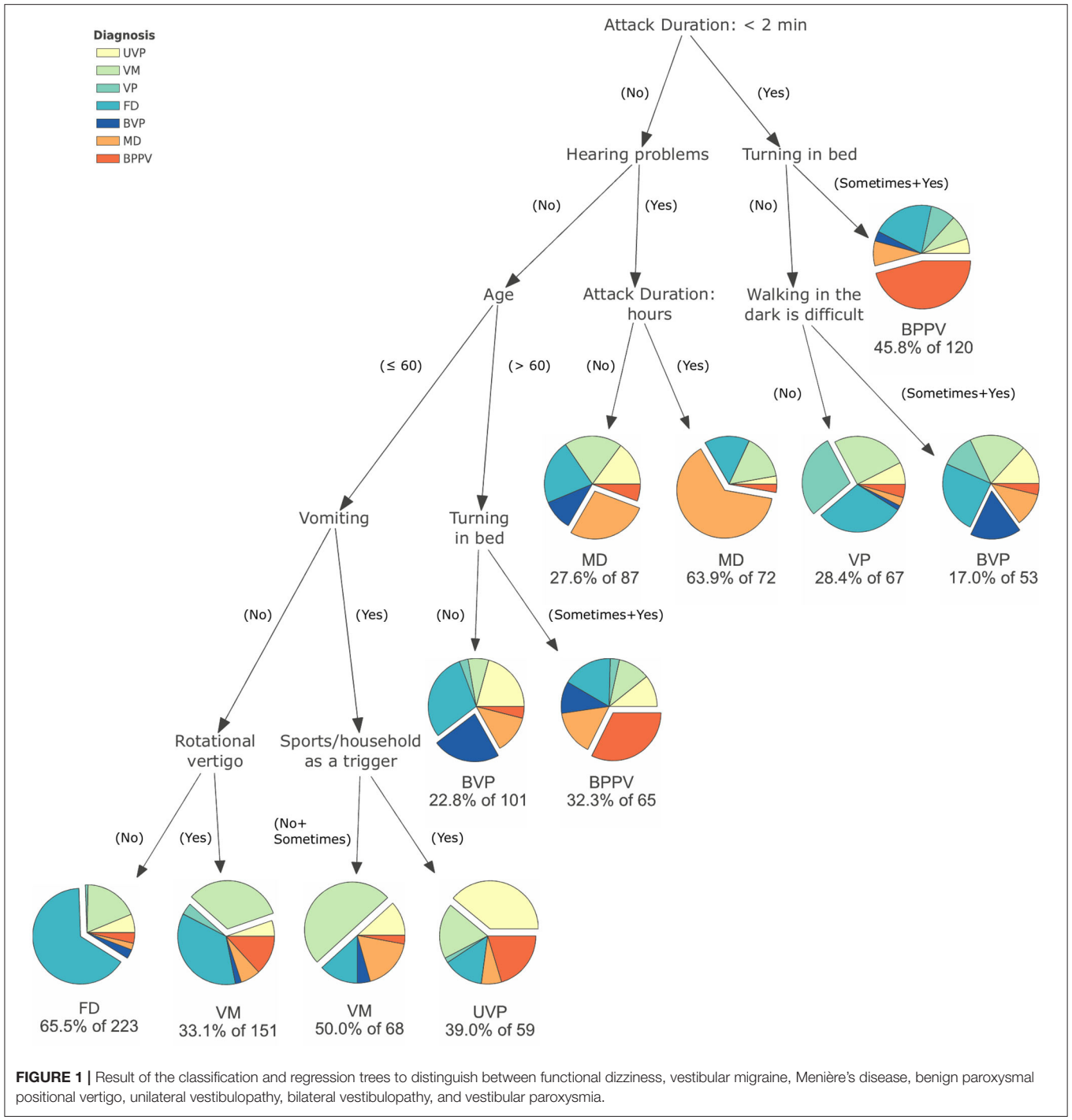

FIGURE 1 | Result of the classification and regression trees to distinguish between functional dizziness, vestibular migraine, Menière's disease, benign paroxysmal positional vertigo, unilateral vestibulopathy, bilateral vestibulopathy, and vestibular paroxysmia.

accuracy using modern machine learning methods (68). Recent research aimed to alleviate the drawback of procedural opacity and develop explainable artificial intelligence (69), but their work was based on pattern recognition and has not been applied to data sets with numeric, ordinal, or categorical data yet. Thus, further research is required to identify if such an approach might be appropriate for a clinical decision-making system in specialized areas like vestibular disorders.

Studies using transparent learning methods, like CART, are scarce. A study from 2000 also applied classification trees for differentiation of vestibular disorders (70). They identified hearing loss, duration of the disease, frequency of attacks, severity of rotational vertigo, onset and type of hearing loss, and occurrence of head injury at onset of vertigo as important variables for diagnostic classification. These variables are similar to the ones presented here, although some variables were not

TABLE 3 | Comparison of the classification of the classification and regression trees algorithm with the diagnosis made at the German Center for Vertigo and Balance Disorders (DSGZ) for functional dizziness (FD), vestibular migraine (VM), Menière's disease (MD), benign paroxysmal positional vertigo (BPPV), unilateral vestibulopathy (UVP), bilateral vestibulopathy (BVP), and vestibular paroxysmia (VP).

		Diagnosis by DSGZ							Diagnostic parameters			
		FD	VM	MD	BPPV	UVP	BVP	VP	SENS (%)	SPEC (%)	PPV (%)	NPV (%)
Diagnosis by classification algorithm	FD	146	41	5	8	14	7	2	42.2	57.8	65.5	34.5
	VM	63	84	22	22	16	6	6	39.1	60.9	38.4	61.6
	MD	30	28	70	7	15	9	0	49.3	50.7	44.0	56.0
	BPPV	36	17	20	76	13	11	12	56.7	43.3	41.1	58.9
	UVP	8	11	4	12	23	0	1	20.2	79.8	39.0	61.0
	BVP	43	17	19	6	28	32	9	48.5	51.5	20.8	79.2
	VP	20	17	2	3	5	1	19	38.8	61.2	28.4	71.6

Performance is described by SENS, sensitivity; SPEC, specificity; PPV, positive predictive value, and NPV, negative predictive value.

TABLE 4 | Variable importance of the 20 most relevant variables to differentiate between the seven different vestibular diagnoses (functional dizziness, vestibular migraine, Menière's disease, benign paroxysmal positional vertigo, unilateral vestibulopathy, bilateral vestibulopathy, and vestibular paroxysmia).

Variables	Mean decrease in accuracy	Root node[#]
Vomiting	1.41	744
Age	1.02	978
Hearing problems	0.93	682
Turning in bed as a trigger	0.92	343
Attack duration: <2 min	0.76	1,105
Rotational vertigo	0.67	338
Getting in and out of bed is difficult	0.63	138
Attack duration: hours	0.61	446
Nausea	0.57	481
Positional maneuver as a trigger	0.38	223
Ear pressure	0.29	321
Walking in the dark is difficult	0.27	780
Walking on sidewalks is difficult	0.24	504
Ear noise	0.20	72
Attack duration: several days	0.16	491
Provocational nystagmus	0.16	46
Gait disturbance	0.16	268
Bending over as a trigger	0.16	62
Eye movement disorder	0.14	52
Headache	0.12	31

The estimation of importance measures was based on random forests with 10,000 trees. The mean decrease in accuracy was based on permutation in importance.
[#]Root node indicates how often a variable was used to split the root node (higher frequencies indicate higher relevance for the classification).

surveyed in the present study (e.g., association to trauma). Another study used boosted decision trees to identify two different feature sets, one for general practitioners and one for experts (71). All these studies reported a higher accuracy than our study, as they used a one-vs.-all classification approach, which results in better accuracy but is less precise than our approach. To put this into context, a one-vs.-all approach has an implicit minimal accuracy of 50% as the classification problem is reduced to a dichotomous choice. In contrast to this, our algorithm aims at distinguishing between seven different vertigo syndromes simultaneously, yielding a minimal accuracy of $1/7 = 14\%$. Thus, the overall accuracy of 42.2% is a notable improvement.

Limitations

In our study, we used data from a patient registry of a tertiary referral center for balance disorders, which is not representative for patients presenting with vertigo and dizziness in primary or secondary care. Patients visiting specialized units are usually a selection of severe or chronic patients with a long history of disease or unsuccessful therapy. This may explain the low accuracy of our findings. However, this registry is one of the largest data collections of information on vestibular disorders, including rare forms, and belongs to one of the most comprehensive and valid sources for clinical phenotyping. In addition, there are patients with overlapping syndromes (e.g., VM and MD). This overlap may pose a challenge for the current diagnostic approach as these patients may present with a set of symptoms not characteristic for the assigned diagnosis. Furthermore, certain syndromes occurring in the emergency setting, like vestibular TIA or stroke, or other rare syndromes, like vestibular schwannoma, are not sufficiently represented in the patient registry. To be applied in a real-world setting, these vestibular syndromes should be incorporated in the algorithm, for example, by including expert knowledge or data sets from emergency departments. To further improve the diagnostic algorithm for the classification of common vestibular disorders, results from basic clinical vestibular testing (such as the clinical head impulse test or positioning maneuvers) and a modification of symptomatic categories need to be incorporated into the model.

There are several shortcomings of CART as opposed to other methods. Firstly, trees are not very robust to small changes in the data, i.e., a small change can result into a different tree. Furthermore, CART cannot handle non-random missing values in an adequate way. A common approach to handle missing

values is based on surrogate splits, which cannot be applied here, as the best surrogate candidates have the same cause of missingness, e.g., the decision of the physician that a certain measurement is not necessary for diagnosis (72). Secondly, trees cannot compete with complex ensemble methods in terms of prediction accuracy alone. A study using real and simulated data sets showed that the accuracy of the best single-tree algorithm is on average about 10% less than that of a tree ensemble (21).

However, we are confident that, for the purpose of the current study, CART represents the most transparent method to develop an algorithm for diagnosing vestibular disorders. The main advantage of tree-based models is that they mirror human decision-making. Thus, the identified tree can act as a blueprint for taking and structuring patient records.

Conclusion

The presented algorithm used a transparent and easily applicable approach for categorizing different common vestibular syndromes based on eight key questions. It may be helpful for the initial triage of patients but needs to be followed by a basic clinical exam of vestibular and ocular motor functions to improve the accuracy of the diagnostic classification. To evaluate if the identified algorithm might be a basis for a simple-to-use algorithm in a primary care setting, further studies are required.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: an application form has to be sent to the Scientific Committee of the German Center for Vertigo and Balance Disorders. Requests to access these datasets should be directed to ralf.strobl@med.uni-muenchen.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethic committee of the medical faculty, LMU Munich, Munich, Germany. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RS contributed to the drafting/revising of the manuscript for content, including writing, study concept/design, interpretation of data, and acquisition of data. MG contributed to statistical analysis and revision of the manuscript for content, including writing, and study concept. AZ contributed to the interpretation of data and revision of the manuscript for content. DH contributed to the development of the concept and revision of the manuscript for content. FF contributed to the interpretation of data, revision of the manuscript for content, and medical writing. EG contributed to the drafting/revision the manuscript for content, including medical writing and study concept/design. All authors contributed to the article and approved the submitted version.

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Clinical Subtypes and vHIT Parameters in a Population With Bilateral Vestibulopathy

Fiorella Mancino-Moreira[1†], *Almudena Rueda*[2], *Jonathan Esteban-Sanchez*[1] *and Eduardo Martin-Sanz*[1,3*†]

[1] Department of Otolaryngology, University Hospital of Getafe, Madrid, Spain, [2] Department of Neurology, Getafe University Hospital, Madrid, Spain, [3] Department of Medicine, School of Biomedical Sciences and Health, Universidad Europea de Madrid, Madrid, Spain

Objective: To evaluate the different peripheral, neurological, genetic, and systemic etiologies of bilateral vestibulopathy (BVP) and the value of vHIT in the diagnostic process.

Materials and methods: A retrospective case review was performed on 176 patients diagnosed with BVP in a tertiary referral center, between 2010 and 2020. Inclusion criteria comprised imbalance and/or oscillopsia during locomotion and horizontal angular VOR gain on both sides <0.8. We classified patients into different groups according to (1) their fulfillment of the Barany guideline for bilateral vestibulopathy (2) the definite etiology of BVP and (3) the four clinical subtypes distributed in our population (recurrent vertigo with BVP, rapidly progressive BVP, slowly progressive BVP, and slowly progressive BVP with ataxia). Medical history of vertigo, hypoacusis or migraine, and family background of imbalance and/or oscillopsia were assessed. Horizontal, posterior, and superior semicircular canal angular VOR gain was registered along with saccadic parameters such as velocity, and dispersion of the saccades' latency values.

Results: Barany's Society diagnostic criteria for BVP was accomplished in 89 patients. Among our patients, 13.6% had migraines in their medical history and the idiopathic group accounted for 50% of the population. All four clinical subtypes were found in our population, slowly progressive bilateral vestibulopathy without vertigo was the most frequent one. A percentage of our population could not be categorized into any of the former subtypes, many of these patients were diagnosed with BVP after suffering a single vertigo episode. Lower vHIT gains were found in those patients with Barany's criteria for BVP and oscillopsia was significantly more prevalent in this group.

Conclusions: Bilateral vestibulopathy manifests with very different patterns representing a very heterogeneous condition. The distribution of the clinical subtypes and Barany's criteria are a useful clinical tool to differentiate groups of patients. The vHIT can serve as an initial tool for identifying patients with BVP. The finding of bilateral vestibular involvement in a clinically suspected unilateral vestibulopathy should be considered in some patients.

Keywords: bilateral vestibular hypofunction, bilateral vestibulopathy, vHIT, head impulse test, subtypes

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****Correspondence:***
Eduardo Martin-Sanz
emartinsanz@gmail.com

[†] These authors have contributed equally to this work and share first authorship

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INTRODUCTION

Bilateral vestibulopathy (BVP) is a heterogeneous clinical condition characterized by a hypofunction of the vestibular nerves or labyrinths on both sides (1). Partially reduced or absent function of the vestibular organs and/or vestibular nerves results in different levels of impairment or total loss of the major vestibular functions: posture and balance control, gaze stabilization, and spatial orientation (2).

Spatial disorientation, oscillopsia, diminished dynamic visual acuity (DVA), and balance problems are the main deficits reported by patients with BVP, particularly in darkness and on uneven ground.

BVP is a disorder with different clinical pictures (combined or isolated deficits of the otolith and semicircular canal functions), it remains a diagnostic challenge, and therefore, its often under or misdiagnosed.

The most frequent etiologies of BVP include ototoxicity, Meniere's disease, infectious diseases, and genetic disorders (3). However, the etiology remains undetermined in 20–51% of the patients (4). Its estimated prevalence in adults is 28/100,000, and it accounts for 4–7% of dizziness/vertigo (5).

Four different clinical subtypes have been described as follows (3): (I) recurrent vertigo and BVP (II), rapidly progressive BVP (III), slowly progressive BVP, and (IV) BVP with neurological deficits. In a recent study (3) most non-idiopathic BVP patients presented a clinical subtype that would be expected due to its etiology.

Nevertheless, so far we know, there is no correlation between the clinical subtypes and vestibular test findings, but it is believed that stratifying the patient population may facilitate the identification of underlying causes for which there may be a genetic predisposition (6).

Many challenges are met when establishing the diagnosis of BVP. Currently, many different diagnostic tests are used for vestibular evaluation, such as the caloric test, rotatory chair tests, video head impulse test (vHIT), vestibular-evoked myogenic potentials (VEMP), DVA test (7).

In 2017, the Committee for the International Classification of Vestibular Disorders (ICVD) of the Barany Society proposed the diagnostic criteria of BVP (8). However, some controversy remains, and at this moment, no diagnostic standards regarding some clinical and vestibular test findings are available.

The vHIT is a test of the angular vestibulo-ocular reflex (VOR). A bilaterally reduced or absent angular VOR function has been included in the diagnostic criteria for BVP in the consensus document of the Classification Committee of the Bárány Society (8).

Recently Batuecas-Caletrio et al. (9) described that BVP patients with scattered saccades were more prone to oscillopsia independent of their gain values, suggesting that the degree of synchronization of the saccades in successive head impulses can be considered a useful measurement of compensation.

We aim to review our series of BVP patients, analyze their etiologies, identify and characterize the clinical subtypes, and correlate with audio-vestibular function and familiar aggregation.

MATERIALS AND METHODS

This is a retrospective study. Patients with a suspicion of BVP were included between January 1, 2010 and June 30, 2020.

Inclusion criteria were (1) a vHIT response from the six semicircular canals, below normal established limits for age (10, 11) and/or (2) the presence of unsteadiness when walking or oscillopsia during quick head movements and/or (3) worsening of unsteadiness in darkness and/or on uneven ground.

Clinical and demographic variables such as age, sex, personal and familial history, presence of imbalance, and/or oscillopsia were recorded.

The patients were then categorized depending on the fulfillment of the diagnostic criteria for BVP proposed by the Barany Society (8).

The patients were also classified in the four clinical subtypes previously described: (I) recurrent vertigo with BVP (II), rapidly progressive BVP (III), slowly progressive BVP, and (IV) BVP with neurological deficits (such as ataxia, polyneuropathy).

The protocol for this study was approved by the institutional review board at our hospital. The research was under the World Medical Association Declaration of Helsinki.

Audiometry

Audiometry was performed in a soundproofed booth (IAC mini 250), and the findings were reported in auditory thresholds of each frequency elicited (0.5, 1, 2, 3, 4, 6, and 8 kHz) and pure-tone averages (PTAs), which were computed by taking the average of the four frequencies (0.5, 1, 2, and 3 kHz).

Video Head Impulse Test

We evaluated the dynamic function of the horizontal semicircular canals using the vHIT (GN Otometrics; Denmark). Fast, short, and unpredictable head impulses were performed in random horizontal directions while the subject was seated in front of the ground-fixed target and was instructed to maintain his/her vision continually fixed on the target during the test. Eye and head velocities were acquired with a sampling frequency of 250 Hz, and we calculated the hVOR gain from an average of 20 head impulses performed over a range of velocities from 100–250°/s.

The first pair of vertical canals was evaluated next. To do this, the patient's head was rotated 40° to the right to align it with the left-anterior/right-posterior plane. Patients were directed to continue staring at the same earth-fixed target as before. Brief, abrupt, forward, and backward head impulses were made to stimulate the left anterior semicircular canal and the right posterior semicircular canal, respectively. After 20 impulses in each direction, the second pair of vertical canals were evaluated. To do this, the patient's head was rotated 40° to the left while they continued staring at the same earth-fixed target as was used before to align the right-anterior/left-posterior plane. Forward and backward head impulses stimulated the right anterior semicircular canal and the left posterior semicircular canal, respectively.

The presence or absence of saccades, their latency, and amplitude were registered for each exploration. To assess the

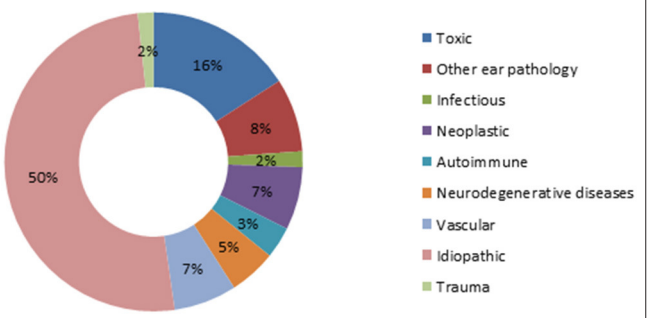

FIGURE 1 | Distribution of etiologies in the study's population.

dispersion of the saccades' latency values, we used the PR score described by Rey Martinez et al. (12).

PR score is a quantitative variable that ranges between 0 and 100; when PR is close to zero, saccadic responses are said to be gathered, and when it is nearing 100 they are said to be scattered.

Statistical Analyses

All data were stored and analyzed in an SPSS file version 25 (SPSS Inc.; Chicago, IL, USA). All tests were two-tailed, and $p < 0.05$ were considered significant. A χ^2 test with Bonferroni's correction for multiple comparisons was performed to assess the differences in BVP detection. We conducted χ^2 *post-hoc* tests based on adjusted standardized residuals (13).

Mann-Whitney *U*-test and Spearman's ρ were used to detect differences between measures within each series.

A multivariant ANOVA test was used to compare the auditory thresholds for every frequency and VOR gain between cohorts of patients.

RESULTS

In this study, 176 patients were initially included. The mean age of the patients was 61.70 ± 14.9 (range 14-90) at the time of the inclusion in the study.

Of our population, 56.8% were male and 43.2% female. The imbalance was perceived by 160 patients (96.2% of the population), while 76 (43.2%) experienced oscillopsia.

Etiology

In our global population, the etiology for BVP was determined in 87 patients (50%). Meniere's disease, ototoxicity, and cerebellar infarction were the most prevalent diagnosis in those known etiologies. **Figure 1** shows the complete distribution of etiologies in the study's population.

Of our population with Meniere's disease (MD), three cases had a synchronic MD, and seven cases developed bilateral metachronous MD.

Four patients of our MD population had unilateral disease with confirmed BVP, and all patients fulfilled the diagnostic criteria for definite MD. All those patients had a positive electrocochleography in both ears.

The group of neurodegenerative diseases consisted of nine patients with cerebellar atrophy, six of them with a confirmed CANVAS diagnosis.

Of our population, 117 patients (66.5%) had valid information regarding the familial history of chronic vertigo or disequilibrium. Fourteen patients had a first-degree relative, and five patients a second-degree relative. This makes 16.3% of patients with a prior family history, considering just those patients with relevant information of their familial background.

Among our patients, 13.6% had migraines in their medical history.

General vHIT Parameters

Considering the total population, the mean gain was 0.60 ± 0.17 and 0.42 ± 0.18 for both better and worse horizontal semicircular canals. Mean values were 0.53 ± 0.27 and 0.43 ± 0.23 for better and worse posterior semicircular canals, respectively. Superior semicircular canals had 0.62 ± 0.27 and 0.49 ± 0.22 on their better and worse side, respectively.

A 41.6% of our population developed covert saccades and 87.6% had overt saccades on the worse side, while 32.6 and 66.4% of our patients had covert saccades and overt saccades, respectively, on the better side. There were statistically significant differences between both sides, either for covert saccades (Chi-square: 30.31; $p = 0.001$) or overt saccades (Chi-square: 15.06; $p = 0.001$).

The mean overt saccades latencies were 218.4 ± 65.1 and 226.3 ± 62.74 ms for the worse and better side, respectively. The mean covert saccades latencies were 112.32 ± 26.5 and 109.5 ± 28.26 ms for the worse and better side, respectively. We didn't find any statistically significant differences between both sides, either in overt or covert saccades latencies ($p < 0.05$).

The PR score had a mean value of 46.45 ± 26.89 on the better side and 50.38 ± 27.57 on the worse side, without statistically significant differences.

Auditory Results

The PTA of the global population was 40.26 ± 29.17 and 32.96 ± 24.19 for the worse and better side, respectively. No correlation was found between the PTA and VOR gain values.

Clinical Subtypes of BVP

Slowly progressive BVP without vertigo, followed by recurrent vertigo with BVP, was found in 24.4% ($n = 43$) and 21.0% ($n = 37$), respectively. In the former subtype, idiopathic BVP was most accounted for, while Meniere's disease was the most frequent diagnosis in the latter. In 17% of our population ($n = 30$), a rapidly progressive BVP was diagnosed, due in many cases to ototoxicity, while 14.9% of our cases ($n = 26$) developed BVP with neurological deficit.

A significant percentage of our population could not be categorized in any of the former subtypes. Many of them were diagnosed with BVP after suffering a single vertigo episode (22.7%, $n = 40$), so a new subtype was added (**Figure 2** and **Table 1**).

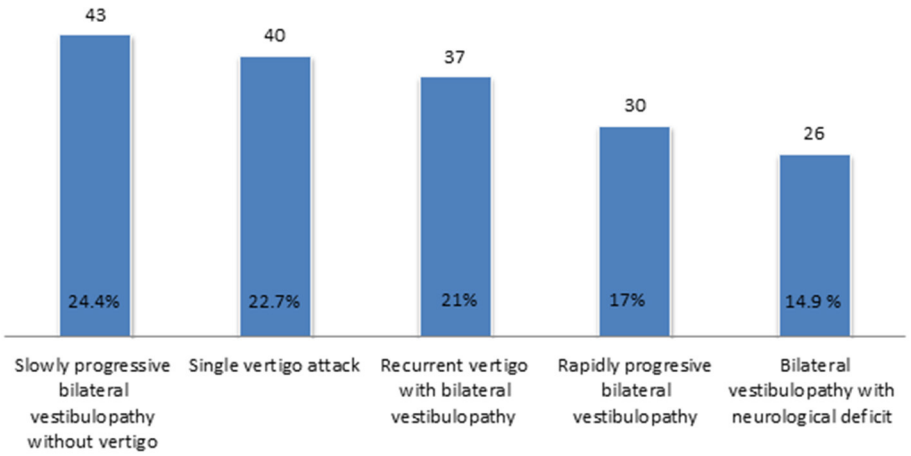

FIGURE 2 | Clinical subtypes.

TABLE 1 | Distribution of etiologies with respect to the clinical subtypes.

	Slowly progressive bilateral vestibulopathy without vertigo	Single vertigo attack	Recurrent vertigo with BVH	Rapidly progresive BVH	BVH with neurological deficit (ataxia, polyneuropathy)
Toxic	4	2	5	16	1
Other ear pathology	2	0	12	0	0
Infectious	1	0	0	1	1
Neoplastic	8	1	0	2	1
Autoimmune	2	0	1	2	1
Neurodegenerative diseases	2	0	0	0	7
Vascular	0	3	2	2	5
Idiopathic	24	33	17	5	10
Trauma	0	1	0	2	0
Total	43	40	37	30	26

Regarding the vHIT parameters, the gain in the better horizontal semicircular canal was significantly higher in the single vertigo episode group compared to the other clinical subtypes ($p < 0.05$) (**Figure 3A** shows vHIT examples according to clinical subtypes).

The time of evolution differed significantly depending on the clinical subtypes of BVP (**Figure 3B**). The rapidly progressive BVP and the single vertigo attack subtypes had a shorter time of evolution and differed significantly from the rest of the groups.

Barany Criteria

Eighty-nine patients (50.6%) fulfilled the diagnostic criteria for BVP proposed by Barany's society. The rest of our population fulfilled the diagnostic criteria for probable BVP.

The time of evolution was 41.67 ± 56.53 and 34.29 ± 50.09 months for those patients with and without Barany's criteria for BVP, respectively. We didn't find significant differences regarding the time of evolution between both groups.

The global distribution of different diagnoses did not differ significantly between both groups (Chi-square: 7.53; $p = 0.48$), but idiopathic cases were significantly less prevalent ($p = 0.024$) in those patients who fulfilled Barany's diagnostic criteria for BVP. **Figure 4** shows the distribution of the different diagnoses according to Barany's diagnostic criteria for BVP.

The imbalance was equally distributed in both groups of patients (Chi-square: 0.002; $p = 0.962$). Oscillopsia was significantly more prevalent in the group of patients that fulfilled Barany's criteria (Chi-square: 5.138; $p = 0.023$).

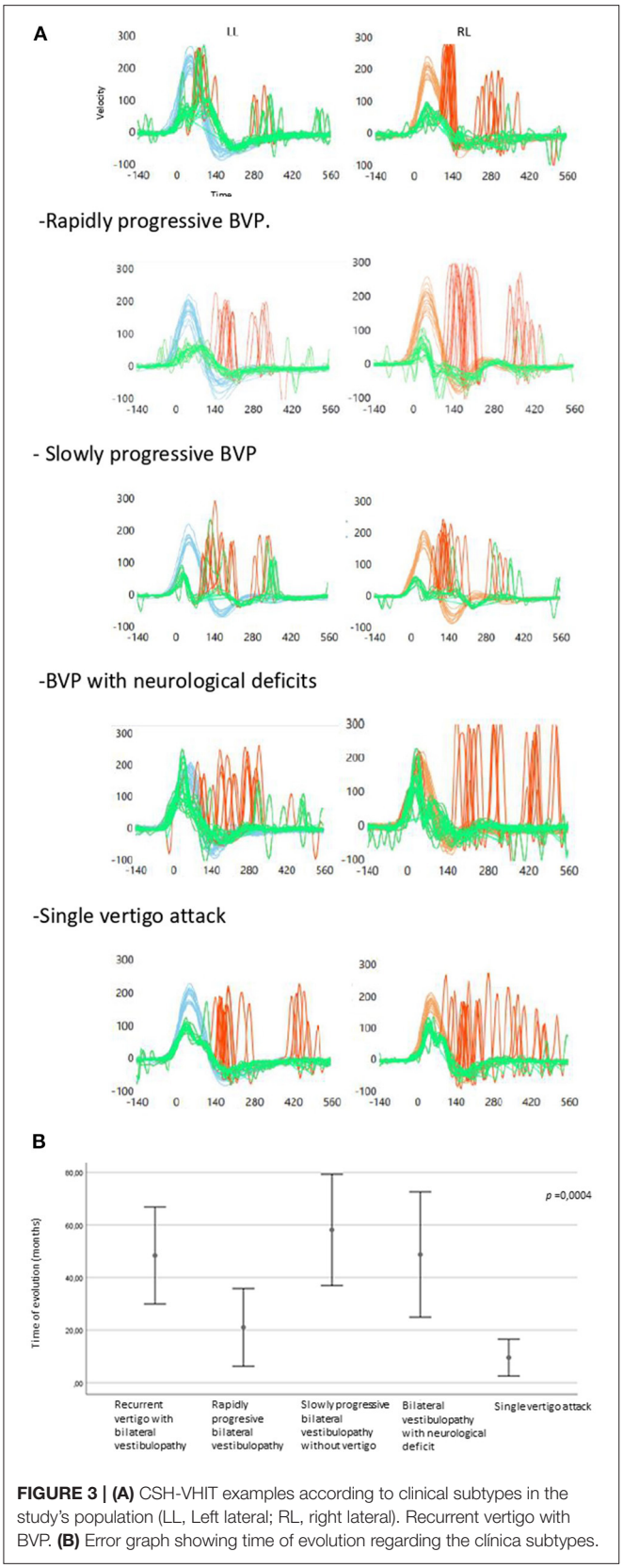

FIGURE 3 | (A) CSH-VHIT examples according to clinical subtypes in the study's population (LL, Left lateral; RL, right lateral). Recurrent vertigo with BVP. **(B)** Error graph showing time of evolution regarding the clínica subtypes.

The gain of the worse horizontal semicircular canal was significantly lower in those patients with Barany's criteria for BVP compared to the probable BVP group ($p < 0.001$). No other vHIT parameters, such as the gain in the posterior and superior canal, PR score, the presence of saccades, or its latency, either in the better or the worse side, showed significant differences depending on Barany's criteria. **Figure 5** shows the gain for each canal on vHIT concerning Barany's criteria.

Neither the PTA nor the auditory thresholds for every frequency elicited differed significantly depending on the fulfillment of Barany's criteria for BVP.

DISCUSSION

This study mainly focuses on the difficulties inherent in diagnosing a BVP in our population of 176 patients.

We had nearly half of our population with an unknown diagnosis, which makes idiopathic cases still be the barrier to demolish.

The most important variations in idiopathic cases among the different series are mainly explained by the population's heterogeneity, and the clinical setting in which the patients are seen. Being a neurological or an otorhinolaryngological department may influence the type of patients referred to and the medical follow-up performed. These variations may explain the different percentages of MD when comparing our population to other similar studies (4). Bilateral MD rarely presents with each ear simultaneously but rather sequentially with the second ear involvement occurring many years after the first (14). This fact is represented in our population, with three and seven cases of synchronic and metachronous MD, respectively.

Four patients of our MD population had definite unilateral disease based on Barany's guidelines. All those patients had a positive electrocochleography in both ears, suggesting the endolymphatic hydrops as possible but not confirmed, the underlying cause of their BVP.

The finding of bilateral vestibular involvement in a clinically suspected unilateral vestibulopathy is still one of the most challenging diagnoses and has significant pathophysiological implications. Recovery from vestibular disorders, regardless of the etiology, is partly mediated by this process of vestibular habituation (15), controlled by the central vestibular system.

A unilateral vestibular schwannoma with bilateral vestibular loss was found in 12 patients. Lucieer et al. (3) reported four similar patients, and this condition was also described by Pinna et al. (16). They described 12.7% of their population of 511 vestibular schwannomas, with contralateral hyporeflexia, and 6.5% with a total areflexia. Although some authors attribute contralateral hydrops as the possible explanation for this BVP, the possibility of a habituation process in slow-growing vestibular schwannomas should be noted.

Habituation of vestibular nystagmus has been observed among ballet dancers, pilots, and figure skaters. This fact could be linked to our three cases of symptomatic BVP developed after unilateral vestibular neuritis. Immediately following unilateral

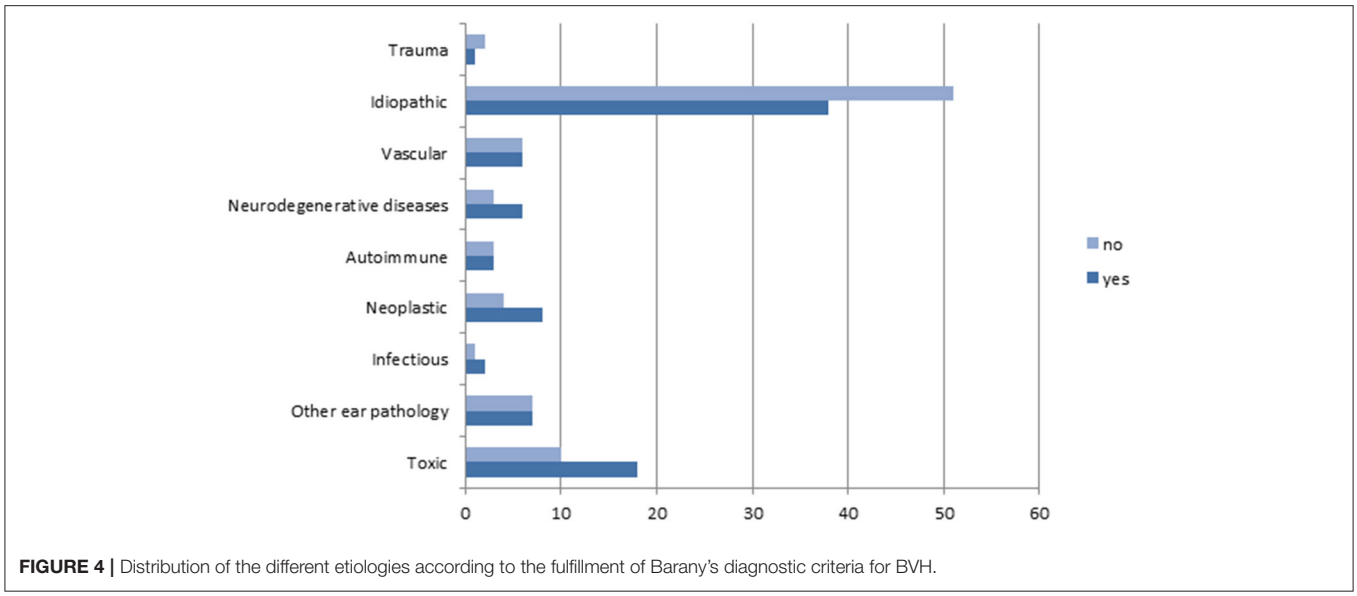

FIGURE 4 | Distribution of the different etiologies according to the fulfillment of Barany's diagnostic criteria for BVH.

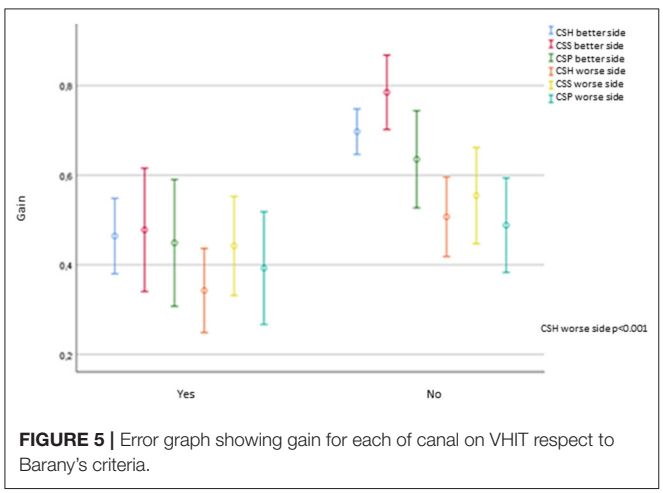

FIGURE 5 | Error graph showing gain for each of canal on VHIT respect to Barany's criteria.

vestibular deafferentation, there is asymmetric nuclei activity where the intact side becomes initially increased, after which cerebellar inhibition depresses the activity from the vestibular nuclei bilaterally (17). Although cerebellar inhibition is supposed to be transitional, our finding raises the question of whether a unilateral vestibular loss can develop asymptomatic chronic BVP. In our three vestibular neuritis cases, all developed significant vestibular damage on one side with moderate affection of the contralateral one, thus developing BVP, which fulfilled Barany's diagnostic criteria.

The group of patients who fulfilled Barany's criteria for BVP had a trend toward a longer time of evolution, although those differences were non-significant. This together with the significant lower horizontal gains and the higher proportion of patients with oscillopsia could lead us to the conclusion that those patients will possibly develop a complete BVP syndrome, ulteriorly.

Regarding the vHIT parameters, our results are similar to those of other BVP populations with a significant decrease of the different semicircular canals gain and the consequent development of corrective saccades (1, 9).

The diagnostic criteria for BVP based on the consensus document of the Classification Committee of the Bárány Society specify that bilaterally reduced or absent angular VOR function documented by a bilaterally pathological horizontal angular VOR gain of <0.6, measured by vHIT or scleral search-coil technique, is mandatory for definite diagnosis (1). However, since the introduction of vHIT in clinical practice, the initial evaluation has been expanded to all six canals (1). Both new approaches (providing a more accurate degree of dysfunction and a specific pattern) present new insight to better understand the different degrees of disability and handicap known to occur in that population of patients (9).

Nevertheless, further analysis of caloric response or horizontal angular VOR gain upon sinusoidal stimulation on a rotatory chair might contribute to a better understanding of our BVP patients.

Although our findings elicited that after head impulses toward the worse side, the PR was higher than after those toward the side where a higher gain value was obtained, those results were non-significant. Our results are similar to those found by Batuecas et al. (9), but they found significant differences in the PR score between both sides. Those differences could be explained by the differences in etiologies or the time of evolution in each population.

All previously described clinical subtypes were identified in our population. Slowly progressive BVP without vertigo and recurrent vertigo with BVP groups were the most prevalent. Our findings are similar to those described by Lucieer et al. (3) either in the idiopathic or in the non-idiopathic cases. Nevertheless, in our population, a significant proportion of our patients developed BVP after a single episode of vertigo. Those

patients had a significantly shorter time of evolution and a significantly higher horizontal semicircular canal gain in the better side compared to the rest of the groups except the slowly progressive BVP without vertigo.

Thirty-three out of 40 patients in the former group were idiopathic, although the clinical presentation was suggestive of an acute vestibular syndrome, such as vestibular neuritis. All those patients complained about unsteadiness or oscillopsia and had bilaterally diminution of the horizontal semicircular canal gain. Nevertheless, a significantly greater proportion of the latter group did not fulfill Barany's criteria for BVP. In our opinion, this fact together with the shorter time of evolution prompts us to consider the possibility of a transitory BVP.

Dix and Hood (18) described a significant percentage of their population of 274 patients with vestibular neuritis, with bilateral abolition of both caloric and rotational response. A mechanism of vestibular habituation suppression was suggested with a possible mechanism of central suppression.

About 20% of patients with a chronic stable unilateral vestibulopathy will continue to experience chronic postural imbalance and oscillopsia, the same symptoms that all patients with BVP, symptoms which constitute the so-called syndrome of chronic vestibular insufficiency (19).

A 66.5% of our population had valid information regarding the familial history of chronic vertigo or disequilibrium. We had a 16.3% of patients with a clinical presentation and family history of disequilibrium, suggestive of genetic predisposition. Unfortunately, no genetic investigations were performed, and most of those patients were idiopathic.

A clear limitation is the retrospective nature of the study. There is frequently an absence of data on potential confounding factors if the data was recorded in the past. It may be difficult to identify an appropriately exposed cohort and an appropriate comparison group. Further studies are needed and should address the issue to conduct prospective studies with appropriate comparison groups.

CONCLUSIONS

BVP is a heterogeneous condition with a high proportion of idiopathic cases.

The distribution of the clinical subtypes and Barany's criteria are a useful clinical tool to differentiate groups of patients, and therefore predict its evolution.

The finding of bilateral vestibular involvement in a clinically suspected unilateral vestibulopathy should be considered in some patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by institutional review board at our hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

FM-M and EM-S contributed to planning of the study, acquisition of data, statistical analyses, interpretation, and writing of the manuscript. AR and JE-S contributed to the planning of the study and acquisition of data. All authors contributed to the article and approved the submitted version.

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frontiers in Neurology

ORIGINAL RESEARCH
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Radiological Configuration of the Vestibular Aqueduct Predicts Bilateral Progression in Meniere's Disease

David Bächinger[1,2], *Bernhard Schuknecht*[3], *Julia Dlugaiczyk*[1,2] *and Andreas H. Eckhard*[1,2]*

[1] Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Zurich, Zurich, Switzerland, [2] University of Zurich, Zurich, Switzerland, [3] Medical Radiological Institute MRI, Zurich, Switzerland

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***Correspondence:**
Andreas H. Eckhard
andreasheinrich.eckhard@usz.ch

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Objective: Meniere's disease (MD) progresses from unilateral to bilateral disease in up to 50% of patients, often chronically and severely impairing balance and hearing functions. According to previous studies, 91% of bilateral MD patients demonstrate bilateral hypoplasia of the endolymphatic sac (ES) upon histological and radiological examination of their inner ears. Here, we seek to validate a radiological marker for ES hypoplasia that predicts the risk for future progression to bilateral MD in individual patients.

Methods: Patients with unilateral MD and radiological evidence for ES hypoplasia in either the clinically affected inner ear (cohort MD_{uni}-hp_{uni}) or both inner ears (cohort MD_{uni}-hp_{bi}) were included. Given our hypothesis that ES hypoplasia critically predisposes the inner ear to MD, we expected progression to bilateral MD only in the MD_{uni}-hp_{bi} cohort. To investigate eventual progression to bilateral MD, clinical, audiometric, and imaging data were retrospectively collected over follow-up periods of up to 31 years.

Results: A total of 44 patients were included in the MD-hp_{uni} ($n = 15$) and MD_{uni}-hp_{bi} ($n = 29$) cohorts. In line with our radiology-based predictions, none (0/15) of the MD-hp_{uni} patients exhibited progression to bilateral MD, whereas 20/29 (69%) MD-hp_{bi} patients have already progressed to bilateral MD. Using the Kaplan–Meier estimator, bilateral disease progression would be observed in 100% of MD-hp_{bi} patients 31 years after the initial diagnosis with an estimated median time to bilateral progression of 12 years. The nine MD-hp_{bi} patients who, so far, remained with unilateral disease demonstrated a median time since initial (unilateral) MD diagnosis of only 6 years and are thus still expected to progress to bilateral disease.

Conclusion: Progression to bilateral MD adheres to predictions based on the radiological presence or absence of ES hypoplasia. This prognostic tool, if validated by prospective long-term studies, will provide clinically relevant information about a patient's future disease burden and will help to select more personalized treatment regimens.

Keywords: endolymphatic hydrops, endolymphatic sac, vestibular aqueduct, prognosis, MRI, CT, imaging

INTRODUCTION

Meniere's disease (MD), a chronic inner ear disorder, causes fluctuating vestibular and auditory symptoms and exhibits a highly variable disease course among patients (1, 2). Most severely affected are the 10–50% of patients (3–5) in whom the disease progresses to bilateral MD, often years to decades after its initial (unilateral) manifestation. Due to bilateral vestibulopathy, patients with bilateral MD experience chronic debilitating vestibular symptoms, such as oscillopsia and imbalance, and a broad range of cognitive and emotional impairment (6). Moreover, bilateral MD often leads to severe to profound hearing loss with unserviceable speech discrimination (7, 8). A biomarker for future bilateral disease would allow clinicians to identify those patients, to preemptively counsel them about the expected disease course, and to personalize therapy regimens in clinically meaningful ways.

In this study, radiological evidence for endolymphatic sac (ES) hypoplasia (9, 10), i.e., the suspected etiopathology in approximately 30% of MD patients, with the designated endotype "MD-hp" (11), was used to prognosticate disease laterality. In a recent human temporal bone study, either of two pathologies of the ES, i.e., degeneration or developmental hypoplasia, was consistently found in cases with clinical MD (9). These ES pathologies ("endotypes") can be linked to the pathogenesis of endolymphatic hydrops, a histopathologic and radiologic marker of MD (9, 12). The subtype of ES pathology correlates with the course of the vestibular aqueduct, which–in contrast to the ES epithelium–can be visualized in clinical imaging (10). In MD, the course of the vestibular aqueduct can therefore be used as a radiologic surrogate marker for the underlying subtype of cellular ES pathology (10). Using this marker, it has been shown in clinical patients that the pathologic endotypes are associated with differing clinical phenotypes (11). In MD-hp patients, one or both inner ears may exhibit ES hypoplasia. However, to date, it is not clear whether ES hypoplasia critically predisposes to MD. Here, we hypothesized that progression to bilateral MD only occurs in patients with bilateral ES hypoplasia (cohort MD_{uni}-hp_{bi}) but not in those with unilateral ES hypoplasia (cohort MD_{uni}-hp_{uni}).

MATERIALS AND METHODS

Ethics Approval

This study was approved by the local ethics committee (KEK-ZH-Nr. 2016-01619/2019-01006) in accordance with the Declaration of Helsinki and its amendments. Informed consent has been obtained from all participants.

Abbreviations: ATVA, angular trajectory of the vestibular aqueduct; cVEMP, cervical vestibular-evoked myogenic potential; ES, endolymphatic sac; Gd-MRI, gadolinium-enhanced MRI; HRCT, high-resolution CT; MD, Meniere's disease; MD-dg, Meniere's disease associated with a degeneration of the endolymphatic sac (and a normal vestibular aqueduct); MD-hp, Meniere's disease associated with a developmental hypoplasia of the vestibular aqueduct and endolymphatic sac.

Study Design and Participants

Patients from an interdisciplinary tertiary neurotology center fulfilling the following inclusion criteria were included into the study between August 2019 and December 2020: (i) patients with an initial diagnosis of unilateral definite MD (1, 13), (ii) radiological evidence for uni- or bilateral ES hypoplasia (endotype MD-hp), (iii) endolymphatic hydrops in the affected ear (see next paragraph), and (iv) age $\geq$18 years. The time point of first MD manifestation was defined by the first reported episode of spontaneous vertigo lasting >20 min or by the first audiometrically documented hearing loss, which matched the MD diagnostic criteria (1, 13). Patients with secondary Meniere's syndrome due to a known pathology were excluded (14, 15). Bilateral progression was defined as recurrence of vertigo attack(s) and audiometrically documented hearing loss in the second ear (16).

Temporal Bone Imaging

At the time of initial clinical work-up, 3 Tesla gadolinium-enhanced MRI (Gd-MRI) of the temporal bones was performed using a 32-channel phased array coil to visualize endolymphatic hydrops (17, 18) and to exclude other intra- or retrolabyrinthine pathology. Endolymphatic hydrops grading was performed separately for the cochlea and the vestibule (18) by an experienced neuroradiologist (BS). In some patients, additional high-resolution CT (HRCT) of the temporal bones was performed, e.g., to exclude a dehiscence syndrome.

Vestibular Aqueduct Measurements and Patient Endotyping

The angular trajectory of the vestibular aqueduct (ATVA) was determined for each inner ear in HRCT data if available or in Gd-MR imaging data (3D real inversion recovery sequence). An ATVA with an angle α_{exit} >140° indicated ES hypoplasia, and an α_{exit} <120° indicated a normal ES (**Figure 1**), as defined previously (9, 10). Patients with α_{exit} >140° and <120° on the clinically affected and the non-affected side, respectively, were assigned to the MD_{uni}-hp_{uni} cohort, whereas those with an α_{exit} >140° on both sides were assigned to the MD_{uni}-hp_{bi} cohort.

Statistical Analysis

Values are reported as absolute numbers (percentage) or means with standard deviation (SD) and range. Continuous variables were analyzed using a two-tailed Student's *t*-test for independent samples. For binary variables, a Fisher's exact test was performed. A $p < 0.05$ was considered as statistically significant. The Kaplan–Meier method was used to quantify the percentage of patients with progression to bilateral MD. The Log-rank (Mantel–Cox) test was used to compare Kaplan–Meier curves. Follow-up times were censored for all cases at the time when the final analysis was initiated (January 2021). Statistical analyses were performed using Prism for Apple Macintosh, version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Clinical Features of the Study Cohorts

A total of 44 patients were included between August 2019 and January 2020. From those, 15 patients were assigned to the MD-hp$_{uni}$ cohort, and 29 patients to the MD-hp$_{bi}$ cohort based on the radiological criteria defined above (**Table 1**). In 35/44 (80%) patients, HRCT was available and used for vestibular aqueduct measurements. In the remaining 9/44 (20%) patients, Gd-MRI was used for vestibular aqueduct measurements. Male to female ratio, mean age at MD onset (time point of initial diagnosis), and range of disease duration did not significantly differ between both cohorts (**Table 1**). Among the 44 MD-hp patients, 14 (32%) had a positive family history for MD, 3 (7%) had migraine, and none had a known autoimmune disorder.

Progression to Bilateral MD Is Exclusively Observed in the MD-hp$_{bi}$ Cohort

Within 30 and 31 years from onset of MD, none (0/15) of the MD-hp$_{uni}$ patients and 20/29 (69%) MD-hp$_{bi}$ patients progressed to bilateral MD, respectively ($p = 0.001$; **Figure 2**, **Table 1**). In MD-hp$_{bi}$ patients, the estimated median time to bilateral progression was 12 years. The proportion of patients with bilateral MD was estimated to increase to 43% after 10 years and to 90% after 30 years with unilateral MD, respectively (**Figure 2**).

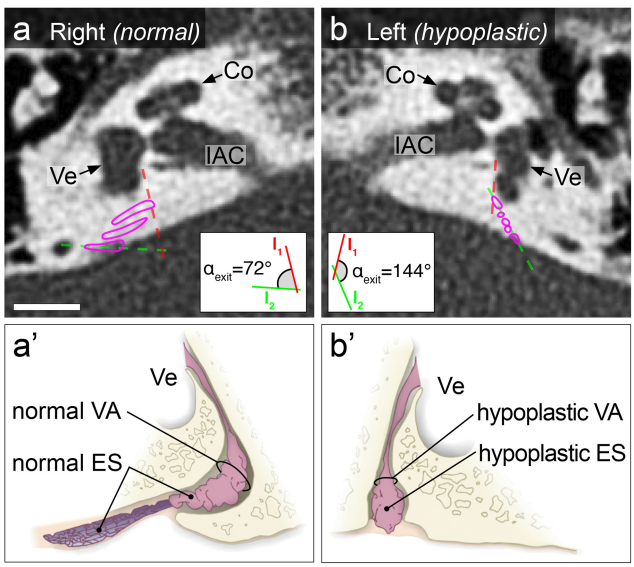

FIGURE 1 | Illustration of normal and hypoplastic ES morphology in temporal bone HRCT from an MD$_{uni}$-hp$_{uni}$ patient. Axial plane CT images show a normal, bent course of the 2D-reconstructed vestibular aqueduct on the right (magenta outlines in **(a)**; ATVA with $\alpha_{exit} = 72°$), indicating the presence of a normal ES. The panel below shows a drawing of a normal ES located within a normal vestibular aqueduct **(a')**. On the left side, an abnormal, straight course of the vestibular aqueduct was found (magenta outlines in **(b)**; ATVA with $\alpha_{exit} = 144°$), indicating a hypoplastic ES. The panel below shows a drawing of a hypoplastic ES located within a hypoplastic vestibular aqueduct **(b')**. This patient had a 9-year history of left-sided MD. Insets show the geometric measurement results for the ATVA, according to previously described methods (10). Co, cochlea; ES, endolymphatic sac; IAC, internal auditory canal; VA, vestibular aqueduct; Ve, vestibule. Scale bar: 5 mm. **(a',b')** are adapted from Eckhard et al. (9), under the terms of the Creative Commons CC BY license (http://creativecommons.org/licenses/by/4.0/).

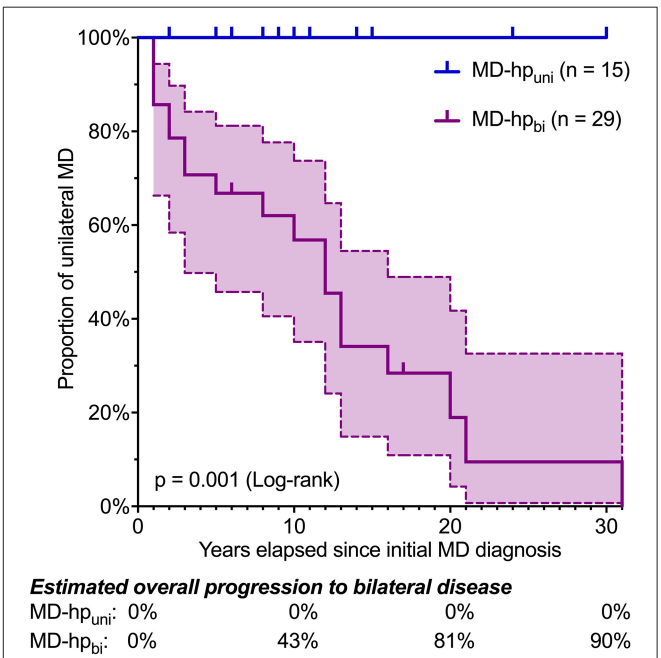

FIGURE 2 | Kaplan–Meier plot for progression to bilateral MD in the MD-hp$_{uni}$ and MD-hp$_{bi}$ cohorts. Ticks indicate censored cases. In the MD-hp$_{bi}$ cohort, median time to bilateral progression was 12 years. None of the MD-hp$_{uni}$ patients developed bilateral disease within 30 years. Dashed lines indicate 95% confidence intervals.

TABLE 1 | Demographics and clinical characteristics of the MD-hp cohorts.

	MD-hp ($n = 44$)	MD-hp$_{uni}$ ($n = 15$)	MD-hp$_{bi}$ ($n = 29$)	MD-hp$_{uni}$ vs. MD-hp$_{bi}$
Male to female ratio, no. (%)	37:7	13 (87%):2 (13%)	24 (83%):5 (17%)	$p = 0.99$
Mean age at onset, years (SD)	39.0 (11.6)	39.1 (7.8)	39.0 (13.2)	$p = 0.96$
Mean disease duration, years (SD, range)	14.7 (12.1)	10.7 (7.7, 2–30)	16.8 (13.7, 1–31)	$p = 0.12$
Unilateral to bilateral MD ratio, no. (%)	24 (55%):20 (45%)	15 (100%):0 (0%)	9 (31%):20 (69%)	$p < 0.0001$

Prospective Observation of Bilateral Disease Progression in an MD_{uni}-hp_{bi} Patient

This male patient, who was followed up in our neurotology center between age 53 and 58, was prognosticated to develop bilateral MD based on temporal bone HRCT imaging signs for bilateral ES hypoplasia (**Figures 3a,b**). The patient initially was seen at age 53 with complaints of monthly, spontaneous vertigo episodes (up to 6 h), accompanied by hearing loss and fullness in his right ear. Pure tone audiometry at the time showed moderate sensorineural hearing loss at low and high frequencies ("peak pattern") on the right side (**Figures 3c,d**). Inner ear Gd-MRI demonstrated grade 1 cochleovestibular hydrops in the right ear (**Figures 3e,f**), supporting the clinical diagnosis of right definite MD. The patient was started on betahistine (48 mg twice daily). Over the following 5 years, in which he was followed up at 6–12-month intervals, he reported only one more vertigo attack. During this time, right-sided hearing progressively deteriorated to a moderate sensorineural hearing loss that affected all frequencies [**Figure 3c**, age 53–58 (I)]. A left-sided age-appropriate high-frequency sensorineural hearing loss was observed during that time [**Figure 3d**, age 54–58 (I)]. At age 58, the patient again experienced weekly, hours-long spontaneous vertigo episodes as well as a new hearing loss in his previously unaffected left ear. Pure tone audiometry at that time demonstrated a new low-frequency hearing loss in the left ear (**Figure 3d**), and sequential Gd-MRI of the inner ears showed a new cochlear hydrops grade 1 in the left ear (**Figures 3g,h**). The clinical diagnosis was revised accordingly to bilateral definite MD. Of note, a further progression of high-, but not low-frequency sensorineural hearing loss was observed for the right ear supporting the notion of an anticipated age-related decline in the right ear, whereas vertigo attacks now originated from the left ear [**Figures 3c,d**, age 58 (I) and 58 (II)].

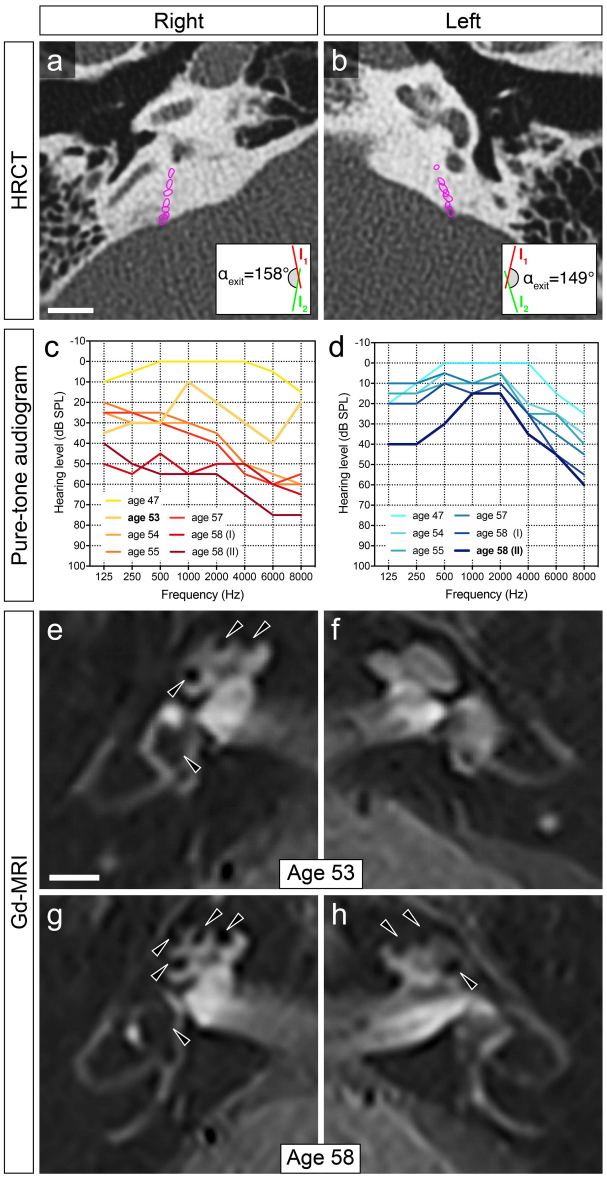

FIGURE 3 | Exemplary clinical case progressing to bilateral disease. **(a,b)** In this patient, ATVA measurements on temporal bone HRCT images performed at age 53 determined angles (α_{exit}) consistent with bilateral ES hypoplasia (2D-reconstructed course of the vestibular aqueduct outlined in magenta). Scale bar: 5 mm. **(c,d)** Audiogram data (air conduction thresholds) before MD first manifested (age 47), after onset of unilateral (right) MD [ages 53–58 (I)], and shortly after progression to bilateral disease [age 58 (II)]. **(e,f)** Gd-MRI at the time point of initial MD diagnosis (age 53) demonstrated grade 1 (18) cochleovestibular hydrops (black arrowheads) in the right inner ear. **(g,h)** Sequential Gd-MRI, after clinical and audiometric data indicated progression to bilateral MD (age 58), demonstrated grade 1 cochleovestibular hydrops in the right and left inner ears. Scale bar: 5 mm.

DISCUSSION

MD-hp patients statistically have a 25% risk for developing bilateral disease, which is 5-fold higher than other MD patients (11). Thus, providing MD-hp patients with a personalized prognosis, instead of a mere statistical one-in-four chance for bilateral disease progression, would advance the clinical management of this patient group, which is at highest risk for a severe disease course, in a clinically relevant manner.

MD-hp patients are distinguished by a developmentally rudimentary, i.e., hypoplastic, ES (**Figure 1b′**), which has been initially described in sporadic MD cases (19–22) and was more recently demonstrated as a consistent finding in about 30% of pathology cases with a clinical MD diagnosis (9)—now designated as the MD-hp patient group (11). Pathophysiologically, the absence of normal ion transport mechanisms in the hypoplastic ES epithelium is believed to be the key pathology that causes disturbances of the inner ear fluids and, ultimately, endolymphatic hydrops and clinical symptoms (9, 23). ES hypoplasia is consistently associated with a hypoplastic bony VA, which can be radiologically visualized and was established as a clinical surrogate marker (ATVA) for the presence of ES hypoplasia (10).

This present preliminary and previous (11) data suggest that the radiological ATVA marker has absolute positive and negative

predictive power for making this personalized prognosis. If ultimately validated by long-term prospective studies, this would allow clinicians (i) to counsel patients about aspects of their future disease course, in particular about the long-term impacts on hearing and balance functions; (ii) to make better decisions on whether ablative therapies, such as intratympanic gentamicin or vestibular neurectomy, can be considered; and potentially also (iii) to screen family members and children from MD-hp patients for their risk of developing uni- or bilateral MD. Furthermore, the ATVA marker enables to define clinically more homogenous subgroups of MD patients for future (genomic) studies.

In bilateral MD, non-ablative treatments, such as intratympanic steroids, should be preferred over ablative treatments, which should be avoided (16). Using the ATVA marker, progression to bilaterality can be anticipated in MD-hp_{bi} patients and virtually excluded in MD-hp_{uni} patients. Therefore, this clinical tool may allow to obviate iatrogenic bilateral vestibular loss and/or hearing loss due to ablative treatments in MD-hp_{bi} patients. On the other hand, in MD-hp_{uni} patients with an infinitesimal risk for bilateral progression, ablative treatments, such as intratympanic gentamicin administration, may be liberally used.

ES surgery is commonly considered as a non-ablative treatment of MD (16). It should be noted, however, that in MD-hp patients, the ES is hypoplastic and anteriorly displaced. These anatomical abnormalities impede the surgical approach of the ES *via* the commonly used transmastoid route; furthermore, the hypoplastic ES occasionally does not exit the temporal bone at all. Of note, the surgical non-visualization rate of the ES during ES surgery corresponds to the percentage of MD-hp patients among all MD patients (11, 24). Although there is lacking evidence for a beneficial effect of ES surgery (25), this "non-ablative" surgical treatment–if considered–may therefore be particularly avoided in MD-hp patients.

Notably, among other proposed markers for predicting bilateral MD (2, 18), cervical vestibular-evoked myogenic potential (cVEMP) metrics were retrospectively found to be altered, with high specificity and sensitivity, in clinically yet unaffected contralateral ears 24–288 months before clinical symptoms manifested (26, 27). The actual time course of these cVEMP changes and their earliest appearance during the presymptomatic phase remain to be investigated. The present radiological (ATVA) marker may potentially identify inner ears at risk even before any MD-associated functional changes occur, i.e., in early childhood, when the temporal bone anatomy is matured. However, its validity for predicting MD in asymptomatic individuals with ES hypoplasia needs to be investigated.

Regarding the potential clinical use of the ATVA marker, the following points should further be considered: (i) the time interval from initial MD diagnosis to bilateral affection is highly variable among patients (4, 5, 11, 28, 29) and cannot be predicted with the ATVA marker; (ii) the measurement of an α_{exit} of $<120^{\circ}$ in the contralateral ear does not with absolute certainty exclude progression to bilateral disease, since a different MD-causing process can affect this ear (i.e., another MD endotype) (9, 11), although this remains hypothetical; and (iii) the determination of the ATVA on both sides in Gd-MRI (3D IR sequence) data requires systemic (i.v.) delivery of the Gd-contrast agent.

In conclusion, ongoing observations will determine whether the promising preliminary data hold up and whether both study cohorts remain "true to prediction," thus ultimately validating the ATVA marker as a prognostic tool for bilateral disease progression in MD-hp patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission Zürich, KEK-ZH-Nr. 2016-01619/2019-01006. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study was concepted and designed by DB and AE. Acquisition and analysis of data were performed by DB, BS, JD, and AE. DB and AE drafted the manuscript. All authors critically reviewed and revised the manuscript.

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Distinct MicroRNA Profiles in the Perilymph and Serum of Patients With Menière's Disease

Matthew Shew[1], Helena Wichova[2], Madeleine St. Peter[2], Athanasia Warnecke[3] and Hinrich Staecker[2]*

[1] Department of Otolaryngology Head and Neck Surgery, Washington University School of Medicine in St. Louis, St. Louis, MO, United States, [2] Department of Otolaryngology Head and Neck Surgery, University of Kansas School of Medicine, Kansas City, KS, United States, [3] Department of Otolaryngology Head and Neck Surgery, Hannover Medical School, Hanover, Germany

Hypothesis: Menière's disease microRNA (miRNA) profiles are unique and are reflected in the perilymph and serum of patients.

Background: Development of effective biomarkers for Menière's disease are needed. miRNAs are small RNA sequences that downregulate mRNA translation and play a significant role in a variety of disease states, ultimately making them a promising biomarker. miRNAs can be readily isolated from human inner ear perilymph and serum, and may exhibit disease-specific profiles.

Methods: Perilymph sampling was performed in 10 patients undergoing surgery; 5 patients with Meniere's disease and 5 patients with otosclerosis serving as controls. miRNAs were isolated from the serum of 5 patients with bilateral Menière's disease and compared to 5 healthy age-matched controls. For evaluation of miRNAs an Agilent miRNA gene chip was used. Analysis of miRNA expression was carried out using Qlucore and Ingenuitey Pathway Analysis software. Promising miRNAs biomarkers were validated using qPCR.

Results: In the perilymph of patients with Menière's disease, we identified 16 differentially expressed miRNAs that are predicted to regulate over 220 different cochlear genes. Six miRNAs are postulated to regulate aquaporin expression and twelve miRNAs are postulated to regulate a variety of inflammatory and autoimmune pathways. When comparing perilymph with serum samples, miRNA-1299 and−1270 were differentially expressed in both the perilymph and serum of Ménière's patients compared to controls. Further analysis using qPCR confirmed miRNA-1299 is downregulated over 3-fold in Meniere's disease serum samples compared to controls.

Conclusions: Patients with Ménière's disease exhibit distinct miRNA expression profiles within both the perilymph and serum. The altered perilymph miRNAs identified can be linked to postulated Ménière's disease pathways and may serve as biomarkers. miRNA-1299 was validated to be downregulated in both the serum and perilymph of Menière's patients.

Keywords: Meniere disease, microRNA, liquid biopsy, perilymph, biomarker

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****Correspondence:***
Matthew Shew
mshew@wustl.edu

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INTRODUCTION

Menière's disease is a chronic debilitating disorder of the inner ear, characterized by fluctuating episodes of vertigo, hearing loss, tinnitus, and aural pressure (1). Menière's disease affects 250–500 per 100,000 people and has repeatedly been shown to negatively impact patient's quality of life comparable to many other more common chronic medical diseases (2, 3). Since Prosper Menière first recognized this debilitating disease over 150 years ago, we have made significant strides to further understand the pathophysiology of Menière's disease. Most notably, 75 years ago post-mortem temporal bone analysis demonstrated endolymphatic hydrops as a pathologic correlate to Menière's disease (4). However, despite our best efforts, there remains a significant knowledge gap in the pathophysiology, diagnosis, and management of Menière's disease. While post-mortem studies have offered invaluable information and insights (5), we still have a limited understanding of what is occurring on a molecular level in patients with active Menière's disease, particularly in the early stages of the disease and other inner ear disorders with fluctuating symptoms.

The inner ear is a complex, delicate, fluid-filled structure that lies deep within the temporal bone of the skull. Unfortunately, a biopsy equivalent of the inner ear is not feasible, and diagnosis of the different diseases often rely on various forms of hearing performance testing. However, hearing performance testing such as the audiogram, do not always accurately reflect the true underlying pathology (6). As a result of these shortcomings, there is significant interest in obtaining a "liquid biopsy" equivalent of the inner ear. Many investigators are analyzing the components of inner ear fluid to gain insights into what may be occurring on a molecular level in active inner ear disease states (7–12).

Diagnosis of Menière's disease is categorized as "probable" or "definite" based on a composition of fluctuating and variable clinical criteria established by the Bárány Society and adopted by the American Academy of Otolaryngology Head and Neck Surgery (13, 14). New diagnostic methodology has aided to refine the diagnosis of Menière's disease using objective electrophysiologic testing and/or imaging, however testing is not always reliable and thus not routinely recommended (15). Unfortunately, there is no single diagnostic test for Menière's disease that serves as the gold standard. This is a result of our limited knowledge of the underlying pathology resulting in Menière's disease. Multiple pathophysiology mechanisms have been postulated including genetic (16), vascular (17, 18), immunologic (18–20), aberrant aquaporin expression (21), or a combination of causes.

Evaluation of micro RNA (miRNA) perilymph profiles in inner ear disease may help define the hearing loss on a molecular level (11). Inner ear diseases from otosclerosis to profound sensorineural hearing loss (SNHL) exhibit distinct miRNA profiles (22, 23). miRNAs are 19–23 base pair single stranded RNA sequences that regulate post translation gene expression through messenger RNA (mRNA) silencing (24). miRNA molecules are conserved throughout multiple species, have been identified in tissue and fluids throughout the body, and are known to play a significant role in various pathologies including cancer and other neurodegenerative diseases (25, 26). miRNAs have also been shown to play a key role in inner ear development and show distinct expression patterns in patients with hearing loss compared to healthy controls, making them an intriguing biomarker (27–30). In previous work, we preliminary established Meniere's disease could be diagnosed based on perilymph miRNA profile alone, further validating miRNAs as a potential biomarker either through the perilymph or serum of patients (31).

In the current study we analyzed the miRNA profiles of patients with Menière's disease, collected at the time of labyrinthectomy, and compare their profiles to patients with otosclerosis. With the goal of identifying potential Menière's specific biomarkers, we compared the differentially expressed miRNAs within the perilymph and compared it to serum miRNA profiles in patients with active bilateral Menière's disease and healthy age matched controls.

METHODS

Human perilymph sampling was approved by the University of Kansas Human Studies Committee and Institutional Review Board (Study00142630). All experiments performed were in accordance with relevant guidelines and regulations. Patients were recruited for perilymph sampling if they were undergoing a surgical procedure where the inner ear was already being opened. miRNA perilymph profiling was performed using two disease classes, Menière's disease and otosclerosis. For Menière's disease, perilymph was collected at the time of labyrinthectomy for patients with intractable symptoms and who failed hearing conservative management. Perilymph sampling in patients with a healthy and normal ear is not currently feasible, therefore we used otosclerosis patients as a control since they have minimal SNHL. Patients with SNHL secondary to otosclerosis were not included. All patients received standard of care and underwent surgical treatment with either stapedotomy or labyrinthectomy. When the patient's inner ear was opened for their indicated procedure, a small sterile glass capillary tube was used to collect ∼2–5 μL volume of perilymph (11).

In the second part of this project, we sought to evaluate if the perilymph profile of Meniere's disease is also reflected in the serum of patients. An overlap would indicate that miRNAs may serve as a promising biomarker for Menière's disease. In order to evaluate serum miRNA profiles, human serum was collected, and miRNAs were isolated from five patients with active bilateral Menière's disease. Serum miRNA profiles from Menière's disease were compared to five healthy age matched controls.

Patient Selection and Audiometry

All patients underwent pure tone audiometry prior to their respective procedures. Air and bone conduction thresholds were determined, and a CT scan of the temporal bone was performed for all patients with diagnosis of otosclerosis. Only patients with conductive hearing loss (CHL) and radiologically confirmed otosclerosis were included in the CHL control group ($n = 5$). For the Meniere's disease cohort, patient's required the diagnosis of "definite Meniere's" based on the Bárány Society

and adopted by the American Academy of Otolaryngology Head and Neck Surgery (13, 14). This included documented low to mid frequency SNHL, episodic vertigo that lasts 20 min to 12 h, and aural symptoms (fullness and/or tinnitus). Patients were diagnosed with bilateral Meniere's disease if they fit "definite Meniere's," where symptoms lateralized to both ears (tinnitus, aural fullness, pressure) and documented low frequency SNHL in both ears at some point in their Meniere's history. A total of five patients ($n = 5$) underwent labyrinthectomy for poorly controlled Menière's disease who had failed hearing conservative therapy including dietary control, diuretics, betahistine, and/or endolymphatic sac decompression. Patients were not considered for intratympanic gentamycin injections.

Perilymph Sampling During Stapedectomy

The skin of the external auditory canal was injected with 0.5 ml of 1% lidocaine + 1:100,000 epinephrine. Using a round knife, a cut was made in the skin of the external canal and small tympanomeatal flap was carefully elevated medially revealing the middle ear structures. Using the Omniguide^{TM} CO_2 laser with a power setting of four watts, 0.1 s single bursts, the stapes superstructures were removed. Using the laser, a rosette fenestration was made in the stapes footplate. Upon making the fenestration, perilymph could be seen coming out from the vestibule. We then removed excess perilymph with a sterile glass capillary tube. After successful collection of perilymph fluid, the stapes footplate fenestration was enlarged to accommodate the stapes prosthesis. The surgery was then completed in a standard fashion.

Perilymph Sampling During Labyrinthectomy

Through a post auricular incision, a standard mastoidectomy was performed and the horizontal semicircular canal was identified. The horizontal semicircular canal was carefully blue lined with a diamond drill and opened on one posterior end to allow free flow of perilymph out of the inner ear. The perilymph sample was obtained using a sterile glass capillary tube. A standard labyrinthectomy was performed following collection of adequate sample.

Human Serum Sampling

Five patients with bilateral active Menière's disease based on 2015 Barany Society diagnostic criteria and five age matched healthy controls were enrolled. Six ml of whole blood was collected from each participant and transferred to tubes containing EDTA. The samples were then spun down at 2,000 ×g for 15 min. The serum layer was then pipetted off and frozen at −20°C. Once all 10 samples were collected, microRNA was isolated from the serum using Qiagen miRNeasy Serum/Plasma Kit (Cat No./ID 217184).

MicroRNA Analysis

Perilymph collection has been previously described (11, 23). Total RNA was extracted with Trizol reagent (Thermofisher, cat #15596018) and purified by centrifuging with a phase lock heavy gel (Tiagen, cat # WMS-2302830). MicroRNA from serum samples was extracted as described above. Samples from both perilymph and serum were analyzed with an Agilent RNA6000 Pico kit using an Agilent Bioanalyzer 2100 yielding on average 0.5–2 ng of total RNA per sample. Samples were processed and analyzed with an Affymetrix miRNA 4.0 array to determine the presence of micro RNAs as per manufacturer instructions. The Affymetrix miRNA 4.0 array interrogates all miRNA sequences listed in miRBase Release 20; interrogating 30,434 mature miRNAs from 203 organisms of which 2,578 are from humans. The arrays were background corrected, normalized, and gene-level summarized using the Robust Multichip Average (RMA) algorithm. In order to ascertain which miRNAs were significantly expressed in each array, for each miRNA probe in the array, a detection p-value was computed based on a Wilcoxon Rank-Sum test of the miRNA probe set signals compared to the distribution of a GC matched background signal comprising of anti-genomic probes in the same array. The detection p-values were adjusted for multiple hypothesis testing (FDR) using the Benjamini and Hochberg method. These analyses were performed using Affymetrix Expression Console Software. miRNAs with a normalized log2 signal intensity ≥ 7 and an adjusted detection p-value (FDR) ≤ 0.05 were considered significantly expressed in the assay.

To further understand miRNA interaction within the human cochlea as it relates directly to Menière's disease, we approached analysis in a two-step process. First, we compared the perilymph miRNA profiles between patients with Menière's disease and otosclerosis (control) to differentiate which miRNAs are unique to Menière's disease using Qlucore software (Qlucore Inc., Lund Sweden). The miRNA expression data were then analyzed using Ingenuity Pathway Analysis (IPA) software (Qiagen Bioinformatics, Redwood City, CA). In order to further understand miRNA interactions within the human inner ear, we used IPA software to predict potential interactions with genes expressed in a normal hearing human cochlea (GEO Series accession number GSE128505) (10, 29). Secondly, in order to identify miRNAs that are clinically relevant, we screened the identified miRNA-mRNA interactions for genes that have been identified to play a pathologic role in Meniere's disease in either animal models or population studies (30–33). Key miRNAs identified were then compared the miRNA expression levels in the serum of patients with Meniere's disease and controls. MicroRNA data from serum samples was analyzed by microarray as described above with a focus on miRNAs that had been identified as differentially expressed in serum.

Serum miRNA qPCR Validation Analysis

After running microRNA arrays from the serum samples, residual RNA from the samples was validated by Q-RT-PCR. Two serum samples were excluded from further processing due to low RNA concentration, one control and one from the Meniere's cohort. A second sample from the Meniere's cohort was excluded from analysis due to genomic DNA contamination, leaving 4 samples in the control group and 3 in the Meniere's group. cDNA was synthesized using the miScript II reverse transcription kit (Qiagen, cat#218161). qPCR was then performed on the BioRad CFX using the miScript SYBR Green miRNA PCR system (Qiagen, cat#218073). All

TABLE 1 | Audiometric data for both patient cohorts.

	Meniere's disease	Otosclerosis
Number of patients	5	5
Age (years) (Mean ± Std)	66.6 ± 18.1	52.2 ± 10.6
PTA (dB) (Mean ± Std)		
Air	69.2 ± 18.8	58 ± 6.7
Bone	60.8 ± 23.2	30.4 ± 8.6

reactions were performed in triplicate and the Ct value was determined using the threshold calculated by the BioRad software. Mir-191-5p was used for normalization based on its stable expression across treatment and control samples. The universal primer from the miScript SYBR Green miRNA PCR kit (Qiagen, cat#218073) was used as the reverse primer for each reaction. MiScript primer assays for hsa-miR-191-5p (5′ CAACGGAATCCCAAAAGCAGCTG 3′) and hsa-miR-1270 (5′ CTGGAGATATGGAAGAGCTGTG 3′) were used as forward primers for qPCR (Qiagen, cat#218300). The hsa-miR-1299 forward primer sequence was 5′ CTGGAATTCTGTGTGAGGG 3′. Fold change was calculated using the $2^{-\Delta\Delta CT}$ method (32).

RESULTS

Perilymph miRNA Profile in Meniere's Disease vs. Otosclerosis (CHL/Controls)

In order to identify which miRNAs are unique to Meniere's disease, we compared the perilymph miRNA expression profiles between Meniere's disease ($n = 5$) and otosclerosis ($n = 5$) (**Table 1**). One significant challenge is that perilymph sampling in patients with normal hearing is not currently feasible. Therefore, patients with otosclerosis serve as our control, given this pathology is largely exclusive to the stapes footplate. To maximize this patient population as controls, we excluded patients with SNHL secondary to advanced otosclerosis. Using gene expression analysis, we identified 16 unique miRNAs that were significantly downregulated in patients with Menière's disease (**Table 2**).

The 16 miRNAs altered in Menière's disease were screened for targets in a human cochlear cDNA library using IPA software. Analysis was only limited to miRNA-mRNA prediction interactions that have either been experimentally proven or "high probability" based on paired nucleotide sequencing between miRNA and mRNA. The 16 miRNAs unique to Meniere's disease were predicted to interact with over 220 genes within the cochlea. To focus our search, we only analyzed proposed gene candidates that have been proven to play a pathologic role in Meniere's disease through either animal models or human genetic population studies. Aberrant aquaporin regulation have been consistently linked to Menière's disease in both experimental models and population studies (33, 34). Altered regulation of both pathways have been linked to Menière's disease as well (18, 35–37). Six of the 16 miRNAs are predicted to regulate aquaporin gene expression (**Table 3**). Twelve of the 16 miRNAs are predicted to regulate either inflammatory or autoimmune regulatory pathways (**Table 4**).

TABLE 2 | MiRNAs significantly downregulated in Menière's disease vs. otosclerosis perilymph.

Micro RNA	*T*-Test
hsa-miR-4296	−2.146997624
hsa-miR-6827-3p	−2.156687123
hsa-miR-3658	−2.205593839
hsa-miR-219b-5p	−2.22463251
hsa-miR-1243	−2.231753534
hsa-miR-4715-3p	−2.258995064
hsa-miR-5700	−2.277212392
hsa-miR-4746-5p	−2.298984529
hsa-miR-542-3p	−2.307117824
hsa-miR-1299	−2.332907361
hsa-miR-1255a	−2.438288476
hsa-miR-6853-5p	−2.443161671
hsa-miR-518f-3p	−2.521748132
hsa-miR-620	−2.556715548
hsa-miR-5192	−2.680462648
hsa-miR-424-3p	−3.009047712

TABLE 3 | MiRNAs unique to Meniere's disease perilymph that are postulated to directly regulate aquaporin gene expression.

Micro RNA	Aquaporin
hsa-miR-219b-5p	AQP2
hsa-miR-1243	AQP4
hsa-miR-4746-5p	AQP2 + AQP4
hsa-miR-1299	AQP2
hsa-miR-1255a	AQP3 + AQP4
hsa-miR-1270	AQP4

TABLE 4 | MiRNAs differentially expressed in Meniere's disease perilymph and their inflammatory pathway targets.

Micro RNA	Inflammatory pathway targets
hsa-miR-542-3p	KRTAP6-3, LINC01169, R3HDM2
hsa-miR-6853-5p	POLR2J2/ POLR2J3, NME4
hsa-miR-424-3p	OTOR, HIGD1A, RNF13
hsa-miR-1243	RNF13, LYRM2, RPS16, RAP1B
hsa-miR-5700	TNFRSF11B, RAP1B, ELOC
hsa-miR-518f-3p	RAP1B
hsa-miR-4746-5p	NMNAT2, STEAP2
hsa-miR-1255a	RPS16, NMNAT2
hsa-miR-4715-3p	SCP2
hsa-miR-1299	SCP2, ATP5PO
hsa-miR-3658	ATP5PO, SCP2
hsa-miR-1270	SCP2, STEAP2

Evaluating Human Serum miRNA Profiles for Potential Biomarkers

An ideal candidate Menière's disease biomarker would be identifiable within both the perilymph and serum of patients, thereby allowing us to evaluate the function of the inner ear based on a serum sample. We collected and isolated miRNAs in the serum of patients with bilateral active Menière's disease ($n = 5$) and age matched controls ($n = 5$). Using miRNA array analysis, we identified 79 alternately expressed miRNAs in the serum of patients with Menière's disease compared to controls. miRNA-1299 and−1270 were uniquely and differentially expressed in the perilymph and serum of patients with Menière's disease, making both promising biomarkers. Both miRNA-1299 and-1270 were linked to inflammatory and autoimmune pathways. miRNA-1299 can be linked to aquaporin expression.

To further evaluate if either miRNA-1299 or−1270 could be utilized as a Menière's disease biomarker, qPCR was utilized to validate if both were unique and exclusive to Meniere's disease serum. Using the $2^{-\Delta\Delta CT}$ method, miRNA-1299 downregulated 3.13-fold in the serum of patients with Menière's disease compared to controls. However, miRNA-1270 had similar expression levels in the serum of both patient cohorts (fold change = 0.923).

DISCUSSION

There is a need for a surrogate marker for Meniere's disease, to not only serve as a diagnostic marker but also help further elucidate what may be occurring on a molecular level. This study is one of the first studies to describe the miRNA profiles in patients with Menière's disease. By evaluating and comparing miRNA perilymph and serum profiles in patients with Menière's disease to controls, miRNA-1299 was identified exclusively in patients with Menière's and may serve as a promising disease specific biomarker.

miRNAs have shown significant potential as a reliable biomarker for otherwise difficult to diagnose diseases (26, 38, 39). miRNAs are small non-coding RNAs that downregulate gene expression through various negative feedback loops (39). Using IPA software, we are able to identify potential miRNA—mRNA interactions in a human cochlear cDNA library to gain further insight into various aberrant pathways in Menière's disease. However, this type of analysis is limited. We can only interrogate and infer mRNA regulation, and we do not directly identify genes involved with Menière's disease (24). Despite this limitation, we know miRNAs play a key mechanistic role in various inner ear pathologies such as sudden hearing loss and inner ear development (26, 27, 29, 30). Additionally, miRNAs have been identified within all main cellular compartments, exosomes, and cell free components throughout the body and have been successfully used as biomarkers for other neurodegenerative diseases like Alzheimer's and Parkinson's (25, 36, 37). Finally, in previous work, we found miRNAs exhibit inner ear disease specific profiles and that various inner ear pathologies can be diagnosed based on miRNA profiles alone (11, 22, 23). Taken together, miRNAs offer an intriguing biomarker for Menière's disease.

We identified 16 unique miRNAs that were significantly downregulated in the perilymph of patients with Menière's disease. While these 16 miRNAs were proposed to regulate over 220 different genes within the cochlea, we narrowed our focus to gene pathways that have been shown to play a pathologic role in Menière's disease. Aquaporins are a group of intramembrane proteins that serve as water channels in various cellular processes and have been shown to potentially play a key pathogenic role in Menière's disease (21, 40). In guinea pig models, endolymphatic hydrops can be induced through administration of vasopressin, resulting in upregulation of aquaporins (33). In humans, AQP2 and AQP3 have been shown to be aberrantly expressed in genetic population studies (34). It was promising that not only unique miRNAs were identified in Menière's patient cohorts, but that their genetic interactions are linked to pathways that have been identified in Menière's disease. IPA software predicted that miRNA-1255 and−1243 target to AQP3 mRNA, and miRNA-4746 and−1299 are target AQP2 mRNA. Compared to previous miRNA perilymph studies on sudden and progressive sensorineural hearing loss, there was no overlap observed in the 16 significant miRNAs identified in this study (29, 30).

Twelve of the 16 miRNAs unique to Menière's disease are directly predicted to regulate inflammation and/or autoimmune pathways. Recent research supports the link between inflammation, cellular degeneration, and the pathogenesis of Menière's disease (16). By analyzing the perilymph proteome in patients with Menière's disease, Lin et al. discovered 38 proteins that were differentially expressed in patients with Menière's disease and strongly linked to tissue injury and inflammation (10). Several pathologic mechanisms have been proposed for Menière's diseases involving inflammatory pathways including viral-induced inflammation (10), otitis media induced inflammatory products and toxins (41), circulating immune complexes leading to increase cellular permeability (37, 42), genetic predisposition to altered NF-KB mediated inflammatory response (43), distinct and altered cytokine profiles (44), and autoimmunity (37, 45). Together, these studies suggest a strong correlation between Meniere's disease and an aberrant inflammation cascade. Genes that were predicted to be targeted by two or more of the twelve miRNAs associated with inflammation included *TNFRSF11B, RAP1B, NMNAT2, RPS16, R3HDM2, STEAP2, and SCP2*. Altered TNF expression has been proposed to play a significant role in Meniere's disease and TNF-α inhibitors are being preliminarily investigated as a therapeutic intervention (45, 46). From our identified miRNAs, miRNA-1299 was identified as the most promising biomarker for Menière's disease since it is differentially regulated in both perilymph and serum of Menière's patients, and is predicted to target *R3HDM2, SCP2,* and *ATP5PO* mRNA.

While perilymph and serum miRNAs have been uniquely linked to Menière's disease and miRNA-1299 shows potential as viable disease biomarker, there are several limitations to this study. First, by analyzing miRNAs within the perilymph, we are studying a small subset of miRNAs that are limited to the extracellular exosomes or cell free miRNA and not

evaluating tissue directly (47). Secondly, although promising miRNAs were identified, the small sample size severely limits statistical analysis and ability to draw stronger conclusions. Future prospective studies will need to increase the sample size and evaluate these miRNA biomarkers as compared to other inner ear pathologies that can present simultaneously with Menière's disease, such as sensorineural hearing loss. For example, future studies will need to compare Meniere's miRNA profiles to patients with known isolated genetic causes of SNHL, such as patients with the DFNA9 mutation. Third, we anticipate that larger cohorts may also reveal that different subtypes of Meniere's disease as suggested by epidemiological studies (48). Furthermore, if these miRNA biomarkers remain promising in larger studies, future research endeavors will need to validate their mechanism of action. As further samples are collected for disease specific biomarkers, it will be critical to not only collect perilymph and serum samples from the same patients, but also compare miRNA disease profiles in patients with unilateral and bilateral active Menière's disease. Finally, statistically significant miRNAs identified in this study are different from our machine learning studies (31). We hypothesize differences are likely attributed to machine learning methodology, which is focused on miRNA complex pattern matching and not solely differences in single miRNA expression levels. Approaches in this study helped find statistically significant miRNAs based on expression alone, whereas machine learning may find a subset of miRNAs and how their expression pattern, both upregulation and downregulation, may be interconnected and unique to various inner ear pathologies. Future work will need to further understand the validity of both methodological approaches and how they can help identify single miRNAs as compared to assay like expression patterns of miRNA biomarkers for inner ear pathologies.

CONCLUSION

The pathophysiology and disease mechanisms driving Menière's disease remain unclear. While a direct tissue biopsy of the inner ear is not feasible, perilymph sampling has shown significant promise as a "liquid biopsy." In this study, Menière's disease not only demonstrated a unique and distinct miRNA profile compared to controls, but unique miRNAs can be directly linked to proven or well-accepted aberrant disease mechanisms underlying Menière's disease. miRNA-1299 is differentially expressed in the perilymph and serum of patients with Menière's disease, predicted to regulate both aquaporin and inflammatory disease mechanisms linked to Menière's disease, and may ultimately serve as a promising biomarker. While definitive conclusions are limited by a small sample size, miRNA profiling in patients with Menière's disease is a feasible methodology to further elucidate what may be occurring a molecular level and identify future biomarkers to diagnose, prognose, and guide treatment.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE176560.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Kansas Medical Center Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MS, HW, MSP, and HS planned and performed the experiments. MS, HW, and HS gathered patient perilymph and serum samples. MS, HS, HW, AW, and MSP contributed to the interpretation of data and drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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Recurrent Vestibular Symptoms Not Otherwise Specified: Clinical Characteristics Compared With Vestibular Migraine and Menière's Disease

Julia Dlugaiczyk[1], Thomas Lempert[2], Jose Antonio Lopez-Escamez[3], Roberto Teggi[4], Michael von Brevern[5] and Alexandre Bisdorff[6]*

[1] Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Zurich, University of Zurich, Zurich, Switzerland, [2] Department of Neurology, Schlosspark-Klinik, Berlin, Germany, [3] Otology and Neurotology Group CTS 495, Department of Genomic Medicine, Centre for Genomic and Oncological Research (GENyO) Pfizer-Universidad de Granada-Junta de Andalucía, Granada, Spain, [4] ENT Department, San Raffaele Scientific Institute, "Vita e Salute" University, Milan, Italy, [5] Private Practice of Neurology and Department of Neurology, Charité, Berlin, Germany, [6] Clinique du Vertige, Centre Hospitalier Emile Mayrisch, Esch-sur-Alzette, Luxembourg

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***Correspondence:**
Julia Dlugaiczyk
julia.dlugaiczyk@usz.ch

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Despite the huge progress in the definition and classification of vestibular disorders within the last decade, there are still patients whose recurrent vestibular symptoms cannot be attributed to any of the recognized episodic vestibular syndromes, such as Menière's disease (MD), vestibular migraine (VM), benign paroxysmal positional vertigo (BPPV), vestibular paroxysmia, orthostatic vertigo or transient ischemic attack (TIA). The aim of the present international, multi-center, cross-sectional study was to systematically characterize the clinical picture of recurrent vestibular symptoms not otherwise specified (RVS-NOS) and to compare it to MD and VM. Thirty-five patients with RVS-NOS, 150 patients with VM or probable VM and 119 patients with MD were included in the study. The symptoms of RVS-NOS had been present for 5.4 years on average before inclusion, similar to VM and MD in this study, suggesting that RVS-NOS is not a transitory state before converting into another diagnosis. Overall, the profile of RVS-NOS vestibular symptoms was more similar to VM than MD. In particular, the spectrum of vestibular symptom types was larger in VM and RVS-NOS than in MD, both at group comparison and the individual level. However, in contrast to VM, no female preponderance was observed for RVS-NOS. Positional, head-motion and orthostatic vertigo were reported more frequently by patients with RVS-NOS than MD, while external vertigo was more prevalent in the MD group. At group level, the spectrum of attack durations from minutes to 3 days was evenly distributed for VM, while a small peak for short and long attacks in RVS-NOS and a big single peak of hours in MD were discernible. In general, vertigo attacks and associated vegetative symptoms (nausea and vomiting) were milder in RVS-NOS than in the other two disorders. Some patients with RVS-NOS described accompanying auditory symptoms (tinnitus: 2.9%, aural fullness and hearing loss: 5.7% each), migrainous symptoms (photophobia, phonophobia or visual aura in 5.7% each) or non-migrainous headaches (14%), but did not fulfill the diagnostic criteria for MD

or VM. Absence of a life time diagnosis of migraine headache and attack duration of <5 min were further reasons not to qualify for VM. In some RVS-NOS patients with accompanying ear symptoms, attack durations of <20 min excluded them from being diagnosed with MD. These findings suggest that RVS-NOS is a stable diagnosis over time whose overall clinical presentation is more similar to VM than to MD. It is more likely to be composed of several disorders including a spectrum of mild or incomplete variants of known vestibular disorders, such as VM and MD, rather than a single disease entity with distinct pathognomonic features.

Keywords: recurrent vestibular symptoms not otherwise specified, benign recurrent vertigo, Menière's disease, vestibular migraine, Bárány Vestibular Symptoms grid, episodic vestibular syndrome

INTRODUCTION

The Bárány Society began to develop the International Classification of Vestibular Disorders (ICVD) in 2006 (1). Following a systematic categorization of vestibular symptoms (2), diagnostic criteria for the most common episodic vestibular disorders were published, including vestibular migraine (VM) (3), Menière's disease (MD) (4), benign paroxysmal positional vertigo (BPPV) (5), vestibular paroxysmia (VP) (6) and hemodynamic orthostatic dizziness/vertigo (7). Despite this huge progress in international standardization, there are still quite a number of patients whose episodic vestibular symptoms cannot be explained by these or other vestibular disorders (including, but not limited to, third-window syndromes, episodic ataxia, vertebrobasilar TIAs). These recurrent vestibular symptoms of unknown etiology are usually referred to as benign recurrent vertigo (BRV) or recurrent vestibulopathy (RV) (8, 9) for adult patients. Recently, the term "recurrent vertigo of childhood" has been defined for children who do not fulfill the criteria for "vestibular migraine of childhood" (10).

Originally, BRV and RV were defined as recurrent episodes of acute-onset vertigo without cochlear symptoms or signs and not accompanied by other neurological symptoms (8, 9). Slater (8) described the duration of the core event between 1 min and 24 h, often followed by a period of positional vertigo for hours to days. These criteria were modified in subsequent studies. While some authors defined attack duration between "minutes to hours" (11–13), others requested a duration between 5 min to 24 or 72 h (9, 14, 15).

Likewise, symptom quality was defined differently. Some authors (8, 12, 16) excluded patients with head-motion triggered vertigo. Brantberg and Baloh (16), van Esch (13) and van Leeuwen (15) included only patients with spontaneous vertigo, while Pan (17) chose a broader definition of "vestibular symptoms of moderate or severe intensity" not necessarily occurring spontaneously.

Furthermore, the diagnostic value of accompanying symptoms is still an issue of debate. While the original definitions excluded additional cochlear symptoms, some studies showed that around 10 to 26% of patients with recurrent vestibular symptoms of unknown cause report unilateral audiological symptoms associated with an attack (13, 16). Likewise, the role of headaches in the definition of BRV/RV has changed over the years. Slater (8) proposed a link between BRV and migraine, while Brantberg and Baloh (16) distinguished between BRV with and without migraine. With the classification of VM as a separate vestibular disorder, migraine headaches during an attack or a history of migraine became exclusion criteria for BRV/RV (15). Still, around 20% of patients with BRV / RV report non-migrainous headache as an accompanying symptom of vertigo attacks (13, 17).

It is still unknown whether recurrent vestibular symptoms not otherwise specified (RVS-NOS) that do not fulfill any of the criteria of so far established entities are part of the spectrum of established disorders, or a single disease entity with distinct pathognomonic features, or a heterogeneous group of different disorders (18). Since the existing diagnostic criteria for vestibular disorders rely mainly on clinical presentation, the aim of the present study was to systematically categorize *all* vestibular symptoms in patients with RVS-NOS using the Bárány Vestibular Symptoms grid. In line with previously defined diagnostic criteria for vestibular syndromes, we also analyzed symptom intensity, accompanying symptoms during attacks and the temporal profile of the attacks to determine whether there are specific features that help to distinguish RVS-NOS from other diagnoses, mainly MD and VM.

PATIENTS AND METHODS

Patients

The data of the present study was collected in the multi-center, cross-sectional "Vertigo PEVS" study (PEVS = Prospective study on the phenotype of episodic vestibular syndromes) that was performed in six clinical European centers (Luxemburg, Germany, Italy, Spain) between August 2013 and March 2014. Part of the data from VM and MD patients has been published before (19). All patients were interviewed by experienced neuro-otologists (three neurologists and three otolaryngologists) with at least 12 years of clinical practice. The centers were tertiary referral outpatient clinics or vertigo clinics in general hospitals.

In total, 423 patients with an episodic vestibular syndrome were included into the Vertigo PEVS study. For the present paper, detailed analyses were performed for patients with VM, MD and RVS-NOS. The VM group comprised a total of 150 patients suffering from VM (n = 84) or probable VM (pVM, $n = 66$) according to the classification criteria of the Bárány

Society (3). One hundred and nineteen ($n = 119$) patients fulfilled the diagnostic criteria for definite MD according to the American Academy of Otolaryngology—Head and Neck Surgery (AAO-HNS) (20). The AAO-HNS classification for MD was employed as patients were recruited prior to publication of the Bárány Society criteria for MD in 2015 (4). RVS-NOS ($n = 35$) was defined as an episodic vestibular syndrome with at least two episodes of vestibular symptoms according to the Bárány Vestibular Symptoms grid (2) that could not better be explained by another vestibular disorder (including, but not limited to BPPV, VP, MD, VM, vertebrobasilar TIAs or third-window syndromes). Patients fulfilling the criteria for more than one episodic vestibular disorder were excluded from this study, for example, patients diagnosed with MD and VM according to the above mentioned criteria (**Supplementary Material 1**, p. 11).

This study was approved by the local ethics committees of all participating centers. All patients gave written informed consent before entering the study.

Methods

A structured questionnaire was designed to characterize patients' symptoms according to the Bárány Vestibular Symptoms grid (2) (**Supplementary Material 1**). This questionnaire collected all vestibular symptoms reported by patients (vertigo, dizziness, vestibulo-visual symptoms, postural symptoms), the frequency and duration of the attacks and the intensity of the symptoms. Attack duration was defined as a distinct lapse of time during which vestibular symptoms were either continuously present or in case of triggered symptoms, attack duration comprised the time interval during which a specific trigger (e.g., head motion) was able to provoke vestibular symptoms (**Supplementary Material 1**). It also included basic demographic data (age and gender), patient's age at onset of vestibular symptoms and a set of questions to determine the accompanying symptoms occurring during the attacks, that is, vision-related symptoms (photophobia, visual aura, diplopia), hearing-related symptoms (phonophobia, tinnitus, fullness of ear, hearing loss), vegetative symptoms (nausea, vomiting, palpitations, choking), emotional symptoms (anxiety) and headache. Patients were able to choose if accompanying symptoms occurred never, sometimes (<50% of attacks) or mostly (≥50% of attacks) (19).

To characterize the type of headache during the attack, patients were asked to indicate whether headaches were never, sometimes or mostly "hemicranial," "pulsating," "worse on effort" or of "moderate or severe intensity." If patients reported at least two of these features during most of the attacks, the headache was classified as "migraine-type" (19).

In addition to answering the PEVS questionnaire, all patients underwent history taking and a clinical neurotological examination by one of the investigators including, but not limited to, testing for spontaneous, head-shaking and positional nystagmus, smooth pursuit, saccades and head impulse test. A pure tone audiogram was performed in all patients to determine bone and air conduction hearing thresholds. Additional tests to exclude other differential diagnoses were performed at the discretion of each clinician to establish the diagnosis.

Data were entered into an Excel spreadsheet (Microsoft Office 365) and analyzed with GraphPad Prism software (version 9.0.0). For continuous variables (age, age at onset, disease duration, number of different vestibular symptoms or attack durations per patient), one-way ANOVA with Tukey's multiple comparison test was employed. If standard deviations were significantly different between groups, Brown-Forsythe's and Welch's ANOVA with Dunnett's T3 multiple comparisons test were used instead (21). A p-value <0.05 was set to indicate significance. The remaining variables (e.g., relative frequencies of specific symptoms and attack durations) were categorical and analyzed with a Chi square test for three rows (VM, MD, and RVS-NOS). In case of a significant difference ($p < 0.05$), the two-sided Fisher's exact test including the Odds ratio (OR) with 95% confidence interval (95% CI) was performed for VM vs. RVS-NOS, VM vs. MD and RVS-NOS vs. MD. To correct for multiple (i.e., three) comparisons, the adjusted p-value was set to 0.017 for all variables except for attack duration, where p was corrected for 21 possible comparisons ($p < 0.002$). An OR >1 and a 95% CI not including 1 were used as indicators for a correlation between a variable and a specific disorder.

RESULTS

All results are listed in **Supplementary Material 2**. The main focus of this study was laid on features distinguishing RVS-NOS from either VM, MD or both (**Table 1**). In addition, differences between VM and MD were analyzed (**Table 2**).

Age and Gender

The mean age at onset of symptoms was younger for VM (42.43 ± 13.58 years) than for MD (48 ± 13.14 years; ANOVA with Tukey's test for multiple comparisons: $p = 0.0019$). No statistically significant difference was observed for patients with RVS-NOS (47.16 ± 14.24 years) as compared to the other two groups (VM: $p = 0.15$; MD: $p = 0.92$). The mean disease duration on inclusion into the study was >5 years for all three groups. MD patients had a longer history of recurrent vestibular symptoms than those with VM (7.63 ± 8.09 years vs. 5.26 ± 6.59 years; $p = 0.02$), while there was no significant difference between RVS-NOS patients (5.40 ± 6.10 years) and the other two groups (VM: $p = 0.99$; MD: $p = 0.24$).

The proportion of female patients was higher in VM (85%) than in MD (55%) and RVS-NOS (51%; two-sided Fisher's exact test corrected for multiple comparisons: $p < 0.0001$ each; **Tables 1C**, **2A**).

Bárány Vestibular Symptoms Grid

RVS-NOS vs. VM and MD

In general, the spectrum of vestibular symptoms according to the Bárány Vestibular Symptoms grid was broader in RVS-NOS and VM than in MD. First, the average number of different vestibular symptoms per patient was higher in VM and RVS-NOS than in MD. A patient with VM reported 6.03 ± 4.02 (mean ± SD) different vestibular symptoms (median: 5), which was not statistically different from the 5.91 ± 2.81 symptoms (median: 5) experienced by an RVS-NOS patient (two-sided

TABLE 1 | Categorical variables discriminating recurrent vestibular symptoms not otherwise specified (RVS-NOS) from Menière's disease (MD, **Tables 1A,B**), vestibular migraine (VM, **Table 1C**) or both (**Table 1D**).

Variable	MD	RVS-NOS	*P*-value	OR	95% CI
(A) Variables occurring more frequently in RVS-NOS than MD					
Head-motion vertigo (non-spinning) (1.2.2.2)	0%	14%	0.0005	∞	5.41 to ∞
Positional vertigo (transient, that is, <1 min, spinning) (1.2.1.1.1)	0%	11%	0.0023	∞	3.51 to ∞
Orthostatic vertigo (non-spinning) (1.2.6.2)	1%	14%	0.0024	19.67	2.46 to 233.1
Mostly mild attacks	3%	23%	0.0009	8.52	2.64 to 26.44
Positional vertigo (persistent, i.e., >1 min, spinning) (1.2.1.2.1)	3%	17%	0.0047	8.00	2.10 to 30.02
Orthostatic vertigo (spinning) (1.2.6.1)	3%	20%	0.003	7.19	2.03 to 22.78
Palpitations	3%	17%	0.0097	5.95	1.60 to 19.36
Head-motion vertigo (spinning) (1.2.2.1)	8%	29%	0.0023	4.89	1.89 to 12.78
(B) Variables occurring more frequently in MD than RVS-NOS					
Attack duration 1–4 h	66%	17%	<0.0001	9.20	3.46 to 22.97
Nausea	81%	34%	<0.0001	8.00	3.42 to 17.39
Vomiting	46%	11%	0.001	6.67	2.31 to 18.29
Occurrence of severe attacks in patients with mostly mild or moderate attacks	78%	35%	0.027	6.42	1.82 to 19.42
Headache (any type)	41%	14%	0.0043	4.2	1.61 to 10.48
External vertigo (3.1)	59%	26%	0.0009	4.13	1.80 to 10.06
Occurrence of attacks in clusters	59%	34%	0.0125	2.74	1.27 to 6.16
(C) Variables occurring more frequently in VM than RVS-NOS					
Headache (any type)	82%	14%	<0.0001	27.3	10.0 to 68.04
Occurrence of severe attacks in patients with mostly mild or moderate attacks	83%	35%	0.0002	8.80	2.88 to 29.32
Proportion of female patients	85%	51%	<0.0001	5.50	2.35 to 11.86
Nausea	61%	34%	0.0045	3.04	1.45 to 6.64
(D) Variables distinguishing RVS-NOS from VM *and* MD ($p < 0.017$ each)					
Variable	**VM**	**MD**	**RVS-NOS**		
Headache (any type)	82%	41%	14%		
Occurrence of severe attacks in patients with mostly mild or moderate attacks	83%	78%	35%		
Nausea	61%	81%	34%		

*Only those variables occurring at significantly different frequencies between the groups were included (see section Methods for corrected p-values). All variables are listed by descending Odds ratio (OR) and 95% confidence intervals (95% CI). Index numbers of the symptoms according to the Bárány Vestibular Symptoms grid are given in brackets (see **Supplementary Material 1** for an overview of all index numbers).*

Fisher's exact test: $p = 0.99$). On the other hand, only 3.61 ± 2.80 symptoms (median: 2) were described on average per patient with MD, which was significantly less compared to both VM ($p < 0.0001$) and RVS-NOS ($p = 0.0002$). Second, the type of vestibular symptoms on the group level was very similar for patients with RVS-NOS and VM. None of the symptoms from the Bárány Vestibular Symptoms grid occurred at statistically different frequencies between these two groups (**Figures 1**, **2** and **Table 1C**). On the other hand, some symptoms were reported less frequently by patients with MD than those with RVS-NOS (**Table 1A**) and VM (**Table 2A**).

Regarding internal vertigo (part one of the Symptoms grid), positional, head-motion triggered and orthostatic vertigo were experienced more often by patients with RVS-NOS than those with MD (**Table 1A** and **Figure 1**). Non-spinning head-motion triggered vertigo and transient spinning positional vertigo were only reported by RVS-NOS (14 and 11% each), but not by MD patients. Spontaneous spinning vertigo was the most common vestibular symptom in all three disorders occurring at relative frequencies of 60% in RVS-NOS, 54% in MD and 47% in VM (**Supplementary Material 2**). There was, however, no significant difference between the groups (Chi-square test of three rows: $p = 0.32$).

The symptom dizziness (Symptoms grid, part 2) provided no additional information for the discrimination between RVS-NOS and MD (**Figure 2A**). External vertigo (i.e., the false sensation that the visual surround is spinning or flowing) was the only vestibulo-visual symptom (Symptoms grid, part 3) distinguishing RVS-NOS from MD, and the only vestibular symptom that occurred more often in MD (59%) than in RVS-NOS (26%) and VM (37%) (**Figure 2B** and **Tables 1B**, **2B**). None of the postural symptoms (Symptoms grid, part 4) occurred with significantly different frequencies between the groups (**Figure 2C**).

VM vs. MD

As mentioned above, many symptoms of the Bárány Vestibular Symptoms grid, part 1–3, were reported at different frequencies by patients with VM compared to those with MD (**Table 2** and **Figures 1**, **2**). In line with the broader spectrum of symptoms in VM as compared to MD, almost all vestibular symptoms occurred more often in VM than in MD (**Table 2A**), apart from external vertigo, which was more common in MD (**Table 2B**). Of note, there were some symptoms of internal vertigo that

TABLE 2 | Categorical variables discriminating Menière's disease (MD) from vestibular migraine (VM).

Variable	VM	MD	*P*-value	OR	95% CI
(A) Variables occurring more frequently in VM than MD					
Head-motion vertigo (non-spinning) (1.2.2.2)	12%	0%	<0.0001	∞	4.42 to ∞
Positional vertigo (transient, i.e., <1 min, spinning) (1.2.1.1.1)	12%	0%	<0.0001	∞	4.42 to ∞
Visually induced vertigo (non-spinning) (1.2.3.2)	11%	0%	<0.0001	∞	3.79 to ∞
Positional vertigo (persistent, i.e., >1 min, non-spinning) (1.2.1.2.2)	8%	0%	0.0007	∞	2.87 to ∞
Positional vertigo (persistent, i.e., >1 min, spinning) (1.2.1.2.1)	29%	3%	<0.0001	15.54	5.15 to 48.7
Positional dizziness (persistent) (2.2.1.2)	9%	1%	0.0042	11.2	1.76 to 120.4
Orthostatic vertigo (non-spinning) (1.2.6.2)	8%	1%	0.0077	10.26	1.56 to 110.9
Headache (any type)	82%	41%	<0.0001	6.51	3.69 to 11.29
Oscillopsia (head-movement dependent) (3.2.1)	9%	2%	0.0086	6.02	1.56 to 27.02
Orthostatic vertigo (spinning) (1.2.6.1)	17%	3%	0.0005	5.75	2.10 to 15.67
Proportion of female patients	85%	55%	<0.0001	4.83	2.71 to 8.48
Palpitations	14%	3%	0.0027	4.68	1.62 to 12.89
Spontaneous vertigo (non-spinning) (1.1.2)	25%	8%	0.0003	3.70	1.75 to 7.48
Head-motion vertigo (spinning) (1.2.2.1)	23%	8%	0.0007	3.58	1.70 to 7.90
Visually induced dizziness (2.2.3)	18%	6%	0.0029	3.51	1.54 to 8.85
Orthostatic dizziness (2.2.6)	17%	6%	0.0047	3.36	1.46 to 8.49
Head-motion dizziness (2.2.2)	22%	8%	0.0025	3.07	1.50 to 6.24
Exacerbations within attacks	49%	25%	<0.0001	2.81	1.68 to 4.76
Movement-induced blur (3.5)	21%	10%	0.0134	2.42	1.18 to 4.80
Spontaneous dizziness (2.1)	37%	18%	0.0006	2.27	1.53 to 4.75
(B) Variables occurring more frequently in MD than VM					
Attack duration 1–4 h	30%	66%	<0.0001	4.44	2.61 to 7.32
Vomiting	17%	46%	<0.0001	4.30	2.49 to 7.58
Nausea	61%	81%	0.0008	2.63	1.53 to 4.69
External vertigo (3.1)	37%	59%	0.0006	2.40	1.46 to 3.89
Clusters lasting months	12%	33%	0.004	3.73	1.49 to 8.75

Only those variables occurring at significantly different frequencies between the groups were included (see Methods section for corrected p-values). All variables are listed by descending Odds ratio (OR) and 95% confidence intervals (95% CI). Index numbers of the symptoms according to the Bárány Vestibular Symptoms grid are given in brackets (see ***Supplementary Material 1*** *for an overview of all index numbers).*

were only described by patients with VM (non-spinning head-motion vertigo, positional vertigo and visually induced vertigo), but not by those with MD. Postural symptoms (Symptoms grid, part 4) were not significantly different between VM and MD (**Figure 2C**).

Accompanying Symptoms During Attacks

Only accompanying symptoms that are not an integral part of a disease definition were analyzed in the three groups in order to avoid circular argumentation. The only two accompanying symptoms occurring at significantly different frequencies during most attacks (i.e., >50%) in all three groups (**Figure 3** and **Tables 1**, **2**) were nausea (MD: 81%, VM: 61%, RVS-NOS: 34%) and headache of any type (VM: 82%, MD: 41%, RVS-NOS: 14%). In addition, vomiting was more common in MD (46%) than in VM (17%) or RVS-NOS (11%), while palpitations were reported less frequently by MD patients (3%) as compared to the two other disorders (VM: 14%, RVS-NOS: 17%).

Most of the patients did not receive a diagnosis of VM or MD because they did not exhibit any pathognomonic accompanying symptoms (**Table 3**). Headache was experienced by 14% of RVS-NOS patients, however, none of them displayed the characteristic features of migraine-type headaches (see section Methods), indicating that the ICVD criteria were correctly applied during the study. There was a small fraction of RVS-NOS patients with other migrainous symptoms during attacks (14.3% in total; photophobia or visual aura or phonophobia in 5.7% ($n = 2$) each, multiple answers possible; **Figure 3**). These patients did not fulfill the diagnostic criteria for pVM/VM, either because they reported only phonophobia ($n = 2$) or only photophobia ($n = 2$) or because attack duration was <5 min ($n = 2$ patients with visual aura). None of the RVS-NOS patients reported a migraine history, thus excluding pVM. Accompanying auditory symptoms were reported by 8.6% of RVS-NOS patients (tinnitus in 2.9%, fullness of ear and hearing loss in 5.7% each; multiple answers possible). These patients were not diagnosed with MD because attack duration was always <20 min.

Intensity of Attacks

Patients were asked whether they would rate most of their attacks as mild (does not interfere with daily activities), moderate

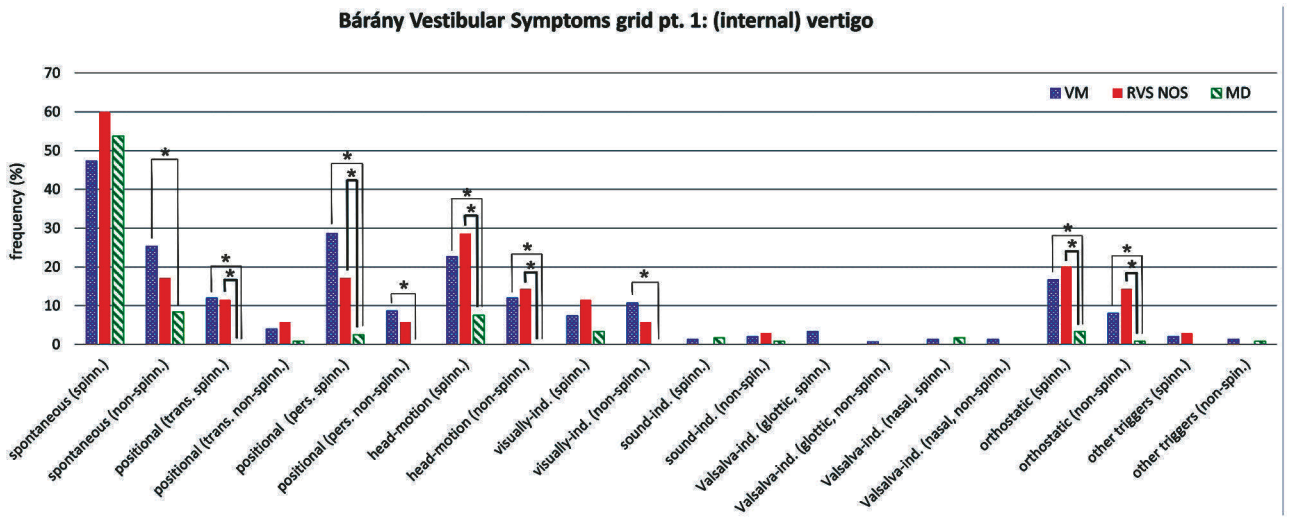

FIGURE 1 | Bárány Vestibular Symptoms grid, part 1: (internal) vertigo. Relative frequencies (%) of the different symptoms are depicted for vestibular migraine (VM), recurrent vestibular symptoms not otherwise specified (RVS-NOS) and Menière's disease (MD). Multiple answers were possible. Symptoms that occurred with significantly different frequencies between groups are marked with an asterisk (*). ind., induced; pers., persistent (≥1 min); spinn., spinning; trans., transient (<1 min).

(interferes with daily activities) or severe (daily activities not possible). Those who reported mostly mild or moderate attacks were asked whether they also experienced severe attacks.

Mostly mild attacks were reported more frequently by patients with RVS-NOS (23%) than MD (3%; two-sided Fisher's exact test: $p = 0.0009$) (**Table 1A**). The occurrence of severe attacks in those patients with mostly mild or moderate attacks was more frequent in patients with VM (83%) and MD (78%) than those with RVS-NOS (35%; see **Tables 1B–D** for statistical analysis). Both observations indicate a less severe attack intensity in RVS-NOS compared to the other two disorders.

Summarizing sections Age and Gender, Bárány Vestibular Symptoms Grid, Accompanying Symptoms During Attacks, and Intensity of Attacks, there were only three features that distinguished patients with RVS-NOS from *both* the MD and the VM groups: less headache of any type, less nausea, and less occurrence of severe attacks in patients with mostly mild or moderate attacks (**Table 1D**).

Temporal Characteristics of Attacks

Attack Frequency and Duration

Regarding attack frequency, patients had to choose one answer in the "Vertigo PEVS" questionnaire (**Supplementary Material 1** and section Rationale of the Present Study). For all three disorders, the most common attack frequency was "≥ 1/month" (i.e., at least one attack per month, but <1 per week), reported by 27% (MD), 26% (VM), and 23% (RVS-NOS) of patients each (**Figure 4A**). While MD patients displayed a single peak for this attack frequency, the distribution was more even in VM, where another 26% chose "≥ 1/week" as most common attack frequency (i.e., at least one attack per week, but <1 per day). A second peak at "≥ 1/year" (i.e., at least one attack per year, but <1 attack within 6 months) was observed for 14% of RVS-NOS patients.

For attack duration, multiple answers were possible (see question 5.1 in **Supplementary Material 1**). On the group level, MD patients displayed a single peak (66% of patients) with an attack duration from one to 4 h (**Figure 4B**). This duration allowed to distinguish patients with MD from VM (OR = 4.4) and RVS-NOS (OR = 9) on the group level (see **Tables 1B**, **2B** for further details). As observed for symptom quality above, the distribution of attack durations was more even across the spectrum of durations for VM and RVS-NOS than for MD. In VM, attack durations ranging from "1–5 min" to "up to 3 days" were evenly distributed, reported by around 30% of patients each. Two peaks were discernible for RVS-NOS ("<1 min": 31% and "5–24 h": 23%).

As more than one answer was possible for attack duration in the "Vertigo PEVS" questionnaire, we sought to identify whether the broad spectrum of attack durations observed for VM and RVS-NOS as groups was also present in the individual patients. On average, patients of all three groups reported between one and two different attack durations (VM: 1.66 ± 1.00; MD: 1.50 ± 0.62; RVS-NOS: 1.22 ± 0.48), and only 16% (VM), 6% (MD) and 3% (RVS-NOS) of patients each described more than two distinct attack durations. Thus, the majority of patients in all three groups have one or two dominant attack durations contrasting with the broad spectrum at the group level for VM and RVS-NOS.

Clusters of Attacks

Periods with many attacks ("clusters") were more common in the MD (59%) than in the RVS-NOS group (34%; two-sided Fisher's exact test: $p = 0.0125$) (**Table 1B**). Clusters lasting for months (instead of weeks) were more often reported by patients with MD (33%) than those with VM (12%, two-sided Fisher's exact test: $p = 0.004$), while no significant difference was

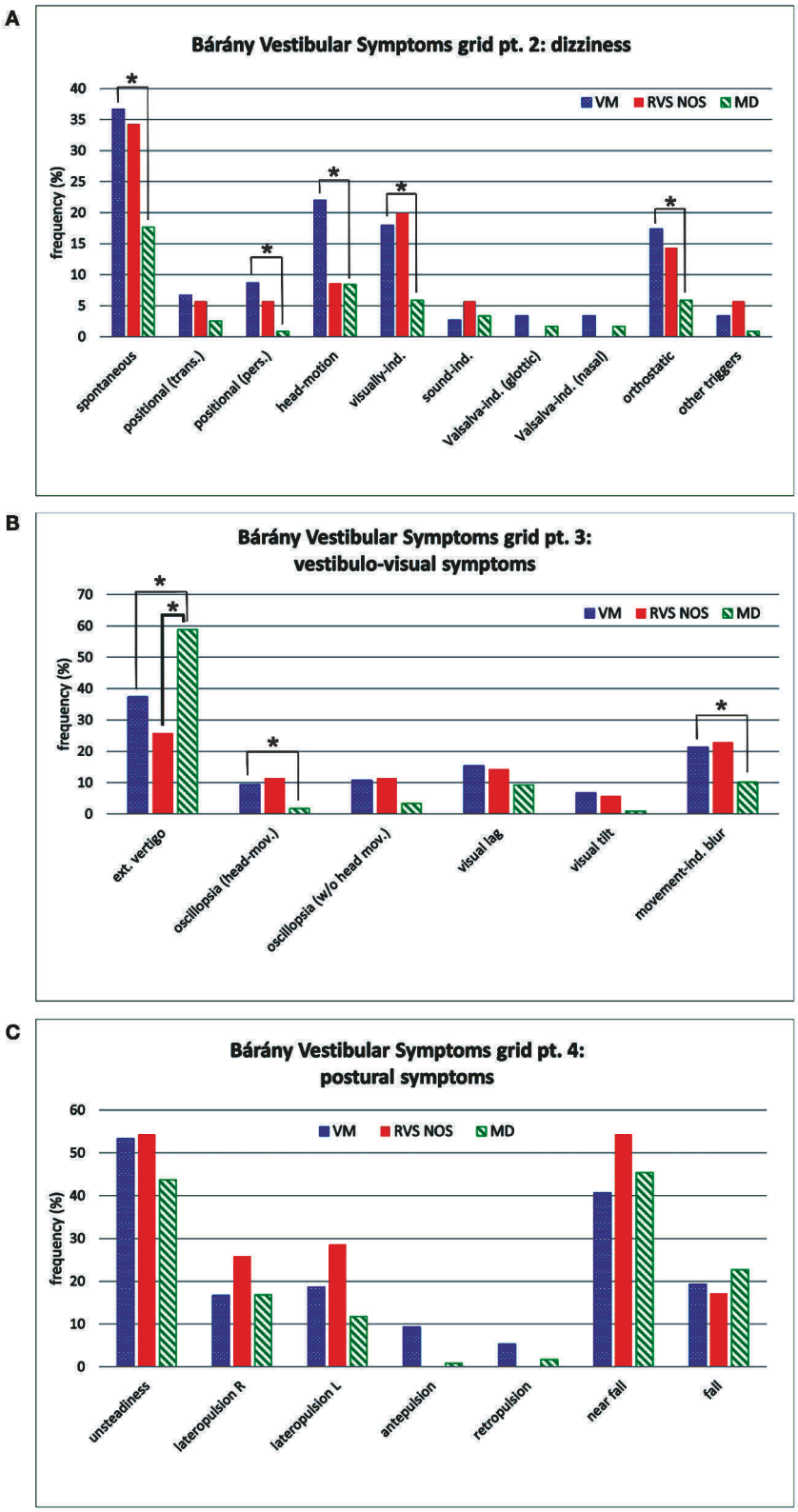

FIGURE 2 | Bárány Vestibular Symptoms grid, parts 2 to 4. **(A)** Dizziness. **(B)** Vestibulo-visual symptoms. **(C)** Postural symptoms. See **Figure 1** for details. Multiple answers were possible. Symptoms that occurred with significantly different frequencies between groups are marked with an asterisk (*). ext., external; ind., induced; L, left; mov., movement; pers., persistent (≥1 min); R, right; trans., transient (<1 min).

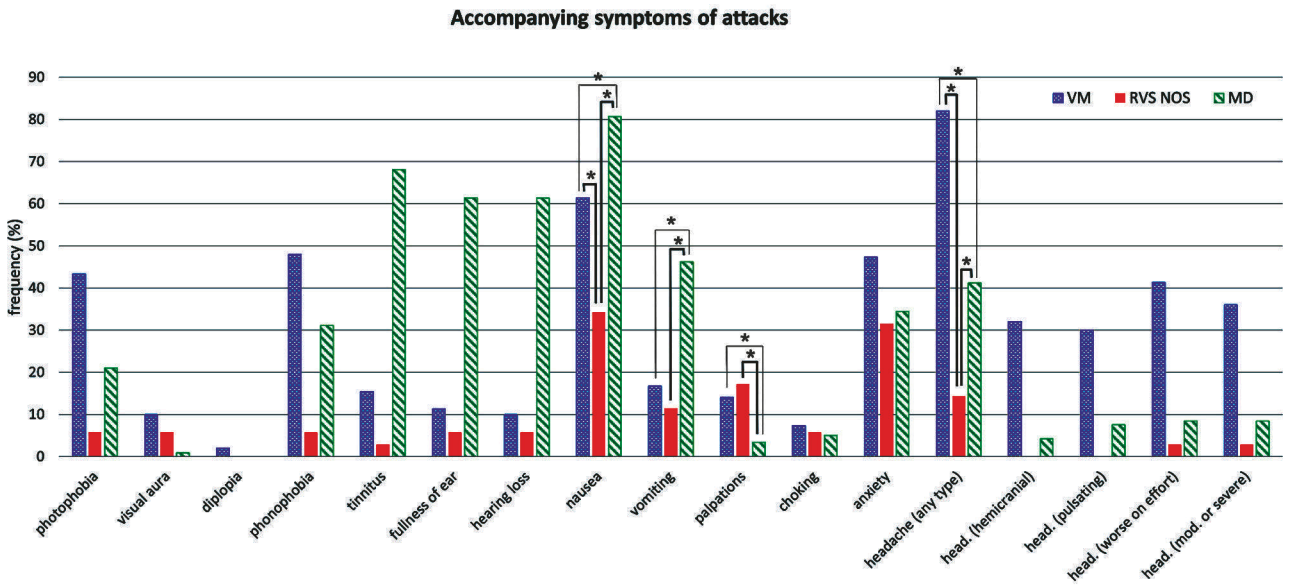

FIGURE 3 | Accompanying symptoms of attacks. See **Figure 1** for details. Multiple answers were possible. Symptoms that occurred with significantly different frequencies between groups are marked with an asterisk (*). head., headache; mod., moderate.

observed between patients with RVS-NOS (8%) and the other two disorders (**Table 2B**).

DISCUSSION

Rationale of the Present Study

Most of the previous definitions of RV/BRV put restrictions on either the quality of vestibular symptoms (only spontaneous attacks of vertigo not precipitated by head movements) or their duration (5 min to 24 or 72 hours) and excluded patients with any accompanying auditory or neurological symptoms (8, 9, 13, 15, 16, 22, 23). On the other hand, studies performed before publication of the diagnostic criteria of VM (3) often did not exclude patients with a history of migraine headache and/or accompanying migrainous symptoms during a vertigo attack (22–24), and many of the cases classified as BRV then, for example, (25), would be diagnosed with VM according to the ICVD criteria today. Thus, the results of these earlier works may be biased by the inclusion of patients considered to have VM according to present criteria.

Here, we decided to classify all those patients as RVS-NOS, whose recurrent vestibular symptoms were not better accounted for by any other recognized episodic vestibular disorder—including VM—regardless of the type of vestibular symptoms, duration of the attacks and presence of accompanying symptoms. We chose the term "recurrent vestibular symptoms not otherwise specified" (RVS-NOS) for these patients in order to indicate that inclusion criteria were different as compared to previous studies. To the best of our knowledge, this is the first international, multi-center study applying the Bárány Vestibular Symptoms grid to a large number of patients with RVS-NOS, MD and VM (305 in total). The aim of this approach was to provide a "real life" picture of those episodic vestibular syndromes that are not captured by any of the current vestibular disease criteria.

TABLE 3 | Reasons why RVS-NOS patients were not diagnosed with vestibular migraine (VM) or Menière's disease (MD) based on accompanying symptoms and duration of attacks.

Variable	RVS-NOS patients (%)
Vestibular migraine	
No accompanying photophobia, phonophobia and/or visual aura	85.7%
No accompanying migraine-type headache	100%
Attack duration < 5 min	48.6%
Menière's disease	
No accompanying auditory symptoms (hearing loss, tinnitus and/or fullness of ear)	91.4%
Attack duration < 20 min	48.6%

Characteristic Features of RVS-NOS

Vestibular Symptoms

One of the most important findings of the present study is the broad spectrum of vestibular symptoms reported by patients with RVS-NOS, both on the group and on the patient level (**Figures 1**, **2**). Spontaneous spinning vertigo was the most common vestibular symptom in RVS-NOS patients (60%). It was however not found in every patient, thus confirming the observation by Pan et al., where spontaneous vertigo was experienced by most (77,8%), but not all patients with BRV (17). Besides spontaneous vertigo, spontaneous dizziness was commonly reported by RVS-NOS patients in our study (34%) and the study by Pan et al. (15%).

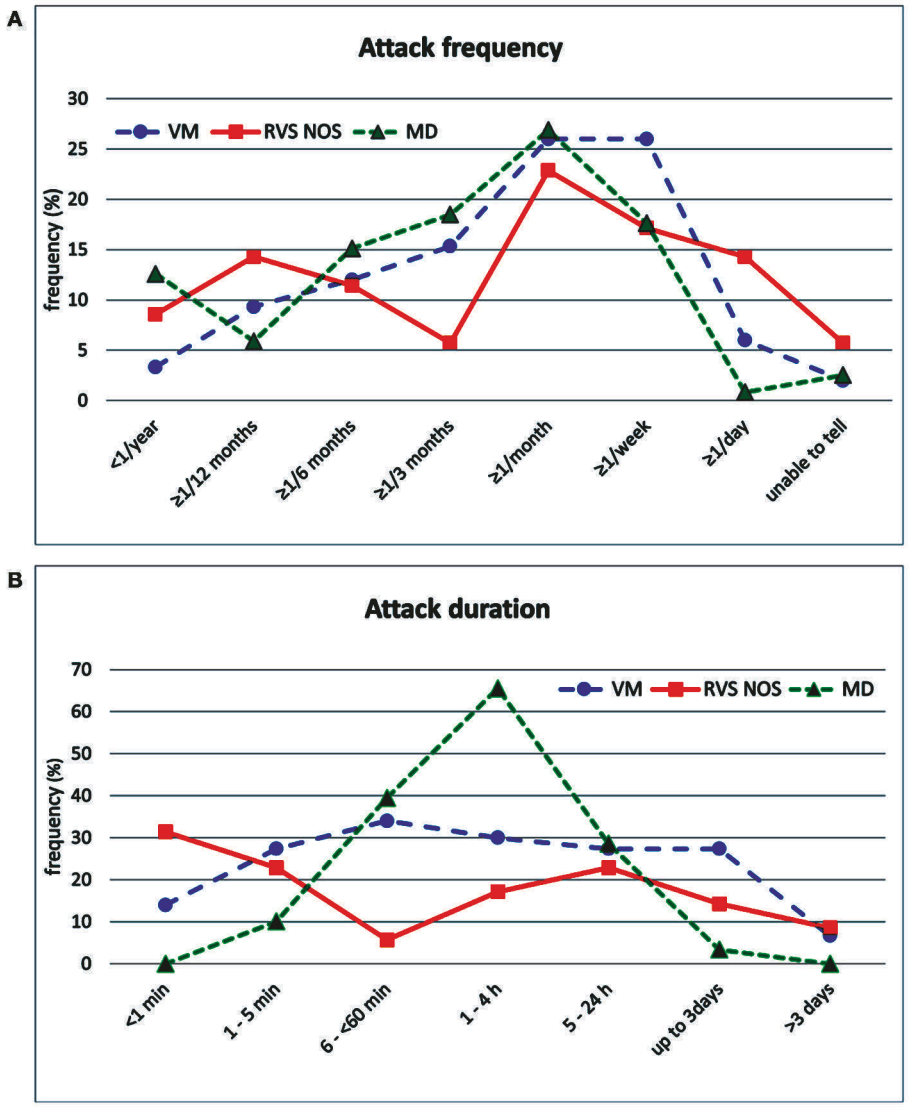

FIGURE 4 | Temporal characteristics of attacks in patients with vestibular migraine (VM), recurrent vestibular symptoms not otherwise specified (RVS-NOS) and Menière's disease (MD) (relative frequencies in %). **(A)** Attack frequency. Only one answer was possible. **(B)** Attack duration. Multiple answers were possible.

While none of the symptoms from the Bárány Vestibular Symptoms grid occurred at different frequencies in RVS-NOS vs. VM in the present study, patients with RVS-NOS experienced certain vertigo types (head-motion, positional and orthostatic) more often than those with MD (**Table 1A** and **Figure 1**). In order to capture this broad spectrum of vestibular symptoms, inclusion criteria for RVS-NOS should comprise all kinds of vestibular symptoms in contrast to previous definitions of BRV and RV that were often restricted to spontaneous (spinning) vertigo (see section Rationale of the Present Study).

Of note, positional vertigo without nystagmus or persistent positional nystagmus not compatible with BPPV have been observed in patients with BRV before (11, 24), and Slater (8) described a period of positional vertigo following the core event of a BRV attack (8). These observations from previous studies might have been contaminated by patients with VM who may present with positional vertigo / nystagmus during an attack as well (26–28). It is possible that some RVS-NOS patients with positional vertigo might actually suffer from BPPV, as the pathognomonic BPPV-type nystagmus may not be visible on every examination (5). On the other hand, diagnosis had to be changed from BRV to BPPV in only 9% of patients after a follow-up of 2 to 8.5 years in two studies (11, 14). In line with these previous observations are the data from our study where the mean disease duration was not different for RVS-NOS patients with attack durations <1 min (4.75 ± 7.73 years), between 1 and 5 min (5.77 ± 8.80 years) and longer than 5 min (6.32 ± 5.28 years). Not to make a BPPV diagnosis over such a long period would be highly unlikely.

The vestibular syndrome of RVS-NOS seems to be stable over time rather than a transitory condition converting into another vertigo syndrome. Previous studies reported that the diagnosis of patients with BRV/RV had to be changed to migraine in only 2 to 7.5% of cases and to MD in only 1 to 4% over median follow-up times between 31 and 63 months (12, 17, 29). This is in line with the results from our study where the mean duration of the vestibular syndrome before the patients' first visit in a neurotology clinic was not significantly different between patients with RVS-NOS (5.40 ± 6.10 years) as compared to VM (5.26 ± 6.59 years) or MD (7.63 ± 8.09 years) indicating a rather stable vestibular syndrome not changing over the years.

Accompanying Symptoms

Accompanying auditory or migraine-type symptoms in ≥50% of attacks were observed in some RVS- NOS patients although all patients fulfilling the criteria for VM/pVM and MD were excluded from this group. 2.9% reported tinnitus, while hearing loss or aural fullness were each present in 5.7%. First, these low rates of accompanying auditory symptoms indicate that the "Vertigo PEVS" questionnaire was applied correctly by the investigators. Second, the fact that some of the patients with RVS-NOS *did* report auditory symptoms without fulfilling the criteria of MD raises the question whether exclusion of auditory symptoms should be part of the diagnostic criteria for RVS-NOS as proposed before (8, 9). Similar rates of accompanying auditory symptoms (3–14%) have been reported for BRV patients without migraine before. Furthermore, the relative frequencies of photophobia *or* phonophobia (5.7% each) and non-migrainous headache (14%) in our study were similar to values from the literature (13, 16, 17, 23).

RVS-NOS patients experienced nausea during ≥50% of attacks less frequently (34%) than patients with VM (61%) or MD (81%); vomiting was less common (11%) than in patients with MD (46%). Both findings indicate a relatively mild attack intensity in RVS-NOS as compared to the other two groups (for details see section Disease Severity). The low prevalence of vomiting in the present study is in accordance with the first description of BRV (8), where none of the patients reported vomiting associated with an attack. In general, frequencies for nausea and vomiting in the present study were lower for all three disorders compared to previous reports (11, 13). This discrepancy is most likely due to the fact that we counted only patients suffering from these symptoms during most of (i.e., ≥50%) the attacks.

No significant difference was observed between the proportion of RVS-NOS, VM and MD patients who experienced anxiety during the attacks (31, 47, and 34%, respectively). This is in line with the study by van Esch et al. (13), who found no difference in Hospital Anxiety and Depression Scores (HADS) between these patient groups (13).

Disease Severity

Several factors in the present study advocate that disease severity was generally milder in patients with RVS-NOS as compared to those with MD or VM. First, the fraction of patients suffering mostly from mild attacks was higher for RVS-NOS (23%) than MD patients (3%). In addition, less patients with mostly mild or moderate attacks experienced also severe attacks in RVS-NOS (35%) than in VM (83%) or MD (78%). Second, clusters of attacks occurred less frequently in the RVS-NOS (34%) as compared to the MD group (59%). Finally, the relatively mild nature of the attacks was reflected by the lower prevalence of nausea and vomiting as compared to the other two disorders (see section Accompanying Symptoms).

Temporal Characteristics of Attacks

At group level, RVS-NOS patients displayed a "two-peak" pattern for attack frequency and duration contrasting the "single-peak" pattern for MD and the "plateau-like" distribution for VM (**Figure 4**). The even distribution of attack durations on the group level for VM resembles the results from previous studies on the duration of headaches and vertigo attacks in VM (30, 31). For RVS-NOS, the second peak was always localized within the lower range of reported values in the present study, that is, attack duration <1 min (31%) and more than one attack per year, but <2 within 6 months (14%).

This observation has several implications. First, it confirms the notion that a subset of patients with RVS-NOS shows a relatively mild disease severity (see above). Second, the high proportion of attacks <1 min (31%) indicates that there is a considerable number of patients with short-lived recurrent vestibular symptoms that cannot be classified as BPPV or VP, confirming observations by Pan et al. (duration <5 min in 22.5%) and Lee et al. (duration <10 min in 6%) (12, 17). Considering the stable course of the vestibular syndrome in these patients (see section Vestibular Symptoms), it is rather unlikely that they will convert into BPPV or VP on the long term. Third-window syndromes are also an unlikely differential diagnosis, as symptoms like sound- and pressure induced vertigo / dizziness were virtually absent in the RVS-NOS group of the present study (**Figures 1**, **2A**).

Of note, a bimodal distribution of attack duration for BRV on the group level has been reported before by Lee et al. ("few minutes": 38.9%; "few hours": 51.4%) and Brantberg and Baloh (1–5 min: 20% and 1–4 h: 30%) (16, 24). In contrast to the broad spectrum of attack durations on the group level in the present study (**Figure 4B**), only 3% of RVS-NOS patients reported more than two different attack durations on the individual patient level. In summary, these distributions of attack duration on the group and individual level indicate that RVS-NOS is—at least currently—a heterogeneous subset of disorders that need to be further characterized in future studies. We propose that there should be no lower limit of attack duration in the definition of RVS-NOS in order to grasp the full spectrum of this multi-facetted disorder.

RVS-NOS in Relation to Other Episodic Vestibular Syndromes

Vestibular Migraine

The different symptoms of the Bárány Vestibular Symptoms grid occurred with similar frequencies in patients with VM and RVS-NOS. In particular, none of these *vestibular* symptoms allowed to distinguish between the two disorders. While previous studies

suggested a link between BRV/RV and migraine (8, 11, 22–25), several findings from the present study indicate that RVS-NOS is *not* just another migraine variant.

First, we observed a balanced gender distribution in patients with RVS-NOS (51% females) in contrast to the well-known female preponderance in VM of 85% in the present study (32–34), confirming the results of the study by van Esch (13). At this point, it should be noted that the female preponderance described in BRV/RV studies before 2012 (8, 23–25) might be due to inclusion of patients that would probably have been diagnosed with VM today (see also Section Rationale of the Present Study). This notion is supported by Brantberg and Baloh, who found a female preponderance only for those BRV patients with a positive migraine history (84% female), while those without migraine displayed a more balanced gender distribution (58%) (16).

Second, the temporal profile of vertigo attacks was different between RVS-NOS and VM in the present study. In summary, patients with RVS-NOS displayed two peaks in the distribution of attack frequencies and duration on the group level in contrast to the "plateau pattern" observed for patients with VM (**Figure 4**). Although the pathophysiological correlate for the two peaks in RVS-NOS is not clear to date (see Section Temporal Characteristics of Attacks), this observation might contribute to a better separation between the two disorders in clinical practice.

Menière's Disease

In general, the clinical presentation of MD patients in the present study was more stereotyped as compared to RVS-NOS: the spectrum of vestibular symptoms was narrower both on the group and on the individual patient level (**Figures 1**, **2**). While a "double-peak" pattern was observed for attack frequency and duration on the RVS-NOS group level, MD patients displayed a single peak for both parameters (**Figure 4**). The marked stereotypic pattern of MD attacks might be due to a common underlying pathology in these patients, such as endolymphatic hydrops (35), which is present on inner ear hydrops MRI in almost all patients with MD (36, 37).

A further characteristic feature of MD in the present study was the relatively high proportion of patients experiencing attack clusters of several months (33%). This has an important implication for designing clinical studies with MD patients: if patients are recruited during a cluster of attacks and then return back to baseline, one may have the illusion of a treatment effect, in particular if the endpoint is defined several months or years after inclusion into the study.

It has been debated whether recurrent vertigo attacks without accompanying hearing loss are a subset of MD ("vestibular MD") (38). In around 20% of cases, MD begins with isolated vestibular symptoms. While 80% of patients develop the full audiovestibular spectrum of symptoms within 5 years, patients with merely vestibular symptoms and without hearing loss over periods of 20 years and more have been described (36, 38). A closer look at the study by Paparella and Mancini reveals, however, that all but one of 51 patients diagnosed with vestibular MD reported aural fullness and 84% suffered from tinnitus. These accompanying symptoms were only encountered in 5.7 and 2.9% of RVS-NOS patients each in the present study. Therefore, it seems unlikely that RVS-NOS is just another subgroup of MD. Nevertheless, a possible conversion into MD cannot be excluded, in particular in those with a disease duration <5 years (63% in the present study).

Possible Causes of RVS-NOS

So, what are the mechanisms behind RVS-NOS? As this is a symptom-oriented study, it can only provide a tentative answer to this question.

We were not able to identify any pathognomonic vestibular symptoms or other operational criteria that clearly separated RVS-NOS from already known vestibular disorders. Therefore, it seems unlikely that RVS-NOS represents a single, so far unrecognized disease entity. The number and the quality of vestibular symptoms in patients with RVS-NOS (both on the group and on the patient level) suggest that part of these patients suffer from a mild form of VM, while the subgroup with auditory symptoms might represent a very mild form of MD. But it probably also includes some as yet unidentified entities, like recurrent spontaneous vertigo with interictal headshaking nystagmus which was only described after our data collection (39).

In summary, these findings indicate that RVS-NOS is a heterogenous group of different disorders with relatively mild clinical presentations—too mild to fulfill the current diagnostic criteria of, for example, VM or MD- and as yet unidentified diseases, one of which was only described after our data collection.

Limitations of the Present Study

This study has several limitations. First, the number of patients with RVS-NOS was quite small compared to those with MD and VM. Studies with larger patient numbers would be desirable in the future to explore whether the results of the present study are representative for RVS-NOS.

Second, patients were recruited before the Bárány Society classifications for MD, BPPV and VP were published. Therefore, the AAO-HNS criteria were applied to identify patients with definite MD, and a customized definition for VP was used (see **Supplementary Material 1**). In particular, probable MD (either according to the AAO-HNS or the Barany Society criteria) was not listed as a separate diagnosis in the PEVS questionnaire. For the purpose of the present study, the Bárány Society criteria for MD, BPPV and VP (both definite and probable) were retrospectively applied to the RVS-NOS group in order to exclude all patients whose symptoms could better be explained by another vestibular disorder.

Third, we only performed a clinical neurotological examination in order to rule out other vestibular disorders, such as BPPV. Additional vestibular tests (e.g., caloric irrigation, video head impulse testing, video-nystagmography) and imaging (e.g., hydrops imaging of the inner ear) were not part of this study. It is unclear to date, whether these examinations have an

additional value in the differential diagnosis of RVS-NOS. For instance, a recent study has helped to identify a specific subset of patients with recurrent spontaneous vertigo whose head-shake nystagmus is clearly different from those of MD and VM patients indicating that the attacks are caused by hyperactivity and asymmetry in the vestibular velocity storage mechanism (39, 40).

Finally, the familial history of auditory or vestibular symptoms was not obtained in the present study. However, both VM and MD show a significant familial aggregation (41, 42), and families with either VM or MD may have individuals with partial syndromes that could fit in the diagnosis of RVS-NOS. Future studies should investigate familial aggregation of RVS-NOS and VM.

Conclusion

The present study suggests that RVS-NOS is a heterogeneous group of vestibular disorders. The stability of symptoms over time indicates that it is most likely not a transition phase before fulfilling the criteria of other well-defined vestibular entities.

There are inherent limitations to what phenotyping of vestibular symptoms may achieve in terms of diagnosis. All vestibular symptoms are non-specific, patterns of symptoms may be more in favor of one or the other entity, but in MD and VM the accompanying symptoms of hearing loss, the audiogram and the other non-vestibular migrainous symptoms determine the diagnostic classification.

Long term follow-up, examination of patients during an attack, future use of biomarkers and possible treatment response may help to further clarify whether RVS-NOS is part of the spectrum of already defined disorders or one or more separate disorders.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité National d'Ethique de Recherche (National Research Ethics Committee, CNER), Luxembourg. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JD and AB analyzed the data and wrote a first draft of the manuscript. All authors designed the study, performed clinical assessment, neurotological examination of study subjects, collected the data, contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.674092/full#supplementary-material

Supplementary Material 1 | "Vertigo PEVS" questionnaire (PEVS = prospective study on the phenotype of episodic vestibular syndromes).

Supplementary Material 2 | Results of the "Vertigo PEVS" questionnaire for patients with vestibular migraine (VM, $n = 150$), recurrent vestibular symptoms not otherwise specified (RVS-NOS, $n = 35$) and Menière's disease (MD, $n = 119$). All results are presented as absolute numbers and relative frequencies in %.

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CASE REPORT
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Case Report: Ménière's Disease-Like Symptoms in 22q11.2 Deletion Syndrome

*Kwang-Dong Choi[1], Jeong-Yeon Kim[1], Seo-Young Choi[1], Eun Hye Oh[2], Hyun-Min Lee[3], Jieun Roh[4] and Jae-Hwan Choi[2]**

[1] Department of Neurology, Pusan National University School of Medicine and Biomedical Research Institute, Pusan National University Hospital, Busan, South Korea, [2] Department of Neurology, Pusan National University School of Medicine, Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, South Korea, [3] Department of Otorhinolaryngology, Pusan National University Yangsan Hospital, Yangsan, South Korea, [4] Department of Radiology, Pusan National University Yangsan Hospital, Yangsan, South Korea

The 22q11.2 deletion syndrome (22q11.2DS), caused by a microdeletion on the long arm of chromosome 22, is characterized by congenital heart disease, hypoparathyroidism, immunodeficiency, developmental delay, and velopharyngeal insufficiency. Anatomic malformations of the middle and inner ears are frequently present, leading to high prevalence of hearing impairment. We present a first case of 22q11.2DS showing fluctuating hearing loss with recurrent vertigo attacks, resembling Ménière's disease. A 38-year-old male known to have 22q11.2DS developed recurrent vertigo, tinnitus, and fluctuating hearing loss in the left ear during a 10-year follow-up period. During vertigo attack, he had spontaneous left-beating nystagmus with downbeat components, but bithermal caloric and video head impulse tests showed normal vestibulo-ocular reflex functions. Sequential pure tone audiograms demonstrated fluctuating sensorineural hearing loss (SNHL) in both ears, which finally progressed to permanent hearing loss in the left ear. Computed tomography imaging of the temporal bone exhibited bilaterally malformed lateral semicircular canals, and delayed 3D-FLAIR sequences revealed cochlear endolymphatic hydrops with dilation of the scala media in the left ear. This case shows that acute vertigo with SNHL can be one of the audiovestibular presentations in 22q11.2DS caused by disturbance of endolymphatic flow.

Keywords: 22q112 deletion syndrome, Ménière's disease, endolymphatic hydrops, case report, vertigo, sensorineural hearing loss

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Pablo Román-Naranjo, Andalusian Autonomous Government of Genomics and Oncological Research (GENYO), Spain

***Correspondence:**
Jae-Hwan Choi
rachelbolan@hanmail.net

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INTRODUCTION

The 22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge syndrome, is caused by a microdeletion on the long arm of chromosome 22 (1). Patients with 22q11.2DS exhibit highly variable phenotypes that include congenital heart disease, hypoparathyroidism, immunodeficiency, developmental delay, and velopharyngeal insufficiency. Anatomic malformations of the middle and inner ears are frequently present, leading to high prevalence of hearing impairment (2, 3). While abnormal morphology of the vestibule and the lateral semicircular canal (LSCC) has been also reported, most patients do not present with vestibular symptoms (4). In this report, we present a first case of 22q11.2DS showing fluctuating hearing loss with recurrent vertigo attacks, resembling Ménière's disease.

CASE REPORT

A 38-year-old male known to have 22q11.2DS presented with recurrent vertigo lasting for hours and fluctuating tinnitus in the left ear. He had a history of neonatal seizure, hypocalcemia due to hypoparathyroidism, and intellectual disability, and underwent cardiac surgery due to congenital heart disease (tetralogy of Fallot) at 15 years of age. On examination, the patient showed characteristic facial features of 22q11.2DS such as hypertelorism, a short and broad nose, and a deeply grooved philtrum. There was no spontaneous nystagmus between vertigo attacks, and bedside head impulse and bithermal caloric tests were normal. The pure tone audiogram (PTA) demonstrated sensorineural hearing loss (SNHL) in the left ear (**Figure 1**). Computed tomography (CT) imaging of the temporal bone revealed bilateral widening of the vestibules, soft tissue densities in the middle ear, and decreased mastoid air cells (**Figure 2A**). The patient was diagnosed with Ménière's disease, and treated with a low-salt diet and diuretics. The vertigo attacks resolved, and follow-up PTA revealed mild improvement of SNHL in the left ear (**Figure 1**).

Five years after his initial presentation, the patient developed recurrent vertigo, hearing loss, and tinnitus in the left ear again. A physical examination demonstrated spontaneous left-beating nystagmus with downbeat components that increased during left eccentric gaze. Bithermal caloric tests were normal, and video head impulse tests showed normal vestibulo-ocular reflex (VOR) gains for all six semicircular canals (SCCs). PTA revealed profound SNHL in the left ear (**Figure 1**). Treatment with diuretics, betahistine, and intratympanic steroid injection to the left ear failed to attenuate the vertigo attacks. A 1-month follow-up PTA revealed additional hearing loss in the right ear without improvement of the left SNHL (**Figure 1**). The patient received an intravenous steroid injection followed by a tapering dose of oral steroids, resulting in a gradual reduction of the frequency of vertigo attacks. At the 1-year follow-up PTA, there was improvement of hearing loss in both ears, particularly in the right ear (**Figure 1**). Since the vertigo attacks recurred after stopping diuretics, the patient was maintained on combination therapy with betahistine and diuretics. During a 3-year follow-up period, he remained symptom-free despite the persistence of left SNHL (**Figure 1**). At the latest follow-up, the patient underwent delayed 3T magnetic resonance imaging (MRI; MAGNETOM Vida, Siemens, Erlangen, Germany) using a 64-channel array head coil, 4 h after intravenous gadolinium (12 ml of gadoterate meglumine, Dotarem; Guerbet, Aulnay-sous-Bois, France) to evaluate the presence of endolymphatic hydrops (EH) (5). We performed the 3D fluid-attenuated inversion recovery (3D-FLAIR) images with the following parameters; field of view (FOV): 160 × 160 mm, repetition time (RT): 7,000 ms, echo time (ET): 303 ms, inversion time (IT): 2,050 ms, matrix size: 256 × 230, flip angle: 120°, number of excitation (NES): 2, and scan time of 6 min 39 s. Axial 3D-FLAIR revealed cochlear EH with dilation of the scala media in the left ear (**Figures 2B,C**), but no cochlear enhancement or vestibular EH.

DISCUSSION

Among the affected genes in 22q11.2DS, deletion of *TBX1* is responsible for the main features of the disease, such as heart problems, hypoplasia of the thymus and parathyroid glands, and velopharyngeal insufficiency (6). *TBX1* is also expressed in the otic vesicle and the periotic mesenchyme during development, and was shown to be necessary for the development of the inner ear in mice (7). Thus, homozygous *TBX1* mutant mice developed inner ear malformations that are characterized by an absent or hypoplastic vestibular apparatus with poorly developed SCCs, and a lack of coiled cochlear duct (6, 7). Likewise, patients with 22q11.2DS frequently present with anatomic malformations of the middle and inner ears in CT imaging of the temporal bone, primarily malformation of the LSCC with an abnormally small bony island, and incomplete partition type II of the cochlea. With regard to the highly prevalent malformations of the vestibule and LSCC in 22q11.2DS, a recent cross-sectional study found that, although vestibular dysfunctions such as caloric hypofunction and abnormal cervical vestibular-evoked myogenic potentials

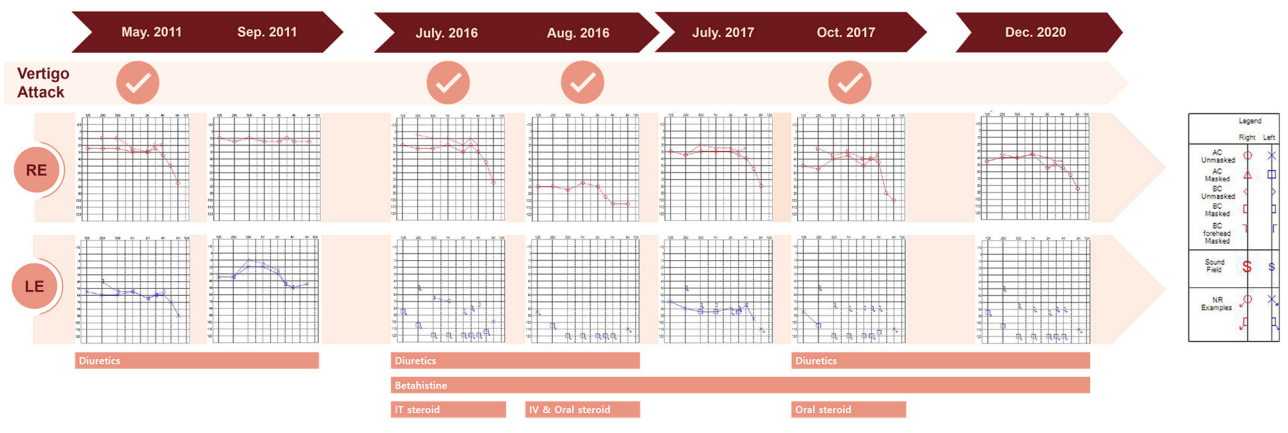

FIGURE 1 | Time course of vertigo attacks, pure tone audiogram results, and treatments. AC, air conduction; BC, bone conduction; IT, intratympanic; IV, intravenous, LE, left ear, NR, no response; RE, right ear.

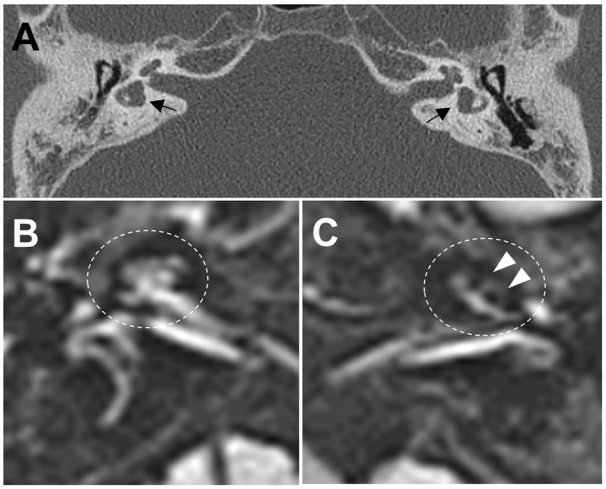

FIGURE 2 | (A) Computed tomography images of the temporal bone demonstrate bilateral widening of the vestibules with an abnormally small bony island of lateral semicircular canals (black arrows) and soft tissue densities in the middle ear, and decreased mastoid air cells. **(B,C)** Axial three-dimensional fluid-attenuated inversion recovery images obtained 4 h after intravenous administration of gadolinium reveal cochlear endolymphatic hydrops (EH) with dilation of the scala media in the left ear (**C**, white arrows), but no EH in the right ear **(B)**.

were common in patients with 22q11.2DS, none had experienced sudden vertigo (4). Postural imbalance has been described in patients with 22q11.2DS, but in most cases, it was associated with general muscle hypotonia or motor delay. Only one case had disequilibrium associated with severe malformations of bilateral LSCCs (8).

Remarkably, our patient presented with Ménière's disease-like symptoms such as whirling-type vertigo with nystagmus, fluctuating hearing loss, and tinnitus. He had bilaterally widened vestibules and LSCCs with an abnormally small bony island, but his VOR functions were spared. These anatomic malformations may have contributed to the development of Ménière's disease-like symptoms in our patient, presumably due to the formation of EH. This hypothesis is supported by the presence of cochlear EH on 3D-FLAIR images, which is concordant with PTA finding showing total deafness in the left ear. Because the patient was maintaining a vertigo-free state with normal VOR functions at the time of MRI examination, vestibular EH might not have been observed. Ménière's disease-like symptoms have been reported in patients with LSCC dysplasia, and histopathologic study revealed EH with hypoplastic endolymphatic sacs (9, 10). MRI evaluation of EH have found enlarged vestibular endolymph in LSCC dysplasia, and a strong negative correlation between the areas of the bony island and the endolymphatic space (11). Likewise, our patient may have had disturbance of endolymphatic flow due to inner ear malformations that caused Ménière's disease-like symptoms. Alternatively, the existence of genetic modifiers interacting with *TBX1* may cause phenotypic variability of audiovestibular dysfunction in 22q11.2DS. A recent study found that non-coding variants in *CRKL* were significantly associated with risk for conotruncal heart defects in individuals with 22q11.2DS (12). Many genes have been related to hereditary non-syndromic hearing loss and Ménière's disease (13–16). It is therefore possible that rare variants in these genes could modify audiovestibular phenotype by interacting with *TBX1* in 22q11.2DS.

Even though it is a rare occurrence, the case described herein shows that acute vertigo with SNHL can be one of the audiovestibular presentations in 22q11.2DS.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

All experiments followed the tenets of the Declaration of Helsinki, and informed consents were obtained after the nature and possible consequences of this study had been explained to the participants. This study was approved by the institutional review boards of Pusan National University Yangsan Hospital.

AUTHOR CONTRIBUTIONS

K-DC conducted the interpretation of the data and wrote the manuscript. J-YK, S-YC, EO, H-ML, and JR contributed to the interpretation and analysis of the data. J-HC conducted the design, conceptualized the study, interpreted the data, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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METHODS
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Using Base-ml to Learn Classification of Common Vestibular Disorders on DizzyReg Registry Data

Gerome Vivar[1,2], *Ralf Strobl*[1,3], *Eva Grill*[1,3], *Nassir Navab*[2], *Andreas Zwergal*[1,4†] *and Seyed-Ahmad Ahmadi*[1,2*†]

[1] German Center for Vertigo and Balance Disorders, University Hospital Munich, Ludwig-Maximilians-University, Munich, Germany, [2] Computer Aided Medical Procedures, Department of Informatics, Technical University Munich, Munich, Germany, [3] Department of Biometry and Epidemiology, Institute for Medical Information Processing, Biometry and Epidemiology, Ludwig-Maximilians-University, Munich, Germany, [4] Department of Neurology, University Hospital Munich, Ludwig-Maximilians-University, Munich, Germany

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Edited by:
Carey David Balaban, University of Pittsburgh, United States

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Marcos Rossi-Izquierdo, Lucus Augusti University Hospital, Spain
Denise Utsch Gonçalves, Federal University of Minas Gerais, Brazil
Marty Slade, Yale University, United States

***Correspondence:**
Seyed-Ahmad Ahmadi
ahmadi@cs.tum.edu

[†] These authors share senior authorship

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Background: Multivariable analyses (MVA) and machine learning (ML) applied on large datasets may have a high potential to provide clinical decision support in neuro-otology and reveal further avenues for vestibular research. To this end, we build base-ml, a comprehensive MVA/ML software tool, and applied it to three increasingly difficult clinical objectives in differentiation of common vestibular disorders, using data from a large prospective clinical patient registry (DizzyReg).

Methods: Base-ml features a full MVA/ML pipeline for classification of multimodal patient data, comprising tools for data loading and pre-processing; a stringent scheme for nested and stratified cross-validation including hyper-parameter optimization; a set of 11 classifiers, ranging from commonly used algorithms like logistic regression and random forests, to artificial neural network models, including a graph-based deep learning model which we recently proposed; a multi-faceted evaluation of classification metrics; tools from the domain of "Explainable AI" that illustrate the input distribution and a statistical analysis of the most important features identified by multiple classifiers.

Results: In the first clinical task, classification of the bilateral vestibular failure ($N = 66$) vs. functional dizziness ($N = 346$) was possible with a classification accuracy ranging up to 92.5% (Random Forest). In the second task, primary functional dizziness ($N = 151$) vs. secondary functional dizziness (following an organic vestibular syndrome) ($N = 204$), was classifiable with an accuracy ranging from 56.5 to 64.2% (k-nearest neighbors/logistic regression). The third task compared four episodic disorders, benign paroxysmal positional vertigo ($N = 134$), vestibular paroxysmia ($N = 49$), Menière disease ($N = 142$) and vestibular migraine ($N = 215$). Classification accuracy ranged between 25.9 and 50.4% (Naïve Bayes/Support Vector Machine). Recent (graph-) deep learning models classified well in all three tasks, but not significantly better than more traditional ML methods. Classifiers reliably identified clinically relevant features as most important toward classification.

Conclusion: The three clinical tasks yielded classification results that correlate with the clinical intuition regarding the difficulty of diagnosis. It is favorable to apply an

array of MVA/ML algorithms rather than a single one, to avoid under-estimation of classification accuracy. Base-ml provides a systematic benchmarking of classifiers, with a standardized output of MVA/ML performance on clinical tasks. To alleviate re-implementation efforts, we provide base-ml as an open-source tool for the community.

Keywords: chronic vestibular disorders, classification, machine learning, multivariable statistics, clinical decision support (cdss), episodic vestibular symptoms

INTRODUCTION

Multivariable statistical analysis (MVA), and modern machine learning (ML) methods have the potential to serve as clinical decision support systems (CDSS) (1–3), including the computer-aided diagnosis (CADx) of vestibular disorders (4–8). In combination with large datasets and multi-site cohorts, MVA/ML classification algorithms allow for investigating interactions between patient variables, which is why recent works advocate that these methods should be used more widely in neuro-otology and vestibular neuroscience (9). However, there is a wide variety of MVA/ML methods available, and recent advances in deep learning (DL) with artificial neural networks (ANN) (10) add to the complexity of the field.

In this work, we followed three clinical three clinical scenarios in the differential diagnosis of vestibular disorders, and defined three respective classification problems with increasing difficulty. We applied a wide variety of MVA/ML/DL methods to investigate the suitability of automated classification for these clinical questions, and to compare the algorithmic outcomes with clinical expert intuition, both from the perspective of supposed task difficulty, and from the perspective of how the algorithms weighted feature importances toward diagnostic classification. For validation, we took advantage of the DizzyReg dataset, a large prospective registry of patients with vestibular disorders (11). The dataset is multimodal and contains three main categories of variables, namely patient characteristics, symptom characteristics, and quantitative parameters from vestibular function tests.

The first classification problem addresses two groups of patients, suffering either from bilateral damage to peripheral vestibular afferents (i.e., bilateral vestibular failure), or functional dizziness without evidence for relevant structural or functional vestibular deficits. Both syndromes present with the chief complaint of persistent dizziness. However, additional symptom features (e.g., triggers, extent of concomitant anxiety and discomfort) may vary considerably. We expected that machine learning can reliably differentiate both disorders based on patient characteristics (e.g., different age spectra), symptom characteristics, and vestibular function test (e.g., head impulse test or caloric testing).

The second classification task is, whether patients with primary functional dizziness (based on psychological triggers and stressors) can be separated against patients with secondary functional dizziness following a preceding organic vestibular disorder (such as acute unilateral vestibulopathy, or benign paroxysmal positional vertigo) (8). This setting is more complex, as patient and symptom characteristics may be similar, but the vestibular function tests may differ.

The third problem is directed to the differentiation of four episodic vestibular disorders, namely benign paroxysmal positional vertigo (BPPV), vestibular paroxysmia (VP), Menière disease (MD) and vestibular migraine (VM). This multi-class problem is supposed to be the most complex, because the demographic characteristics of patients and the spectrum of symptoms can be diverse and may overlap (e.g., between MD and VM), and vestibular function tests may be normal (e.g., in VP or VM).

To investigate classification on these three clinical objectives, we developed base-ml, a comprehensive test-bench for initial ML experimentation on clinical data. With this tool, we aim to provide clinical experts with a better intuitive feeling for the range of ML outcomes that can be expected on the given data. For better transparency, several methods can and should be investigated at the same time, subject to a comparable data pre-processing and cross-validation strategy. To this end, we compare several linear, non-linear and neural-network based ML algorithms, along with a novel graph deep learning method that we recently proposed (6, 12, 13). Following insights from multiple classification experiments for diagnostic decision support in our research over the last few years (4, 6, 13, 14), we also provide a multi-faceted analysis of algorithm outcomes, including an examination of class imbalance, multiple classification metrics, patient feature distributions, and feature importances as rated by the classifiers. To alleviate the implementation burden for multi-algorithm comparison and multivariate evaluation, we provide base-ml as an open-source tool[1] to the vestibular research community, as a starting point for further studies in this direction.

MATERIALS AND METHODS

DizzyReg Registry and Dataset

The objective of the DizzyReg patient registry is to provide a basis for epidemiological and clinical research on common and rare vertigo syndromes, to examine determinants of functioning and quality of life of patients, to identify candidate patients for future clinical research, to integrate information of the different apparative measurements into one data source, and to help understanding the etiology of the vestibular disorders.

The DizzyReg patient registry is an ongoing prospective clinical patient registry which collects all information currently stored in electronical health records and medical discharge

[1] Base-ml source code and documentation: https://github.com/pydsgz/base-ml.

letters to create a comprehensive clinical database of patient characteristics, symptoms, diagnostic procedures, diagnosis, therapy, and outcomes in patients with vertigo or dizziness (11). Study population includes patients with symptoms of vertigo and dizziness referred to the specialized out-patient center for vertigo and balance disorders. Recruitment into the registry commenced in December 2015 at the German Center for Vertigo and Balance Disorders (DSGZ), Munich University Hospital of the Ludwig-Maximilians-Universität. Inclusion criteria into the registry are symptoms of vertigo and dizziness, age 18 years and above, signed informed consent and sufficient knowledge of German.

Questionnaires were issued on first day of presentation to the study center to assess lifestyle and sociodemographic factors as well as self-reported perception of vertigo symptoms, attack duration and the time since first occurrence. Lifestyle and sociodemographic factors assessed using questionnaires include age, gender, education, physical activity, alcohol, smoking, sleep quality. The type of symptoms of patients included: vertigo, dizziness, postural instability, problems while walking, blurred vision, double vision, impaired vision, nausea, vomiting. Concomitant ontological or neurological symptom are documented with a focus on otological symptoms, i.e., hearing loss, tinnitus, aural fullness, pressure, hyperakusis, and neurological symptoms, i.e., headache, type of headache, photo-/phonophobia, double vision, other symptoms (ataxia, sensory loss, paresis, aphasia).

The evolution of symptoms was reconstructed by the frequency and duration of attacks. All aspects of history taking in the DizzyReg follow established concepts such as "So stoned" (15), the "Five Keys" (16) and the "Eight questions" (17). Frequency or time of onset of symptoms was included as a categorial variable with the following categories: "less than 3 month," "3 months to 2 years," "more than 2 years," "more than 5 years," and "more than 10 years." The duration of symptoms is registered in the categories "seconds to minutes," "minutes to hours," "hours to days," "days to weeks," "weeks to months," "continuous."

The registry further collects information on symptoms, quality of life (EQ5D) and functioning (DHI and VAP) in a few standardized questionnaires. Information on triggers is gathered by the respective categories of the Dizziness Handicap Inventory and by elements of the Vertigo Activity and Participation Questionnaire (VAP) (e.g., head movement, position change, physical activity etc).

DHI

The Dizziness Handicap Inventory (DHI) is a well-known and widely used measure to assess self-perceived limitations posed by vertigo and dizziness (18). A total of 25 questions are used to evaluate functional, physical, and emotional aspects of disability. Total score ranging from 0 to 100 is derived from the sum of responses (0 = No, 2 = sometimes, 4 = Yes).

Quality of Life

Health-related quality of life was assessed with the generic EuroQol five-dimensional questionnaire (EQ-5D-3L). This is subdivided into five health state dimensions namely mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, with each dimension assessed in three levels: no problem, some problem, extreme problems. These health states were converted into EQ5D scores using the German time trade-off scoring algorithm (19). The resulting total EQ5D score ranges from 0 to 1 with higher scores indicating better quality of life.

Vertigo Activity and Participation Questionnaire (VAP)

Functioning and participation were assessed based on the Vertigo Activity and Participation Questionnaire (VAP). The VAP is specifically designed for persons with Vertigo and Dizziness and can be used for people of different countries (20–22). The VAP measures functioning and participation in two scales consisting of six items each. Using weights derived from Rasch analysis the first scale has a range of 0–23 points and the second of 0–20 points with higher scores indicating more restrictions.

Data protection clearance and institutional review board approval has been obtained (Nr. 414-15).

Classification Tasks and Cohorts

As mentioned in the introduction, three classification problems with increasing complexity were tested: (1) bilateral vestibular failure vs. functional dizziness; (2) primary vs. secondary functional dizziness; (3) BPPV vs. VP vs. MD vs. VM. **Table 1** provides information about the group cohorts for each task.

Classification Pipeline

A typical machine learning pipeline comprises several steps that interplay toward a high-accuracy prediction (23). After data import, a set of pre-processing routines are applied to patient features, before data is split into several folds for training and testing, using one or several classification algorithms. The classifier performance is evaluated using several quantitative metrics, and finally presented and explained to a clinical expert on vestibular disorders, for a critical review. **Figure 1** presents an overview of our methodological pipeline in this work.

Pre-processing

Multimodal medical datasets commonly pose several challenges for CADx algorithms, including noisy or missing patient features with spurious outliers (24–26), a mixture of categorical and continuous variables (27), and different statistical distribution of variables (23). To account for outliers and different data ranges in DizzyReg variables with continuous distributions, we perform a 90% winsorization which sets extreme values to the 5th and 95th percentiles, before applying a z-transformation (27) which normalizes all variables into a comparable zero-mean and unit-variance data range. Categorical variables are binarized where possible, or represented in form of a one-hot encoding (a.k.a. one-of-K encoding), which creates a binary column for each category and sparsely represents the categories with a value of 1 in the respective column and 0 in all the other columns. To account for missing values, we perform a mean-imputation (24) if $<50\%$ of values are missing in the population, otherwise the feature is omitted from the patient representation.

TABLE 1 | Clinical tasks with respective classes of chronic/episodic vestibular disorders, and respective cohort details.

	Diagnosis abbreviation	*N*	Age mean (s.d.)	EQ5D	DHI	Female/Male
Task 1						
Bilateral vestibular failure	BVF	66	65.0 (17.0)	0.8 (0.2)	46.2 (22.6)	27/39
Functional dizziness	FD	346	47.2 (14.5)	0.8 (0.2)	43.3 (18.4)	178/168
Task 2						
Functional dizziness (Secondary)	FDS	204	52.1 (14.7)	0.8 (0.2)	48.0 (18.8)	130/74
Functional dizziness (Primary)	FDP	151	45.4 (14.6)	0.8 (0.2)	42.6 (17.6)	77/74
Task 3						
Benign Parox. Pos. Vertigo	BPPV	134	57.0 (12.1)	0.8 (0.2)	45.0 (19.6)	88/46
Menière disease	MM	142	53.4 (13.3)	0.9 (0.2)	43.9 (19.8)	78/64
Vestibular migraine	VM	215	44.5 (14.0)	0.8 (0.2)	41.8 (18.6)	145/70
Vestibular paroxysmia	VP	49	51.6 (14.2)	0.9 (0.2)	38.8 (22.5)	20/29

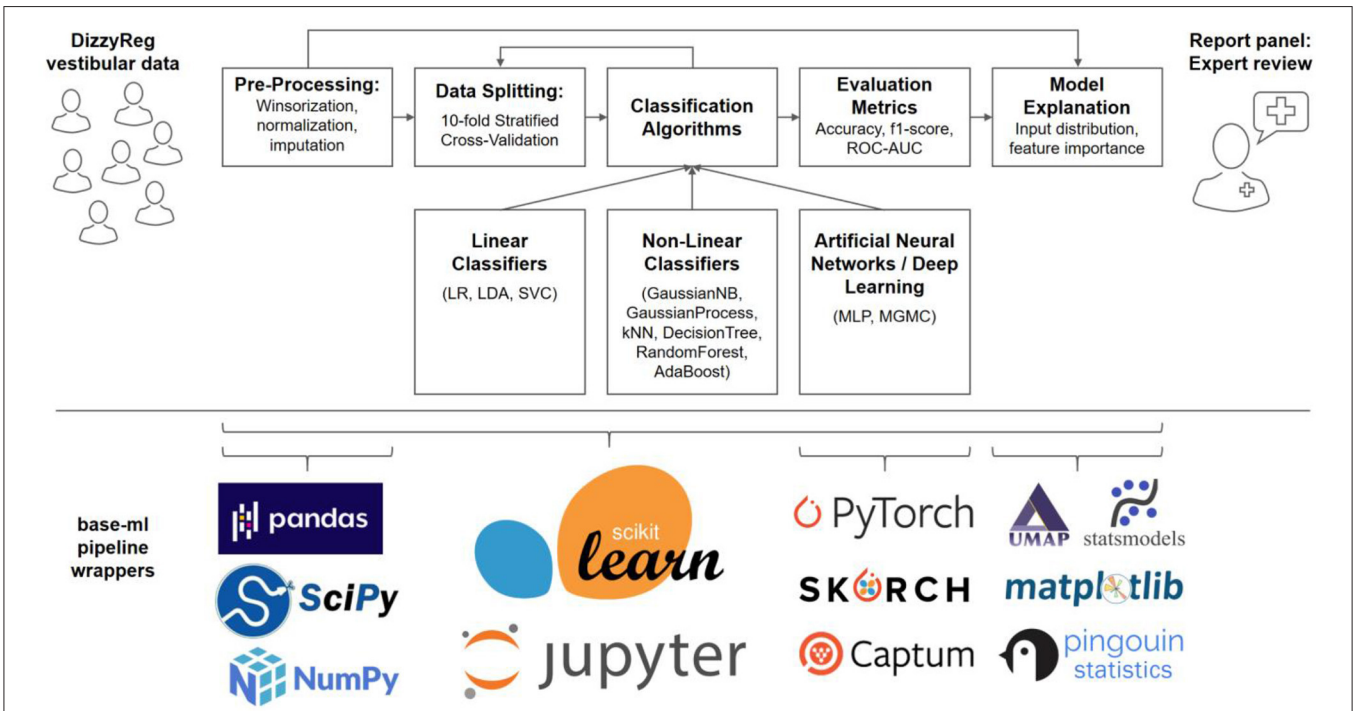

FIGURE 1 | Components and methods of the classification workflow applied to vestibular data in DizzyReg. Raw tabular data is pre-processed and split into 10-folds for stratified cross-validation and for estimation of prospective classification performance. Various linear, non-linear and neural classifiers are repeatedly trained on all folds in the data, the evaluation is performed with various classification metrics. The metrics, along with model explanations, are presented to experts in form of a report panel, who can review the classification outcome and model performance. All pipeline components are implemented in base-ml, a comprehensive software tool which we provide open-source to the vestibular community as a starting point for similar studies. Implemented in Python, and centered around scikit-learn, it comprises various modules for data science, machine learning, descriptive statistics, explainable AI and visualization. Details on base-ml are described in section Base-ml Framework.

Data Splitting

In predictive statistics, in particular in the machine learning community, it is common to assess the prediction performance via hold-out test datasets, which are often randomly sampled and kept separate from the training dataset until the time of pseudo-prospective evaluation (27). Sampling a single test set could result in a biased selection and thus in an overly optimistic or pessimistic test evaluation. To avoid this, it is recommendable to evaluate with multiple test sets, which are sampled either through random shuffling, or through a k-fold splitting. Following common recommendations, we set k to 10 in this work (28). This yields exactly one prediction for each subject in DizzyReg, and exactly ten estimates for the prospective classification performance of each classifier. As recommended by Kohavi in (29), we additionally apply a stratified cross-validation to make sure that each fold has

approximately the same percentage of subjects from each class, which is important especially in the case of class imbalance in the dataset. To ensure that individual classifiers are being trained in a suitable parametrization, we additionally perform hyper-parameter optimization using random search, in a nested cross-validation setup (for details, see section **Appendix C**).

Classification Algorithms and Metrics

Intuitively, ML classifiers try to assign class labels to samples (e.g., patients, represented as multivariable numerical vectors), by fitting separation boundaries between classes in high-dimensional space. Mathematically, these boundaries are expressed in form of a classification function $= f(x)$, which separate the statistical distributions of classes C in the input space X. The past decades of ML research have yielded a diverse set of mathematical models for separation boundaries, and algorithms to fit them to a set of training data X, including linear regression boundaries, rule-based, instance-based, tree-based, kernel-based or Bayesian methods (23), as well as the recent renaissance of artificial neural networks and deep learning (10). Importantly, no single method is guaranteed to perform best on all datasets (30), which is why it is recommendable to test multiple algorithms and let their performances be compared and critically reviewed by a domain expert, instead of deciding on a single algorithm a priori. Therefore, as described in the introduction, we compare several linear, non-linear and neural-network based ML algorithms, along with a novel graph deep learning method that we recently proposed (6, 12, 13). Details on all classifier models and their parametrization are given in section Overview of Selected Classification Algorithms. We quantitatively evaluate the classification performance with three metrics: area-under-the-curve of a receiver-operating-characteristic (ROC-AUC), as well as accuracy and f1-score, defined as (TP/TN/FP/FN denote true or false positives or negatives):

$$\text{Accuracy} = \frac{TP + TN}{N}; \quad \text{f1} - \text{score} = \frac{2\,Prec\,Rec}{Prec + Rec};$$
$$Prec = \frac{TP}{TP + FP}; \quad Rec = \frac{TP}{TP + FN}$$

Model Explanation

A necessary tradeoff in predictive statistics and ML is to choose between model accuracy and model interpretability (31). While linear methods like logistic regression are typically more interpretable, non-linear models, depending on their complexity, are often compared to black boxes. By now, however, "Explainable AI" is a dedicated branch in ML research, and numerous model-specific and model-agnostic methods are available that can partially explain ML prediction outcomes (32). Two common ways to explain model performance is to analyze the distribution of input samples (4, 33), and to analyze feature importance (34), especially in a clinical setting (35).

First, we perform a non-linear mapping of the d-dimensional input distribution after pre-processing onto the 2D plane, and we visualize whether class distributions were already visible in the input data, or whether the input data distribution has unexpected or undesired properties, a technique which has been elucidating in our research before, e.g., in the mapping of posturography data (4). To this end, we utilize "Uniform Manifold Approximation and Projection" (UMAP) (33), a topology-preserving manifold learning technique for visualization and general non-linear dimensionality reduction.

Second, we analyze which patient features contributed to classification outcomes the most, which is a clinically interesting aspect of classifiers. We obtain the "feature importances" for non-ANN-based models and "feature attributions" for ANN-based models. For linear classifiers (see section Linear Classifiers), these can be obtained through the model coefficients (27). For non-linear classifiers (see section Non-linear Classifiers), such as tree-based models, we obtain their feature importance using the Gini-impurity criterion (36). For neural-network based models such as MLP and MGMC (see section Neural Network and Deep Learning Classifiers), we use the Integrated Gradients algorithm (37) and calculate the feature importance by taking the feature attributions of every sample in the training dataset toward their respective ground truth class labels. Obviously, not every classification algorithm yields the same ranking for feature importances. It is argued that a combination of several feature importance rankings can provide more reliable and trustworthy (34). Therefore, for our report to the expert, we aim at presenting a single table with the top 10 most important features for the given classification problem. To merge the feature importance rankings of the different classifiers into a single list, we propose and apply a heuristic for Relative Aggregation of Feature Importance (RAFI), which comprises the following three steps. First, we take the absolute values of all feature importances, to account for algorithms with negative weights (e.g., negative coefficients in linear regression). Second, we normalize the range of importance scores across different classifiers, by computing the percentual importance. Third, we aggregate all normalized global importances by summation, and report the top 10 most important features across all classifiers to the experts for review. In detail, for each feature $\varphi_i \ \left(i \in [1, \ldots, d]\right)$, and across F different classifiers, each with feature importances $I_j(\varphi_i) \ \left(j \in [1, \ldots, F]\right)$, we calculate the global feature importance $I_0(\varphi_i)$ as follows:

$$I_0(\varphi_i) = \sum_{j=1}^{F} \frac{\text{abs}\left(I_j(\varphi_i)\right)}{\sum_{i=1}^{d} \text{abs}\left(I_j(\varphi_i)\right)}$$

Overview of Selected Classification Algorithms

In this work, we apply and compare the outcomes for a total of 11 classification methods, which we chose to represent a wide range of algorithmic approaches. This collection is larger than what is typically encountered in CDSS research, as mentioned, to provide the expert with a better intuitive feeling for the range of outcomes that can be expected on the given data. The algorithms are grouped into three general categories: linear, non-linear, and ANN-based classifiers. Since explaining the inner workings of all methods in detail is out of scope for this work, each algorithm will be outlined only briefly in the following, with its most important parametrizations (if any), and a reference to explanatory material for the interested reader.

Linear Classifiers

As linear classifiers, we apply *Linear Discriminant Analysis (LDA), Logistic Regression (LR)* and *Support Vector Classifiers (SVC)*. All three methods try to fit a set of linear hyperplanes between the *d*-dimensional distributions of the classes. *LDA* [(19), chapter 4.3] models the distribution for each class with a Gaussian and calculates the probability of belonging to a class as the maximum posterior probability in a Bayesian manner. We apply LDA in a default parametrization, without additional regularizations such as shrinkage. *LR* [(19), chapter 4.4] directly learns the posterior distribution of the target class and models it using a sigmoid-activated linear function. We apply LR with simple L2 regularization to avoid overfitting the parameters of the model on the training set. *SVC* (38) is a support-vector machine (SVM) with a linear kernel, which learns a hyperplane that maximizes the gap between the classes, giving slack to key samples ("support vectors") to account for class overlap in the joint distribution. To avoid overfitting, we apply a standard squared l2 penalty term using a regularization parameter of 0.25.

Non-linear Classifiers

Gaussian Naïve Bayes (GNB)

GNB [(19), chapter 6.6.3] is a variant of Naïve Bayes (NB) that allows continuous input features, under the assumption of Gaussian distribution and mutual independence. Class posterior probabilities for new samples are calculated using Bayes Rule. We parametrize *GNB* to estimate class prior probabilities directly from training data, rather than imposing them a-priori.

Gaussian Process Classifier (GP)

GP (39) are a Bayesian alternative to kernel methods like non-linear SVMs. In classification, it models and approximates the class posterior probability as a Gaussian distribution. We set the initial kernel used for GP fitting to a zero-mean, unit-variance radial basis function (RBF), which is then refined during the fitting to training data.

K-Nearest Neighbors Classifier (KNN)

KNN [(19), chapter 2.3.2] classification is an instance-based method, where a sample's class is determined by the majority class label vote of the sample's k-nearest neighbors. We compute similarity as Euclidean distance between two patients' feature vectors, and we use 10 nearest neighbors in the training set to predict the class label of a test input.

Decision Tree Classifier (DT)

DT (36) are a form of rule-based classifiers. A tree represents a hierarchical set of rules or decisions, each decision splitting the feature space in a single feature dimension, using an optimal splitting threshold which is calculated using information-theoretic criteria. Each new sample is passed down the tree, following splitting rules, until a leaf is hit in which a class distribution and majority class is stored. In this work, we use trees with Gini impurity as the splitting criterion, and we allow trees to expand up to a maximum depth of five.

Random Forest Classifier (RF)

RF (40) are an ensemble of multiple decision trees, where each tree is trained using a random subset of training data and a random subset of features. Due to the randomization, the individual trees are highly uncorrelated. Therefore, the ensemble output, which is calculated as an average vote from all trees, weighted by their confidences, is highly robust against various data challenges, such as high dimensional input spaces, noisy data, or highly different data distributions across variables. In this work, we use an ensemble of 10 trees, each with a maximum depth of 5 decision levels.

Adaptive Boosting Classifier (AB)

AB (41), similar to RF, is another ensemble method that combines multiple "weak" classifiers in order to form a much "stronger" classifier. A key difference is the boosting mechanism, i.e., the ensemble is allowed to iteratively add new weak classifiers, which are trained with a higher weight on those input instances that are still being misclassified. In this work, we use decision stubs (i.e., decision trees with a depth of (1) as the weak base classifiers, and we allow the maximum number of classifiers to reach up to 50.

Neural Network and Deep Learning Classifiers

Multi-Layer Perceptron (MLP)

MLP [(19), chapter 11] consider input features as activated neurons followed by one or several fully connected layers (so-called hidden layers) of artificial neurons which weight and sum incoming neuronal connections, before applying a non-linear activation function. The network weights are estimated using the backpropagation algorithm. In this work, we parametrized an ANN with two hidden layers (64 and 32 neurons), and protect every layer against overfitting, as is commonly achieved by applying dropout ($p = 0.3$) (42), followed by batch normalization (43).

Multi-Graph Geometric Matrix Completion (MGMC)

MGMC (13) is a graph-based neural network (GNN) model which we proposed recently, as an extension to our previously published geometric matrix completion approach for multimodal CADx (12). It models the classification problem as a transductive geometric matrix completion problem. Importantly, MGMC is designed to deal with the common problem of missing values in large medical datasets (25), by simultaneously learning an optimal imputation of missing values, along with the optimal classification of patients. MGMC models the patients as nodes in a graph, and computes the edges in the graph through a similarity metric between patients. The similarity is based on a few meta-features (e.g., sex, age, genetic markers etc.), which allows MGMC to span a graph between patients akin to a social network. In previous works, GNNs have shown promising results and a complementary approach in the field of CADx. In this work, we compute multiple patient graphs, each based on similarity measures of a single meta-feature, namely gender (same gender), age (age difference $\pm$ 6 years), EQ5D score (score difference of $\pm$ 0.06), and DHI score (score difference of $\pm$ 11). As advanced model parameters, we use five timesteps for the recurrent graph convolutional network, Chebyshev Polynomials of order five, and

a single hidden layer before the output (16, 32, or 64 neurons, depending on the classification task).

Statistical Methods

The most important features detected by RAFI (cf. section Classification Pipeline) are presented for expert review and interpretation. Each of these features is compared across patient classes via hypothesis tests, to provide a first glance whether there are significant differences across groups. For continuous variables, and in the case of two classes, we first test each variable for normal distribution in each of the patient group with a Shapiro-Wilk test (44). If so, we apply an unpaired two-tailed *t*-test (27), if not, we apply a Mann-Whitney U test (45). For more than two classes, we apply a one-way ANOVA test (27), or a Kruskal-Wallis (46) as an alternative for non-parametric testing, and report the group-level *p*-value. For categorical values, we apply a Chi-squared independence test (47). We report *p*-values for hypothesis tests on all variables, and assume significance at an alpha-level of $p < 0.05$.

Base-ml Framework

As described in the previous sections Classification Pipeline-Statistical Methods numerous methods are necessary to imple1ment a full data science and machine learning pipeline, for a multimodal clinical problem like vestibular classification, and in a multi-site dataset like DizzyReg. Naturally, re-implementing this stack of methods is a time-consuming effort, which should ideally be avoided across research groups. To alleviate future classification experiments similar to this work, and to provide the community with a starting point, we developed base-ml, an open-source Python package[1] provided by the German Center of Vertigo and Balance Disorders. The package can enable a rapid evaluation of machine learning models for prototyping or research. As illustrated in **Figure 1** (lower panel), it is built around scikit-learn (48) as a backbone, which is a reference toolkit for state-of-the-art machine learning and datascience. We complement scikit-learn with various Python modules: *pandas* (49) for data IO and analysis; scipy and numpy (50) for fast linear algebra on array-shaped data; *PyTorch* (51) for implementation of ANNs and more advanced deep learning models like MGMC; *skorch*[2] for integration of PyTorch models into the scikit-learn ecosystem; the Captum[3] library for model interpretability and understanding, which we use for calculation of feature importance in ANNs using Integrated Gradients (37); UMAP (33) for non-linear 2D mapping and visualization of the patients' input distribution; statsmodels (52) and pingouin (53), two Python libraries for descriptive statistics and hypothesis testing; and matplotlib for plotting and scientific visualization. Importantly, using skorch, we enable potential adopters of base-ml to integrate both inductive and transductive neural training workflows and even deep learning models into a comparative benchmark with more traditional ML methods. Skorch combines the ease of use of scikit-learn training workflows and PyTorch's GPU-enabled neural network models. In addition, with base-ml, one can easily evaluate graph-based neural network models.

[2]Scorch source code and documentation: https://github.com/skorch-dev/skorch
[3]Captum source code and documentation: https://github.com/pytorch/captum

RESULTS

The following sections reproduce the classification reports produced by base-ml on the three clinical tasks described in the introduction. It is important to note that base-ml is not restricted to vestibular classification scenarios. As a sanity check for base-ml, regarding classification outcomes, and comparability to baseline results in literature, we perform two additional experiments. Those two base-ml experiments are performed on non-vestibular datasets, i.e., one artificially generated dataset, and one Alzheimer's disease classification dataset, which has been widely studied in literature. To keep the main body of this manuscript dedicated to vestibular analysis, we report on non-vestibular results in the **Appendix**.

Results on Task 1 (Bilateral Vestibular Failure vs. Functional Dizziness)

The results panel for this classification task, as produced by the base-ml framework, is visible in **Figure 2**. The boxplots with metrics illustrate a wide range of classification performances for all classifiers, with an accuracy over the 10 folds between 78.7% ± 6.4% (AdaBoost) and 93.0% ± 3.5% (RF), an f1-score between 0.683 ± 0.144 (DecisionTree) and 0.848 ± 0.091 (GaussianProcess), and an average ROC-AUC between 0.727 ± 0.145 (DecisionTree) and 0.937 ± 0.050 (GaussianProcess), followed closely by a ROC-AUC of 0.921 ± 0.056 (RF). Quantitatively, Gaussian Process classifiers are the top-performing model on this task, and slightly outperform the best-performing neural network model MGMC (mean accuracy/f1-score/ROC-AUC: 90.8%/0.782/0.893). In fact, on this task, even one of the best linear models, LR, performs better than MGMC and almost as good as RF (mean accuracy/f1-score/ROC-AUC: 91.3%/0.831/0.917). The confusion matrices reveal that the group with functional dizziness was detected with a very high sensitivity between 95% (LR) and 98% (MGMC/RF), compared to a much lower sensitivity between 53% (MGMC) and 71% (LR) for patients with bilateral vestibular failure. Notably, hyper-parameter optimization had a positive effect on the outcomes of Task 1, and the average accuracy of all classifiers increased from 87.0 to 89.6% after parameter tuning.

Regarding class imbalance, which is important to consider in context with classification performance, the pie chart (cf. **Figure 2**, bottom left) shows that BVF is strongly under-represented in this DizzyReg subset, at 66 vs. 346 patient samples (16.0% of patients). Finally, the UMAP embedding shows that the FV subjects (colored in yellow) are already clustered and topologically separated from the BVF subjects (colored in purple) at the level of normalized input data. This underlines that the patients have clearly separate characteristics at a feature level, and classifiers have a good chance at fitting decision boundaries between the two groups. The UMAP plot reveals another interesting point, namely that the input data is clearly separated into two clusters, the implications of which are discussed below.

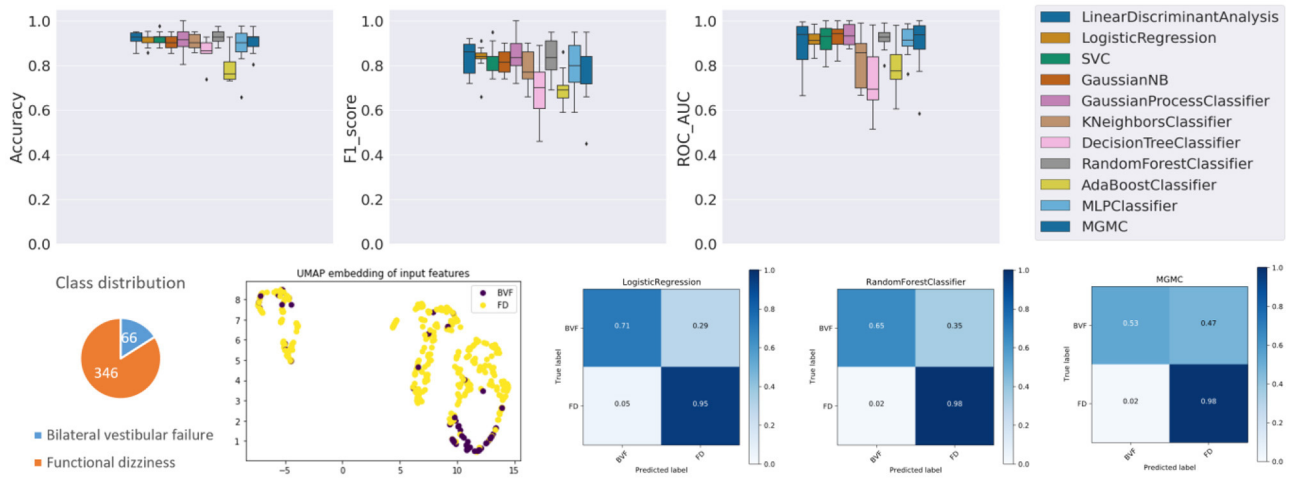

FIGURE 2 | Results panel produced by base-ml for Task 1. It comprises: boxplots for the three classification metrics, accuracy, f1-score and ROC-AUC; a pie chart to highlight potential class imbalances for this task; the UMAP 2D embedding of all patients' input feature vectors; and a more detailed overview of classification outcomes in form of confusion matrices, for three classifiers, LR, MGMC and RF.

The base-ml output also produces **Table 2**, with feature importance scores aggregated with the RAFI heuristic (cf. section Classification Pipeline). Among the top ten features, six features are related to (Video-) Head Impulse Testing (HIT/vHIT; HIT left/right abnormal, vHIT normal result, vHIT gain left/right) or caloric testing, all of which are also statistically significantly different between the two groups at a level of $p < 0.001$. The most important feature is patient age, also with a significantly different expression between the two groups (63.8 ± 15.6 vs. 47.3 ± 14.1 years, $p < 0.0001$). The remaining three features are related to subjective judgement of disability by patients, namely the depression score in EQ5D ($p < 0.001$), a perceived handicap in DHI ($p < 0.01$), and the actual perceived health condition ($p = 0.133$).

Results on Task 2 (Primary vs. Secondary Functional Dizziness)

Compared to task 1, the performance of the 11 classifiers on task 2 is more homogeneous (cf. **Figure 3**), i.e., all classifiers classify with a within a similar accuracy range between 55.2% (DecisionTree) and 62.8% (GaussianProcess), a f1-score range between 0.498 (MLP) and 0.596 (SVC), and ROC-AUC range between 0.571 (DecisionTree) and 0.689 (SVC). Overall, this classification task is dominated by the linear classification algorithm SVC and the non-linear GaussianProcess classifiers, while the DecisionTree and neural network classifier MLP/ANN are the worst-performing algorithms in terms of accuracy and f1-score. The graph neural network method MGMC and RF had an accuracy of 60.6 and 62.2%, both are close to the average accuracy of all classifiers (60.4%). The confusion matrices reveal that LR and RF have an equally high sensitivity for secondary functional dizziness (77%), compared to MGMC (65%), but a comparably lower sensitivity for primary function dizziness (LR/RF: 42%, MGMC: 54%). Notably, hyper-parameter optimization had very little effect on the outcomes of Task 2, as the average accuracy of all classifiers stayed at 60.4% both with and without the parameter tuning.

Again, the lower classification performance could partly be due to class imbalance, i.e., a slight underrepresentation of primary functional dizziness in this DizzyReg subset (42.5% primary vs. 57.5% secondary), however the class imbalance is not as severe as in task 1. The UMAP feature embedding shows that after pre-processing, two clearly separated clusters emerge in the topology of the data. Again, the source for this data separation is not clear and will be discussed further below. However, in the smaller cluster, most of patients are from the group with secondary functional dizziness (purple points), while in the larger cluster, there is a mix of both groups, and this mix is not clearly separable by data topology alone. The classification algorithms still can achieve a certain level of data separability in high-dimensional space, but it is noteworthy that the UMAP embedding reflects that task 2 is more challenging compared to task 1, even before the classifiers are applied.

The top 10 most important features for task 2 (cf. **Table 3**) are largely different from task 1. Expectedly, a normal caloric result (rank 1) and the vHIT gain left/right (ranks 4 and 2) and abnormal HIT result on the right (rank 9) differ in both groups. Patients with primary functional dizziness are younger (rank 3) and tend to drink more alcohol (≥1 drink in the last week, rank 6). One item from the DHI plays an important role for separation, related to problems turning over while in bed (rank 7), and another life quality factor, LIFEQ Q7, i.e., the actual perceived health condition, is relevant as well (rank 8). The duration of vertigo is important as well, in particular whether the duration is between 20 and 60 min (rank 6). Finally, the depression/fear score in the EQ5D questionnaire is relevant (rank 10). All features except EQ5D fear/depression and LIFEQ Q7 are significantly different between the two groups. It is important to note though that multivariable classifiers do not need to depend on univariate feature significance. In high-dimensional

TABLE 2 | Top 10 most important features in Task 1, aggregated over multiple classifiers.

Rank	Feature	Feature Type	Bilateral vestibular failure	Functional dizziness	*P*-Value
1	Age (yrs)	Questionnaire	63.83 ± 15.64	47.33 ± 14.12	<0.0001
2	HIT: right, abnormal	Neurological investigation P1	77.40%	3.40%	<0.0001
3	HIT: left, abnormal	Neurological investigation P1	77.40%	2.30%	<0.0001
4	vHIT: normal result	Apparative tests	14.30%	92.20%	<0.0001
5	vHIT: gain left	Apparative tests	0.8 ± 0.04	0.97 ± 0.12	<0.0001
6	EQ5D: fear, depression	Questionnaire	28.60%	66.40%	<0.0001
7	Caloric: normal result	Apparative tests	31.90%	91.80%	<0.0001
8	vHIT: gain right	Apparative tests	0.71 ± 0.09	0.92 ± 0.15	<0.001
9	DHI: Q21, perceived handicap	DHI	81.20%	92.60%	<0.01
10	LIFEQ: Q7, Actual perceived health condition	LIFEQ	62.51 ± 18.48	58.11 ± 18.9	0.133

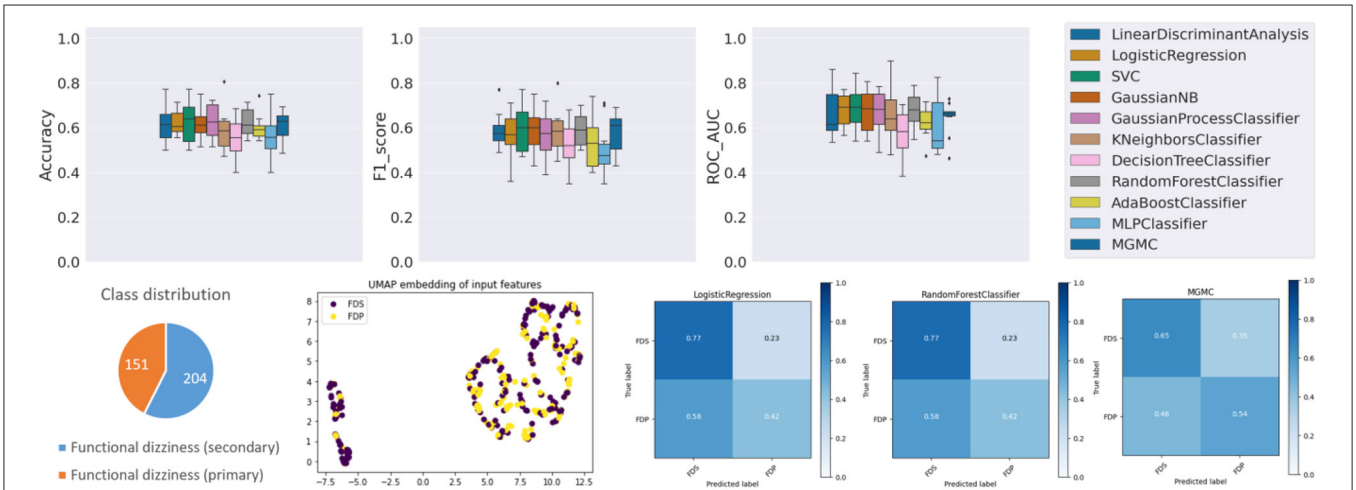

FIGURE 3 | Results panel produced by base-ml for Task 2.

TABLE 3 | Top 10 most important features in Task 2, aggregated over multiple classifiers.

Rank	Feature	Feature type	Functional dizziness (secondary)	Functional dizziness (primary)	*P*-Value
1	Caloric: normal result	Apparative tests	73.10%	96.20%	<0.0001
2	vHIT: gain right	Apparative tests	0.87 ± 0.18	0.92 ± 0.19	<0.0001
3	Age (yrs)	Questionnaire	51.79 ± 13.91	45.61 ± 14.21	<0.0001
4	vHIT: gain left	Apparative tests	0.92 ± 0.13	0.97 ± 0.12	<0.0001
5	Vertigo time: 20–60 min	Questionnaire	13.20%	5.30%	<0.05
6	>= 1 alcoholic drink last week	Questionnaire	43.60%	58.30%	<0.01
7	DHI: Q13, problems turning over in bed	DHI	43.80%	25.70%	<0.001
8	LIFEQ: Q7, Actual perceived health condition	LIFEQ	57.28 ± 19.61	59.34 ± 18.53	0.111
9	HIT: right, abnormal	Neurological investigation P1	13.70%	1.40%	<0.0005
10	EQ5D: fear, depression	Questionnaire	60.0%	70.0%	0.069

space, these two univariately non-significant features may still contribute to a better separation boundary.

Results on Task 3 (BPPV vs. VP vs. MD vs. VM)

Already at first glance (cf. **Figure 4**), and as clinical intuition suggested, task 3 is the most challenging of the three classification tasks. Compared to the average classifier accuracy of task 1 (89.6%) and task 2 (60.4%), the accuracy on task 3 is much lower (48.0%). Individually, the classifiers have an accuracy range between 40.6% (DecisionTree) and 54.3% (LDA), a f1-score range between 0.269 (DecisionTree) and 0.461 (LDA), and a ROC-AUC range between 0.564 (DecisionTree) and 0.764 (LDA). Overall on task 3, linear classifiers, and LDA in particular, classify with

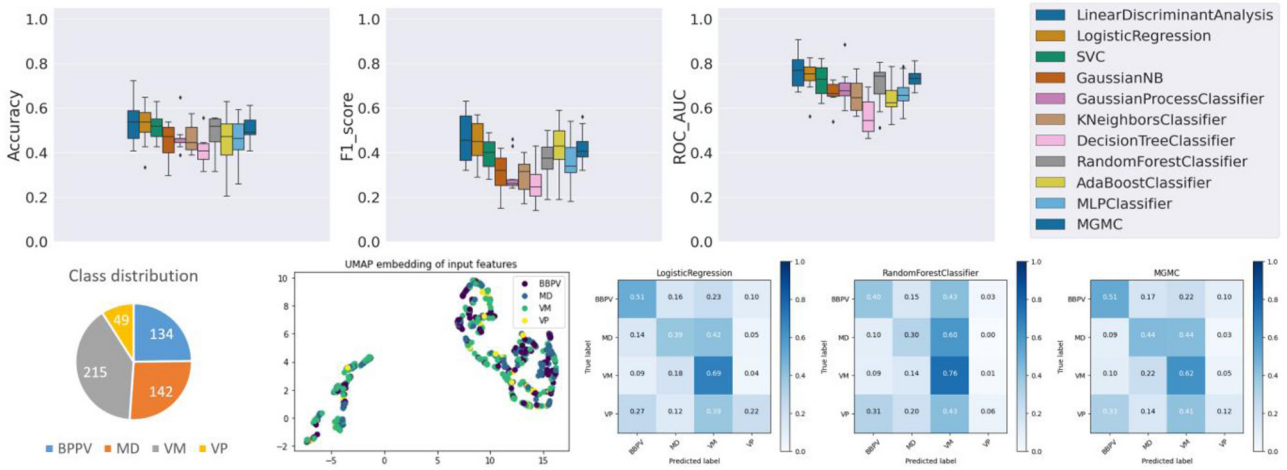

FIGURE 4 | Results panel produced by base-ml for Task 3.

the highest accuracy. The RF classifier, on the other hand, only has an average performance on task 3 (accuracy/f1-score/ROC-AUC: 48.5%/0.372/0.702), in comparison to tasks 1 and 2. The confusion matrices reveal that the disorders VM, BPPV, MD and VP can be classified with a decreasing order of classification sensitivity (e.g., for LR approximately: 70%, 50%, 40%, 20%). On task 3, hyper-parameter optimization had a much higher effect on the classifierd outcomes than in tasks 1 and 2, i.e., after parameter tuning, the average classification accuracy of all models increased from 44.2 to 48.0%.

Class imbalance probably plays a role here as well, as this ordering almost coincides with the class representation in the dataset (VM: 39.8%, BPPV: 24.8%, MD: 26.3%, VP: 9.1%). Looking at the UMAP embedding, the same separation of the data cloud into two clusters is clearly visible, and the four episodic vestibular disorders are visually not clearly separable within the two clusters, which again anticipates the difficulty of the classification task.

Regarding the 10 most important features (cf. **Table 4**), mean patient age ranks on the top (BPPV oldest, VM youngest). Second most important is vertigo time <2 min (which is most frequent in BPPV and VP). Expectedly, several features are related to body relocation, e.g., problems getting into, out of, or turning over inside the bed (DHI Q13, rank 3; VAP Q2, rank 4), bending over (DHI Q25, rank 7), or vertical climbing (VAP Q7, rank 10). Accompanying headache is ranked in 6th position and indicative for VM. There is only one apparative feature relevant for task 3 (normal caloric test, rank 5), with MD being the only group with relevantly abnormal results.

DISCUSSION

In this paper, we have described several approaches for multivariable analysis and machine learning classification of three different patient cohorts from the vestibular registry dataset DizzyReg, i.e., functional dizziness vs. bilateral vestibular failure, primary vs. secondary functional dizziness, and BPPV vs. Menière's disease vs. vestibular migraine vs. vestibular paroxysmia. Clinically, the three tasks were rated with an increasing difficulty and the machine learning classifier performances reflected this grading, with an average accuracy of 87.0, 60.5, and 44.3%, respectively. Using results produced by base-ml, we put these accuracy scores into context with class imbalance, input feature embedddings, confusion matrices and sensitivity scores, as well as tables with the top 10 most important features, aggregated over several classifiers using the proposed RAFI heuristic. In the following, we are going to discuss these results, both from a technical and clinical perspective.

Technical Aspects

The results of the three classification experiments highlight several important points. We believe it to be apparent from the results that it is beneficial to run and benchmark several classification algorithms, ideally from different categories, such as linear, non-linear and neural models. Even a supposedly easy task from a medical perspective does not necessarily lead to a matching classifier performance, depending on which model is used (e.g., 78% classification accuracy in task 1 with Naïve Bayes), hence an a-priori selection could result in too pessimistic an assessment of classification potential using machine learning. Therefore, a wide range of methods in one comprehensive framework might benefit research groups that are new to the field of ML on clinical data. Further, linear models should always be tested along with non-linear and neural network models, as the best linear model (e.g., in task 1, SVC with mean accuracy/f1-score/ROC-AUC: 91.7%/0.819/0.926) may match or even outperform the performance of more complex models, especially if the task has a wide, rather than long data matrix, or if the classes are clearly separable.

Analyzing classifier performance purely using quantitative metrics provides only a narrow view, however. Our analysis reports additionally provide plots on class imbalance, input

TABLE 4 | Top 10 most important features in Task 3, aggregated over multiple classifiers.

Rank	Feature	Feature type	BBPV	MD	VM	VP	*P*-Value
1	Age (yrs)	Questionnaire	56.6±11.4	53.3±13.0	44.7±13.3	51.6±13.6	<0.0001
2	Vertigo time: < 2 min	Questionnaire	44.80%	12.70%	17.20%	71.40%	<0.0001
3	DHI: Q13, problems turning over in bed	DHI	87.90%	47.50%	44.20%	34.70%	<0.0001
4	VAP: Q2, problems to get in/out/turn over in bed.	VAP	93.30%	68.60%	58.50%	49.00%	<0.0001
5	Caloric: normal result	Apparative tests	85.90%	49.50%	84.80%	100.00%	<0.0001
6	Accompanying headache	Questionnaire	16.80%	19.00%	53.50%	15.00%	<0.0001
7	DHI: Q25, bending over increases problems	DHI	76.10%	60.30%	61.20%	61.20%	<0.05
8	DHI: Q6, restricted participation in social activities	DHI	71.40%	82.90%	75.50%	65.30%	<0.05
9	DHI: Q22, increased stress on family/friend relationships	DHI	23.10%	48.90%	45.60%	38.80%	<0.0001
10	VAP: Q7, Vertical climbing (stairs/lift)	VAP	60.00%	64.90%	62.00%	45.70%	0.139

data distribution, and confusion matrices, all of which provide different insights into the experiment. Class representation in the dataset correlated with the sensitivity for each class in all three experiments, which the confusion matrices highlighted. The input data distribution additionally revealed that DizzyReg data in our study had a fundamental separation into two clusters (cf. UMAP embeddings in **Figures 2–4**). At least in task 1 this did not affect classification outcomes to match the clinical intuition, however, for future ML-based studies, this separation would need to be investigated further. Counteracting such a data separation, e.g., with input data transforms (54), or more advanced techniques like domain adaptation (55), could improve classification results further. As such, the results obtained through the base-ml tool provide not only information about which machine learning models to pursue further, but they also indicate starting points regarding the optimization of the input data with classical data science and statistical methods. For clinicians, an important part of the results are the most important features selected by the classifiers, which we present in an aggregated form using the proposed RAFI heuristic. These features will be discussed in more detail and put into a clinical context in section Clinical Implications.

The method presented in this work, and comprised in the base-ml tool have several noteworthy limitations. In general, base-ml is intended as a first screening tool for ML experiments, rather than as a complete ML solution that leads to a trained model for prospective studies and/or deployment. It has been shown previously that hyper-parameter optimization using nested cross-validation can lead to significant improvements of classification performance (6, 12, 13). In our study, while hyper-parameter tuning had no noticeable effects on Task 2, there were noticeable improvements in the average classification outcomes across all models in Tasks 1 and 3. Further, not only the models themselves have hyper-parameters, but every part of the ML pipeline in base-ml could be individually optimized further. This could include alternative input normalization strategies [e.g., power transforms (54, 56)] and imputation methods [e.g., kNN imputation or multiple imputation by chained equations, MICE (57, 58)] or the inclusion of feature selection methods (e.g., based on univariate hypothesis testing), all of which are important toward optimal classifier performance (9). A default treatment made in our experiments, for example, is to discard variables that were recorded for <50% of the population. In clinical practice, however, some variables may be missing because the according examinations or apparative tests were not ordered by the physician, maybe due to time, cost, lack of indication, or expected inefficacy toward diagnosis. In that case, individual rules for variable rejection, imputation and/or normalization may be necessary. For base-ml, we chose to avoid such in-depth treatment, in favor of an ease-of-use at the exploratory stage. However, base-ml is built on top of scikit-learn and already provides an interface to modern deep learning methods with skorch, and explainable AI solutions through Captum. This makes it easy to include many further methods for feature selection, imputation and normalization, as well as further classification explainable AI algorithms (32). However, at a certain level of complexity that aims at deployment rather than exploration, it is recommendable to consider more in-depth analyses and tool, ideally in close collaboration with data science and ML experts, and potentially starting off from insights obtained with base-ml. A particularly interesting avenue is the current research direction of Automated Machine Learning (AutoML), which aims at an optimization of the entire classification pipeline end-to-end (59). Importantly though, small to medium-size datasets might not provide enough data samples to train such complex pipelines. Until more cross-institutional vestibular registry datasets like DizzyReg come to existence, and with sufficient data to apply AutoML, the methods which we wrapped in base-ml and presented in this work still provide a solid starting point for ML-based analysis. As such, and for the time being, we believe these tools to be a valuable contribution for the vestibular research community.

Clinical Implications

Clinical reasoning in the diagnostic differentiation of common vestibular disorders is based on a "mental aggregation" of information from patient characteristics (such as age and gender), symptom characteristics (namely quality, duration, triggers, accompanying symptoms), clinical examination (e.g., positioning maneuvers), and quantitative tests of vestibular function (such as vHIT, calorics) (16). It is an open and relevant question, whether ML-based methods are able to identify features

from a multimodal vestibular patient registry, which resemble this clinical thinking and feature weighting. In the current study, we tested three clinical scenarios of different complexity on the DizzyReg database to further address this issue.

The first classification task represented two groups of patients suffering from chronic dizziness of almost diametrical etiology. In bilateral vestibular failure, imbalance can be directly assigned to an organic damage of vestibular afferents, which is accompanied by a low degree of balance-related anxiety (60, 61), while in functional dizziness the vestibular system is physiologically intact, but the subjective perception of balance is severely disturbed due to fearful introspection (62). It can be expected that ML-based algorithms will predominantly select features as most important for the segregation of both disorders, which represent either measurements of vestibular function or scales for anxiety and perceived disability. Indeed, the top 10 important features exactly meet this assumption with six of them reflecting low and high frequency function of the vestibular-ocular reflex (HIT left/right normal, vHIT gain left/right, bilateral vHIT normal, caloric response normal), and further three features healthy-related quality of life, depression and fear. Furthermore, age was an important differential feature, which is in good accordance to the fact that bilateral vestibular failure appears more frequently in older patients and functional dizziness in younger and mid-aged patients.

In the second classification task, two groups of patients with functional dizziness were compared, who were presumably very similar in their symptomatic presentation, but differed in the evolution of their symptoms: patients with primary functional dizziness, where chronic psychological stress or anxiety is the driving force, and patients with secondary functional dizziness, which develops after a preceding somatic vestibular disorders (e.g., BPPV) due to altered balance perception and strategies of postural control (8). Accordingly, top 10 features for classification included vestibular function tests (such as vHIT gain left/right and caloric response normal). The subtle differences between groups may speak for a partially recovered acute unilateral vestibulopathy or MD as some causes underlying secondary functional dizziness. Furthermore, symptom provocation by position changes in bed may point to BPPV as another vestibular disorder triggering secondary functional dizziness. This findings agree with previous literature (8). Interestingly, patients with primary functional dizziness had higher fear and depression scales, which may indicate a more intense psychological symptom burden. Indeed, previous studies have shown a psychiatric comorbidity in primary functional dizziness in 75 vs. 42% in secondary functional dizziness (63). The more frequent consumption of alcohol in primary functional dizziness may also show that those patients subjectively profit from its relaxing effects to a higher extent than patients with secondary functional dizziness, who have some degree of vestibular deficits, which may exacerbate on alcohol (e.g., partially compensated unilateral vestibulopathy or vestibular migraine).

The third classification task was designed to differentiate common episodic vestibular disorders like BPPV, MD, vestibular migraine and vestibular paroxysmia. Expectedly, a set of features was most indicative for BPPV, namely short attack duration and provocation by position changes. MD as compared to the other vestibular disorders was associated with the highest rate of pathological vestibular function tests (caloric test abnormal). It is well-known that long-standing MD can cause vestibular function deficits (64), while this is less frequent in vestibular migraine (65). The latter was associated with the highest frequency of headache and the youngest mean patient age, in accordance to literature (66). Vestibular paroxysmia was mostly defined by a short-symptom duration. The overall moderate accuracy for classification of the four episodic vestibular disorders can be explained by several factors: (i) one methodological explanation could be that this was a multi-class task, which is more challenging; (ii) despite the exhaustive history taking and examination details for patients recorded in DizzyReg, it is possible that not all relevant information is included. For example, systematic audiological test results are only available for patients with Menière's disease and vestibular migraine, but not for BPPV or vestibular paroxysmia. Therefore, audiological test results could not be generally included in the third classification task as a variable; (iii) there are potential overlaps of symptom characteristics and features. A prominent example is an overlap syndrome of MD and vestibular migraine, which could point toward a common pathophysiology (67); (iv) although the guidelines "International Classification of Vestibular Disorders (ICVD)" of the Barany Society give clear criteria for diagnosis mostly based on history taking, complex clinical constellations such as overlapping syndromes or atypical presentations appear regularly in the practice of a tertiary referral center, which may cause some difficulties in clear-cut classification. Limited classification accuracy may be partly explained by this selection bias, and further testing in primary care settings will be needed; (v) given the difficulty of task 3, the low ML classification performance is neither surprising nor a sign of a failure of ML classification approaches. Instead, our results suggest that ML algorithms, even given considerable data to learn from, may not automatically be able to solve difficult clinical tasks. The wide range of tuned ML algorithm performances presented by base-ml can reveal such difficulty better than a narrow selection of ML results without tuning; (vi) previous studies suggest that expert consensus may not always be unanimous, and may indicate the difficulty of patient diagnosis, despite clear guidelines and diagnostic criteria. For example, authors in (68) tried to validate diagnostic classifications through multi-rater agreement between several experienced otoneurological raters, and an acceptable consensus was achieved only in 62% of the patients. This study indicates that some diagnostic inaccuracy persists in the clinical setting, despite established international classification criteria. This could be taken as a further argument to augment clinical decision making by ML-based support systems.

CONCLUSION

Analysis of large multimodal datasets by novel ML/MVA-methods may contribute to clinical decision making

in neuro-otology. Important features for classification can be identified and aligned with expert experience and diagnostic guidelines. The optimal ML/MVA-method depends on the classification task and data structure. Base-ml provides an innovative open source toolbox to test different methods and clinical tasks in parallel. The multi-faceted presentation of results and explainable AI features, including an identification of clinically relevant features and their statistical analysis, enables clinicians to better understand ML/MVA outcomes, and identify avenues for further investigation. Future research needs to be extended to larger multicenter datasets and new data sources to improve the performance of automated diagnostic support tools.

DATA AVAILABILITY STATEMENT

The data analyzed in this study was obtained from the DSGZ DizzyReg, the following licenses/restrictions apply: The DSGZ provides application forms that must be completed before the data in the DizzyReg may be accessed. Please contact the DSGZ for more details on the application process. Requests to access these datasets should be directed to Ralf Strobl, ralf.strobl@med.uni-muenchen.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board University Hospital Munich Ludwig Maximilian University Munich, Germany. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GV, AZ, RS, and S-AA contributed to conception and design of the study and wrote the first draft of the manuscript. NN and EG contributed to study refinement. RS and GV organized the database. GV and S-AA developed base-ml and performed the data and statistical analyses. S-AA, AZ, NN, and EG provided funding. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX

Appendix A. Supplementary Experiments on TADPOLE Dataset

Data Description

TADOLE (69) is an ADNI-based dataset consisting of imaging-derived features and non-imaging features. The task is to classify whether observations at a baseline timepoint are from healthy normal controls (NC), patients with mild cognitive impairment (MCI), and Alzheimer's disease (AD). It consists of 813 instances (229 NC, 396 MCI, and 188 AD). Imaging features are computed using standard ADNI feature extraction pipelines.

Results and Discussion

We evaluated all models on this dataset as supplementary experiment to understand the strengths and limitations of our proposed model. For our purposes we only look at the F1-score, as this metric is more robust to class imbalance, which is present in TADPOLE. We observe that the best performing models are the hyper-parameter-optimized tree-based models such as Random Forest and AdaBoostClassifier. Furthermore, neural network based models such as MLPClassifier and MGMC yield comparable results but do not outperform other models. We also observe from the confusion matrices that the biggest source of error in most models is to distinguish patients with diagnosed AD from patients with MCI. Likewise, the confusion matrices reveal that models almost never mistake healthy controls with AD patients and vice-versa. Overall almost all models perform comparably, except notable mis-classification rates in KNeighborClassifier and GaussianProcessClassifier. Our obtained classification results of ∼0.6–0.7 F1-score are in line with recent literature, e.g., our previous comparison of MGMC with regular machine learning classifiers [cf. results in (13), not yet computed with base-ml], or RF-based AD classification by Gray et al. (70).

Appendix B. Supplementary Experiments on Generated Dataset

Data Description

To further illustrate the utility of base-ml, we created a synthetic dataset for a binary classification task. We generated 5,000 samples with 20 features of which 10 features are informative and the remaining 10 are uninformative using Scikit-learn (48) (using the built-in function <make_classification>). It is important to note that by design, this classification task has a non-linear separation boundary between the two classes and can therefore not be solved with high accuracy by linear classifier models.

Results and Discussion

As can be seen in **Figure A2**, most non-linear models based on neural networks and properly tuned tree-based models such as Random Forest could yield comparable performance. When looking at the classification accuracy of both MGMC and Random Forest, both perform nearly identically, and with the highest accuracies among all models. As expected, the linear models such as Logistic Regression and Linear Discriminant Analysis obtained the lowest classification accuracy. Overall, we observe that base-ml properly reflects the statistical properties and the difficulty of this artificial classification problem. The source data distributions are not simply separable by topology mapping (see UMAP embedding), and the separation is only resolvable by selected and properly tuned non-linear models – this characteristic would not have been detected by an analysis that was limited to linear models, or less suited non-linear models (e.g., for this dataset: Decision Tree Classifier or AdaBoost Classifier).

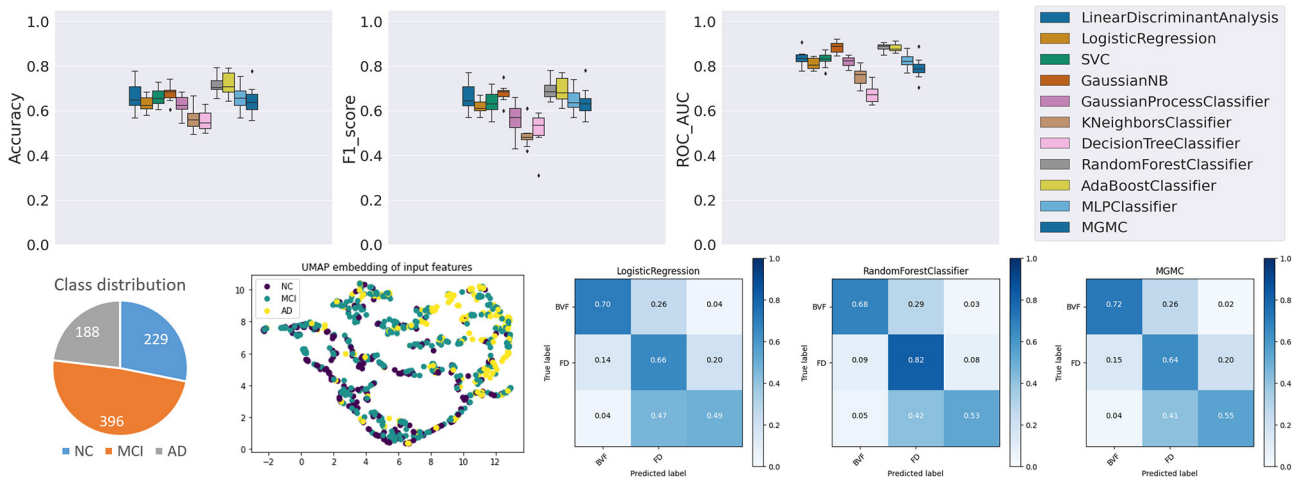

FIGURE A1 | Results panel produced by base-ml for TADPOLE.

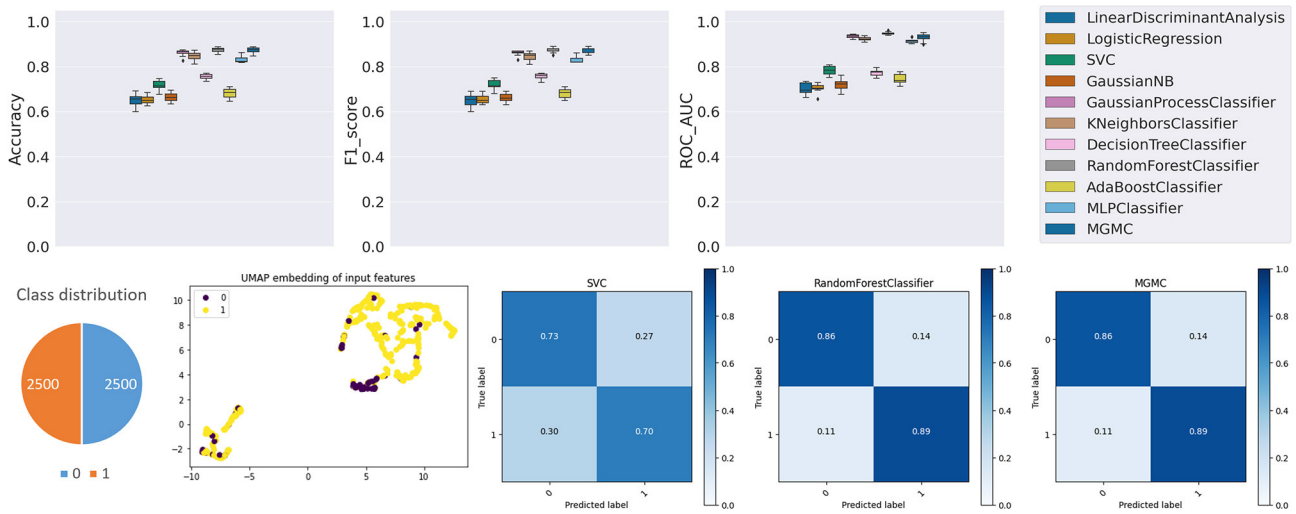

FIGURE A2 | Results panel produced by base-ml for Synthetic data.

Appendix C. Implementation Details: Hyperparameter Search Ranges

To have a more comparable analysis, we selected the best hyperparameters using the validation set, before reporting performance metrics on a with-held test-set (nested cross-validation). We do this by randomly searching the hyperparameter space for 100 iterations for every model and select the best hyperparameters which yields the best validation set classification performance.

For Logistic Regression we used the following hyperparameters (C: randint(1, 11); penalty: {"elasticnet"}, solver: {"saga"}, l1-ratio: uniform(0, 1));

Random Forest (max_depth: {3, None}; max_features: randint(1, 11); min_sample_split: randint(2, 11); bootstrap: {True, False}; criterion: {"gini", "entropy"}, n_estimators: randint(5, 50));

K-Neighbors Classifier (n_neighbors: randint(3, 100); weights: {"uniform", "distance"});

SVC (C: log_uniform(1e−6, 1e+6); gamma log_uniform(1e−6, 1e+6); degree: randint(1, 8), kernel: {"linear", "poly", "rbf"});

Decision trees (max_depth: {3, None}; max_features: randint(1, 11); min_samples_split: randin(2, 11); criterion: {"gini", "entropy"};

Gaussian Process Classifier (kernel: {1∗RBF(), 1∗DotProduct(), 1∗Matern(), 1∗RationalQuadratic(), 1∗WhiteKernel()};

AdaBoostClassifier: (n_estimators: {50, 60, 70, 80, 90, 100}, learning-rate: {0.5, 0.8, 1.0, 1.3});

GaussianNB (var_smoothing: logspace(0, 9, num=100));

Linear Discriminant Analysis (solver: {"svd", "lsqr", "eigen"}; shrinkage: numpy.arange(0, 1, 0.01));

MLP Classifier (learning-rate: {1e−1, 1e−2, 1e−3, 1e−4}; hidden-units: {32, 64, 128}, dropout probability: {0.0, 0.1, 0.2, 0.3, 0.4, 0.5});

MGMC ([cross-entropy, Frobenius-norm, Dirichlet-norm weighting]: uniform(0.001, 1000)).

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