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Vestibular phenotype-genotype correlation in a cohort of 90 patients with Usher syndrome

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Abstract

Usher syndrome has been historically categorized into one of three classical types based on the patient phenotype. However, the vestibular phenotype does not infallibly predict which Usher genes are mutated. Conversely, the Usher syndrome genotype is not sufficient to reliably predict vestibular function. Here we present a characterization of the vestibular phenotype of 90 patients with clinical presentation of Usher syndrome (59 females), aged 10.9 to 75.5 years, with genetic variants in eight Usher syndromic genes and expand the description of atypical Usher syndrome. We identified unexpected horizontal semicircular canal reactivity in response to caloric and rotational stimuli in 12.5% (3 of 24) and 41.7% (10 of 24), respectively, of our USH1 cohort. These findings are not consistent with the classical phenotypic definition of vestibular areflexia in USH1. Similarly, 17% (6 of 35) of our cohort with *USH2A* mutations had saccular dysfunction as evidenced by absent cervical vestibular evoked myogenic potentials in contradiction to the classical assumption of normal vestibular function. The surprising lack of consistent genotypic to vestibular phenotypic findings as well as no clear vestibular phenotypic patterns among atypical USH cases, indicate that even rigorous vestibular phenotyping data will not reliably differentiate the three USH types.

KEYWORDS

atypical Usher syndrome, balance, novel variants, Usher syndrome, vestibular

1 | INTRODUCTION

Usher syndrome is inherited as an autosomal recessive disorder[1-3] with an estimated prevalence based on the clinical presentation of 3.2 to 6.2 per 100 000[4] to more recent estimates as high as one in 6000.[5] Usher syndrome is the most common cause of deaf-blindness, characterized by progressive loss of vision due to retinitis pigmentosa (RP) with varying degrees of hearing loss and dysfunction of the vestibular system. First described in the nineteenth century, the classification of Usher syndrome evolved into three phenotypic types based solely on available diagnostic testing and in the absence of molecular genetic diagnostics. Despite over 100 years of clinical and basic research revealing

phenotypic and genetic heterogeneity of Usher syndrome, the classification of Usher syndrome into phenotypically defined types I, II, and III remains a common practice.[6] Type I is characterized by congenital, profound sensorineural hearing loss (SNHL) and vestibular areflexia; type II by congenital, stable, moderate to severe SNHL with normal vestibular function; and type III by varying degrees of progressive SNHL with variable dysfunction of the vestibular system. The onset of RP is typically pre-pubertal in type I, post-pubertal in type II, and between the second and fourth decade of life in type III.[1]

To date, 12 genes are reported to underlie Usher syndrome, although three of them are disputed.[7-9] Each Usher syndrome gene is associated with one of the three clinical types. However, there are some reported examples of atypical RP,[10] auditory or vestibular manifestations in patients with variants in genes associated with Usher type I[11-17] and type II.[18-24] Some disparity exists because of previous technological limitations in clinical assessment. For example, much of the literature characterizing vestibular integrity in Usher syndrome describes only horizontal semicircular canal function[25,26] or uses age-of-independent-ambulation as an anamnestic proxy for congenital vestibular integrity.[27-30] Contemporary vestibular assessments can independently interrogate the function of all five vestibular sensory organs in the ear: the transducers of linear acceleration, gravity, and thus spatial orientation in the utricle and saccule, and the transducers of angular acceleration in each of the three semicircular canals. Methods such as dynamic posturography allow for a quantitative assessment of functional balance and can examine the contributions of vestibular, visual, and somatosensory cues toward postural stability.

This prospective study included vestibular testing of a group of 90 patients with clinical manifestations of Usher syndrome who had a molecular genetic confirmation of pathogenic variants in known Usher genes. Genotype-phenotype correlations were compared between three groups of patients with variants of eight genes usually associated with either clinically defined type I, type II, or type III Usher syndrome. Noteworthy atypical vestibular findings were observed in 32% (29 of 90) of the Usher syndrome patients in our study. This is consistent with an emerging body of data[14,20,21,31] suggesting that phenotypic boundaries between Usher types should not be assumed based on molecular diagnosis nor should clinical tests be used to infer a likely genotype.

2 | METHODS

2.1 | Participants

Ninety patients (59 females, 31 males) aged 10.9 to 75.5 years (M = 39.35, SD = 15.91) with genetic variants in Usher syndrome genes and clinically confirmed Usher syndrome, hereafter designated as USH1, USH2, and USH3, and no cochlear implantation or middle ear disease were seen between 2005 and 2013 for comprehensive auditory and vestibular testing at the Clinical Center of the National Institutes of Health (NIH) (05-EI-0096, Natural History and Genetic Studies of Usher Syndrome). The study was approved by the Combined Neuroscience Institutional Review Board at the NIH. Written, informed consent was obtained from all patients and guardians of minor patients. Patients underwent a comprehensive ophthalmologic exam at the NIH, which included visual acuity, perimetry, electroretinography, and imaging documenting the presence of RP consistent with Usher syndrome. Visual field and visual acuity are presented in Table 2 and Table S5.

2.2 | Assessments

Criteria for data interpretation and test equipment used for individual tests are presented in Tables S1 and S2 in the supplement. Most patients completed all testing described below, although equipment malfunction and time constraints limited complete assessment of some patients (Table 1).

2.2.1 | Audiologic evaluation

Audiologic evaluation included pure-tone threshold testing by air conduction (0.125, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz) and bone conduction (0.125, 0.25, 0.5, 1, 2, 3, 4 kHz). Pure-tone thresholds were classified for degree and type of hearing loss using a four-frequency (0.5/1/2/4 kHz) pure-tone average (4F-PTA) and three frequency (0.5/1/2 kHz) pure-tone average, respectively (Table S1). Here, we report findings for the ear with the better 4F-PTA.

2.2.2 | Vestibular and balance assessment

Vestibular testing included measurement of the vestibulo-ocular reflex (VOR) elicited by stimulation of the horizontal semicircular canal during bithermal caloric irrigations and sinusoidal harmonic acceleration (SHA) using a rotary chair. The VOR to bithermal air-caloric stimulation (24 and 50°C) was classified as normal, unilateral hypofunction, bilateral hypofunction, or absent. Horizontal semicircular canal reactivity to SHA was recorded at octave frequencies between 0.01 and 0.64 Hz to extend assessment of the VOR beyond the traditional caloric stimulus, which is equivalent to 0.003 Hz. The VOR was interpreted as absent, present with normal gain, or present with reduced gain. All VOR responses were inspected independently by two audiologists to confirm presence of a response, operationally defined as appropriately beating nystagmus with a clear slow and fast phase temporally linked with chair velocity and caloric stimulation.

Cervical vestibular evoked myogenic potentials (cVEMP), which indirectly assess saccular function, were elicited via an air-conducted 0.5 kHz tone burst (Blackman gating, 2 ms rise/fall time, 0 ms plateau) presented monaurally via insert earphones at 100 to 107 dB nHL and a stimulus repetition rate of 5.1/s. Myogenic activity was recorded from surface electrodes placed on the ipsilateral sternocleidomastoid muscle (reference), sternum (active), and forehead (ground). The cVEMP was interpreted based on presence or absence of the P1-N1 response and interaural symmetry of the P1-N1 amplitude.

TABLE 1 Patient characteristics and tests completed for each genetically determined Usher syndrome type

Usher type	Gene	Number of patients	Age in years, mean (SD)	Caloric testing (n)	SHA (n)	cVEMP (n)	SOT (n)
USH1		26	34.5 (15.8)	24	24	20	26
	MYO7A	11	31.7 (17.3)	11	11	9	11
	USH1C	3	37.9 (14.0)	3	3	3	3
	CDH23	6	46.2 (15.2)	4	5	2	6
	PCDH15	5	43.5 (16.6)	5	4	5	5
	USH1G	1	38.9	1	1	1	1
USH2		57	40.8 (15.3)	51	49	39	53
	USH2A	51	40.9 (15.8)	45	44	35	48
	ADGRV1	6	43.1 (12.6)	6	5	4	5
USH3		7	41.8 (18.2)	3	6	6	7
	CLRN1	7	41.8 (18.2)	3	6	6	7

Abbreviations: cVEMP, cervical vestibular evoked myogenic potential; SHA, sinusoidal harmonic acceleration; SOT, sensory organization test.

Functional balance was assessed by the sensory organization test (SOT), a subtest of platform posturography, which provides a measure of postural stability in conditions that rely on somatosensory, visual, or vestibular input. The SOT consists of a series of six conditions (1-6) during which an equilibrium score is calculated through measurement of the patient's sway on a force plate platform. During the first three conditions (1-3) the platform is fixed, and for the other three conditions (4-6) the unfixed platform moves with patient sway. Vestibular-dependent conditions (5, 6) are those where somatosensory and visual stimuli are removed or altered.[32] The vestibular contribution to postural stability was evaluated as normal, reduced, or absent based on the vestibular sensory analysis score (ratio of conditions 5-1). A conditionally-weighted SOT composite score (Table S1) was used to assess overall postural stability and fall risk.

2.3 | Genetic analysis

Genetic variants were identified by Sanger sequencing all the annotated exons of genes associated with Usher syndrome, or from whole-exome sequencing (WES) using Illumina or Applied Biosystems next-generation sequencing (NGS) platforms, or both. Details of Sanger sequencing[33] and NGS[34] have been previously reported. All patients were categorized based upon genotype into one of the following groups: USH1 (variants of *MYO7A*, *USH1C*, *CDH23*, *PCDH15*, or *USH1G*), USH2 (variants of *USH2A* or *ADGRV1*), or USH3 (*CLRN1*).

2.4 | Multiplex ligation-dependent probe amplification assay

In order to determine the copy number variation in *USH2A*, two multiplex ligation-dependent probe amplification (MLPA) probemixes were utilized (SALSA MLPA P361 & P362), whereas for *PCDH15* a single probemix was used (SALSA MLPA P292) according to manufacturer's instructions (MRC Holland, Amsterdam). Briefly, 100 nanograms of DNA for the sample and the references was diluted in 5 μl of low Tris-ethylenediamine tetraacetic acid and denatured at 98°C for 5 min, after which 3 μl of MLPA probe mix and buffer was added at room temperature. The reaction mixture was denatured at 95°C for 1 min and incubated for 16-h at 60°C for probe hybridization with the target sequences. After the hybridization, ligation mixture (32 μl) was added and incubated at 54°C for 15 min for the ligation of the hybridized probes, followed by heat inactivation step at 98°C for 5 min. Furthermore, fluorescent universal primer pair was used for multiplex polymerase chain reaction (PCR) amplification according to the kit protocol. For the analysis of the amplified PCR products, 0.7 μl of each amplified PCR product was mixed with 0.2 μl of GeneScan 500 LIZ dye Size Standard in 9 μl of deionized formamide, which was denatured for 3 min at 86°C, followed by cooling for 2 min at 4°C. The samples were then run on 3730xl DNA Analyzer (Applied Biosystems). The genotype data files were analyzed using Coffalyser.Net software. Three control samples were included with each MLPA probemix run. The two deletions observed were characterized with long range PCR using LA Taq DNA polymerase (Takara, California) to validate MLPA results.

2.5 | Data analysis

Data were analyzed using the SPSS, version 25 (IBM Corp). A one-way analysis of variance was performed to compare age between the genetically classified Usher types and subtypes, with a Tukey's post hoc for multiple comparisons. Because age-related loss of the cVEMP response has been reported,[35] a Mann-Whitney *U* was performed to compare the ages of patients with and without a cVEMP response based on the mutated genes in the USH2 group. Chi-square analysis was performed to identify any significant association between specific USH1 genes and presence vs absence of the VOR during SHA. A linear regression was conducted to investigate the effect of aging on the SOT composite score and a multiple regression was performed to investigate the effects of visual field and visual acuity on condition 4 (vision dependent) of the SOT. Statistical significance was set at $P \leq 0.05$.

TABLE 2 Phenotypic characteristics and genetic variants for USH1 (n = 13) and USH2 (n = 16) patients with atypical vestibular findings

LMG ID	Gene	Allele 1	Allele 2	Age (years)	Degree of HL	Caloric test	SHA	cVEMP	SOT-VEST	Visual field[a]	Visual acuity[b]
USH1											
1687	*CDH23*	c.5237G > A, p.R1746Q	c.7872G > A	58.2	Profound	Absent	**Low**	—	Absent	18	50
1856	*CDH23*	c.3016G > A, p.E1006K	c.3369 + 1G > A	27.4	Profound	—	**Low**	Absent	Absent	19	40
1846	*MYO7A*	c.5392C > T, p.Q1798X	c.4951G > A, p.G1651S	68.5	Profound	Absent	**Low**	Absent	Absent	18	32
1862	*MYO7A*	c.1370C > T, p.A457V	c.401 T > A, p.I134N	21.5	Profound	**BH**	Absent	—	**Low**	126	20
1967	*MYO7A*	c.5428A > T, p.K1810X	c.6025G > A, p.A2009T	26.7	Profound	**WNL**	**WNL**[c]	**Present**	**Low**	56	25
1999[d]	*MYO7A*	c.2904G > T, p.E968D	c.224dup, p.D75EfsX65	47.4	Profound	Absent	**Low**	Absent	Absent	25	100
2000[d]	*MYO7A*	c.487G > A, p.G163R	c.1189G > A, p.A397T	48.9	Profound	Absent	**Low**	Absent	Absent	80	25
2001[d]	*MYO7A*	c.487G > A, p.G163R	c.2904G > T, p.E968D	22.2	Profound	Absent	**Low**	Absent	Absent	85	20
2002[d]	*MYO7A*	c.1189G > A, p.A397T	c.2904G > T, p.E968D	16.1	Profound	Absent	**Low**	Absent	**Low**	137	25
1863	*PCDH15*	c.4733_4736delTCAG; p.V1578AfsX6	**c.92-528C > T**	56.7	**Moderate**	**BH**	Absent	Absent	Absent	140	25
2098	*PCDH15*	c.1737_1738insA, p.A580SfsX9	c.1304A > C, p.D435A	19.3	Profound	Absent	**Low**	Absent	Absent	32	25
1725	*USH1G*	c.113G > A, p.W38X	c.113G > A, p.W38X	38.8	Profound	Absent	Absent	Absent	**Low**	5	40
1920	*USH1C*	c.216G > A	c.216G > A	41.6	Profound	Absent	**Low**	Absent	**Low**	18	63
USH2											
1855	*USH2A*	c.2299delG, p.E767SfsX21	c.6383G > T, p.C2128F	59.8	Moderate	**BH**	WNL	—	WNL	0	LP
1903	*USH2A*	c.920_923dupGCCA, p.H308QfsX16	Exon 27 deleted	75.7	Severe	—	—	—	**Low**	0	HM
1970	*USH2A*	c.1859G > T, p.C620F	c.2276G > T, p.C759F	57.2	Moderate	WNL	WNL	Present	**Low**	10	20
2009	*USH2A*	c.920_923dupGCCA, p.H308QfsX16	c.11864G > A, p.W3955X	58.9	Severe	WNL	WNL	**Absent**	WNL	37	63
2062	*USH2A*	c.2299delG, p.E767SfsX21	c.2299delG, p.E767SfsX21	68.7	**Profound**	—	WNL	—	**Absent**	0	HM
2115	*USH2A*	c.1214delA, p.N405IfsX3	c.15017C > T, p.T5006M	17.0	Moderate	WNL	WNL	**Absent**	WNL	30	32
2142	*USH2A*	c.9469C > T, p.Q3157X	c.13040_13062delinsTCAGAAGTCA, p.T4347IfsX22	28.2	Severe	**UH**	WNL	Present	WNL	140	16
2157	*USH2A*	c.956G > A, p.C319Y	c.15089C > A, p.S5030X	23.6	Moderate	—	WNL	**Absent**	WNL	80	32
2171	*USH2A*	c.2299delG, p.E767SfsX21	—	42.5	Moderate	WNL	WNL	Present	**Low**	11	32
2176	*USH2A*	c.2299delG, p.E767SfsX21	c.4714C > T,p.L1572F	43.1	Moderate	—	—	**Absent**	WNL	13	40
2177	*USH2A*	c.1679delC, p.P560LfsX31	**c.4133_4134dupTC, p.N1379SfsX54**	38.2	Moderate	**UH**	WNL	WNL	WNL	115	32
2193	*USH2A*	c.4758 + 1G > A	c.8584C > T, p.Q2862X	55.1	Severe	**UH**	**Low**	Present	WNL	4	63
2200	*USH2A*	c.1876C > T, p.R626X	**c.4396 + 2 T > G**	48.7	Moderate	WNL	WNL	**Absent**	WNL	20	20
2217	*USH2A*	c.1541 T > C, p.I514T	c.5614_5620del	61.6	Moderate	WNL	WNL	**Absent**	**Low**	10	50

(Continues)

TABLE 2 (Continued)

LMG ID	Gene	Allele 1	Allele 2	Age (years)	Degree of HL	Caloric test	SHA	cVEMP	SOT-VEST	Visual field[a]	Visual acuity[b]
2069	*ADGRV1*	p.S4880fs	p.P194H or p.R2959Q	36.9	Moderate	**UH**	WNL	WNL	WNL	19	40
2219	*ADGRV1*	**c.954_955insAATC, p.Q318NfsX8**	**c.11771delT, p.V3924LfsX11**	29.9	Moderate	**UH**	WNL	Present	WNL	105	32

Note: Bolded text in table identifies novel genetic variants (Allele 1 and Allele 2) and atypical clinical findings. Transcript Accession number: *MYO7A*: NM_000260.4, *USH1G*: NM_173477.5, *PCDH15*: NM_033056.4, *CDH23*: NM_022124.6, *USH1C*: NM_005709.4.

Abbreviations: BH, bilateral hypofunction; cVEMP, cervical vestibular evoked myogenic potential; HL, hearing loss; HM, hand motion perception; SHA, sinusoidal harmonic acceleration; SOT-VEST, sensory organization test-vestibular component; UH, unilateral hypofunction; WNL, within normal limits.

[a]Visual field represents the horizontal extent of the central continuous field in degrees.

[b]Visual acuity measured in Snellen obtained using an ETDRS chart.

[c]Estimated as WNL based on visual inspection of response, data were not saved.

[d]1999, 2000, 2001, and 2002 are members of family LMG353 and phase is known to be in trans-based on sequencing of both parents.

3 | RESULTS

3.1 | Genotype

Twenty-six of 90 (28.9%) patients had deleterious variants in USH1 genes, 57 (63.3%) in USH2 genes, and seven (7.8%) in USH3 genes (Table 1). All had two biallelic deleterious variants apart from the eight patients with only a single variant in USH2A. In two of these eight (25%) patients with only a single causative variant, we were also able to identify a second copy number variant (CNV) of USH2A (Table S6), which was confirmed by long range PCR (Figure S3). Most of the variants are pathogenic or likely pathogenic according to ACMG[36] classification in all but one variant of *ADGRV1* (p.K5421M) in LMG 212, which is predicted to be a variant of uncertain significance. There was not a significant difference in age between patients in each of the three Usher groups (F (2) = 1.64, P = 0.199), nor was there a difference in age within the USH1 subgroups, nor the USH2 subgroups classified by genotype (F (4) = 0.60, P = 0.66; F (1) = 0.95, P = 0.76, respectively).

3.2 | Auditory phenotype

All patients had bilateral SNHL of varying degrees (Figure S1). Twenty-five of 26 (96%) patients in the USH1 group had profound hearing loss and one patient with biallelic mutations in *PCDH15* had bilateral moderate hearing loss. In the USH2 group, the degree of hearing loss was most often moderate (n = 38, 66.7%), although some patients had severe (n = 17, 29.8%) or profound (n = 2, 3.5%) hearing loss. Four of seven (57%) patients in the USH3 group had severe hearing loss, while the remaining three (43%) had profound hearing loss.

3.3 | Vestibular and balance phenotype

The results for individual vestibular assessments are described below for each Usher type. The spectrum of phenotypic and genetic findings for each patient with atypical results is presented in Table 2 and findings for those with typical results are presented in Table S5.

3.3.1 | Caloric testing

An absent VOR to caloric stimulation was documented in 21 of 24 (87.5%) patients with USH1 who had caloric testing. Three (12.5%) patients had a measurable VOR to caloric stimulation; two had bilateral hypofunction (biallelic pathogenic variants in *PCDH15* and *MYO7A*, respectively) and the other had a clinically normal response (biallelic pathogenic variants in *MYO7A*). Forty-five of 51 (88%) patients with USH2 had a normal VOR response to caloric stimulation and six (12%) had reduced VOR responses and a negative history of vertigo. Four patients (8%) with *USH2A* mutations had reduced VOR reactivity; one (2%) had bilateral hypofunction and three (6%) had unilateral hypofunction. Additionally, two patients with *USH2C* mutations (*ADGRV1*) also had

unilateral vestibular hypofunction in response to caloric testing. The VOR to caloric stimulation was variable in the three patients with USH3 (*CLRN1)* who had caloric testing: the response was clinically normal in one, absent in one, and unilaterally reduced (hypofunction) in one (Table S3). Individual caloric response data are shown in Figure 1.

3.3.2 | Rotational vestibular testing

Of the 24 patients with USH1 who completed SHA testing, 14 (58.3%) had an absent response. Ten (41.7%) had measurable VOR gain; one was normal across the rotational frequencies, and the other nine had reduced VOR gain that occurred most often at higher rotational frequencies (Figure 2 and Figure S2). There was no association between the specific USH1 gene and presence vs absence of a VOR response on SHA (χ^2 [8, *n* = 26] = 5.07, *P* = 0.749). The majority (48 of 49, 96%) of patients with USH2 had a clinically normal VOR during SHA. One patient with *USH2A* had reduced VOR gain across three consecutive frequencies. For the six patients with USH3, variable responses were seen in the VOR: one patient (16.7%) had an absent response, one (16.7%) had reduced gain, and four (66.6%) had VOR gain that fell within the normal reference range (Table S3).

3.3.3 | Vestibular evoked myogenic potentials

Nineteen of 20 (95%) patients with USH1 had absent cVEMPs bilaterally and one patient (*MYO7A*) had intact bilaterally symmetric cVEMPs with a normal P1-N1 amplitude. Most (85%) USH2 patients (n = 29 *USH2A* and n = 4 *ADGRV1*) had present and symmetric cVEMP amplitudes; however, six (17%) patients with *USH2A* mutations had absent cVEMPs bilaterally. There was no statistical difference in age between those *USH2A* patients with (11.5-61.7 years, *Mdn* = 35.3, *IQR* = 21.4) and without (17-61.6 years, *Mdn* = 53.0, *IQR* = 37.6) a cVEMP response (*U* = 65, *P* = 0.30). Of the six patients with USH3 who underwent cVEMP testing, three (50%) had an absent response bilaterally, while the other half had present and symmetrical responses (Table S3).

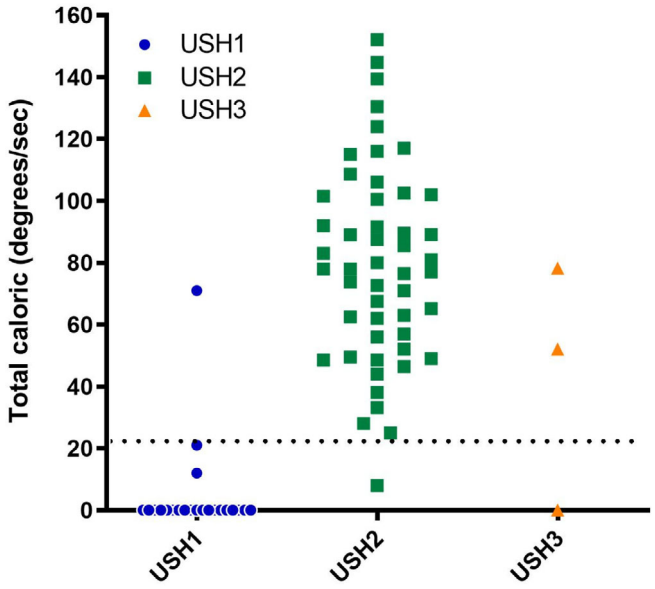

FIGURE 1 Total vestibulo-ocular reflex response to caloric stimulation in degrees per second for all patients with pathologic variants in Usher genes. All symbols falling below the dotted line represent individuals with a bilateral caloric weakness [Colour figure can be viewed at wileyonlinelibrary.com]

3.3.4 | Sensory organization test

The majority of patients across all Usher types had normal postural stability on individual fixed platform test conditions that rely primarily on somatosensory input, with little variability in their results (conditions 1-3; Figure 3). During the eyes-open, unfixed platform condition (4), which is primarily dependent on visual input, postural stability ranged from normal to reduced, including falls, for all three Usher groups (Figure 3). During vestibular-dependent conditions (5 and 6; Figure 3), 21 of 26 (80%) patients with USH1 exhibited excessive postural sway resulting in falls. Five patients with USH1 (19%; three with *MYO7A*, one with *USH1C*, and one with *USH1G* variants) were able to maintain sufficient postural stance without falling on at least one trial during either condition 5 or 6, four of whom had measurable VOR on caloric and/or rotational testing.

Forty-eight of 53 (90%) patients with USH2 had normal vestibular sensory analysis scores on the SOT. Excessive postural sway, resulting in low scores, was documented for five patients with *USH2A* mutations, one of whom fell (restrained by a harness), whereas all five of the patients with *ADGRV1* mutations were able to maintain normal postural stability. Variable performance, ranging from normal postural sway (four of seven patients, 57%) to falls (one of seven patients, 14%), was observed on vestibular-dependent conditions in the USH3 group (Table S3).

The SOT composite score (Figure 3) was reduced and well below the normal age-related fifth percentile for all 26 patients with USH1. Of the 53 patients with USH2 who completed the SOT, 12 (23%) had a reduced SOT composite score, while the remaining 40 patients with USH2 exhibited SOT composite scores that were within normal limits. In the USH3 group, the composite score was reduced in five (71%) of seven patients tested Through a multiple regression analysis, reduced visual field was found to be a significant predictor for poorer performance on condition 4 (*F*[9.26] = 3.43, *P* = 0.001), while aging was found be a significant predictor for poorer overall postural stability as measured by the composite score of the SOT for both the USH1 and USH2 groups, (*F* [7.99] = −2.82, *P* = 0.009) and (*F*[29.74] = −5.45, *P* < 0.0005, respectively). Visual acuity was not a significant predictor for maintaining postural stability in patients with Usher syndrome (*F*[9.26] = −1.12, *P* = 0.26).

4 | DISCUSSION

We provide a contemporary assessment of the vestibular system in a genetically and ophthalmologically confirmed population of

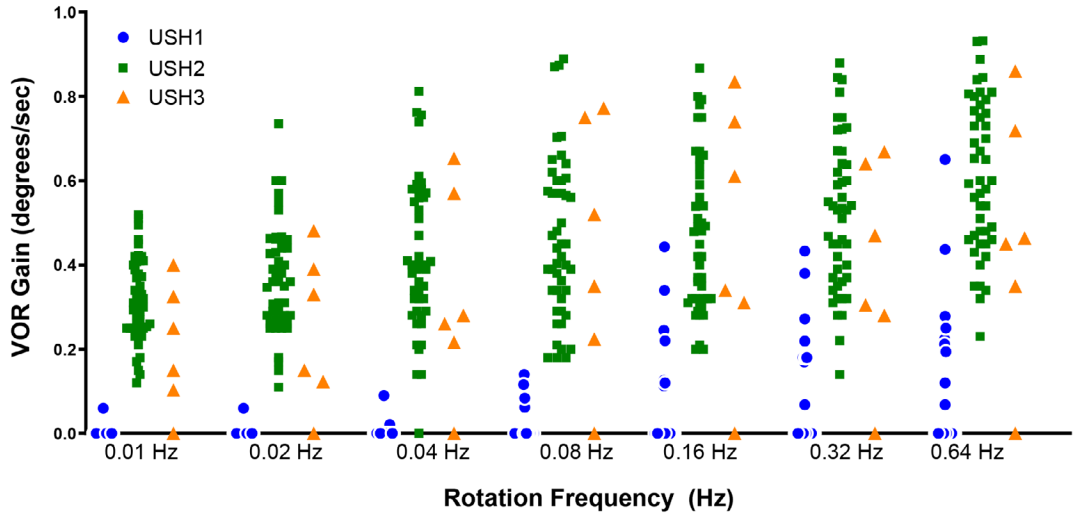

FIGURE 2 Gain of the vestibulo-ocular reflex (VOR) induced during rotary chair testing for individual patients grouped by genetically defined type of Usher syndrome [Colour figure can be viewed at wileyonlinelibrary.com]

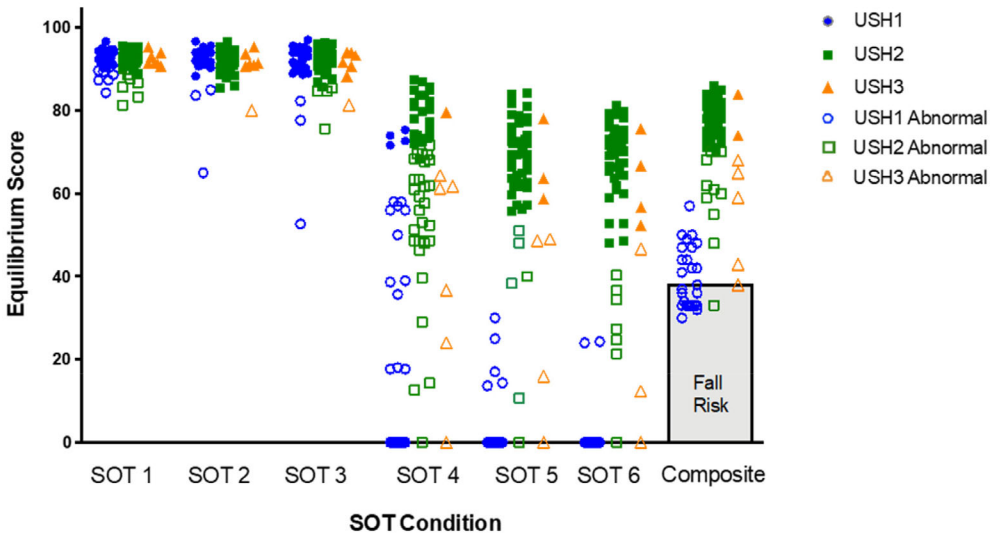

FIGURE 3 Sensory organization test (SOT) equilibrium scores for all six conditions (averaged over three trials) and composite score for all patients. Filled symbols represent scores that fall within the normal range by age, unfilled symbols are scores below the fifth percentile for age. Composite scores that fall within the shaded gray area indicate patients are considered at risk for falls [Colour figure can be viewed at wileyonlinelibrary.com]

90 patients with all three types of Usher syndrome. Previously published studies have employed a limited set of vestibular or balance tests, most commonly the caloric test.[16,31]

Normal saccular function, as measured by the cVEMP test, rarely occurs in patients with USH1. We anticipated that saccular function would be absent based on the classically defined clinical phenotype of profound SNHL and vestibular areflexia. This was the case for all but one of the patients in our USH1 group (LMG1967) who has biallelic pathogenic variants of *MYO7A* and a measurable VOR to both caloric and rotational stimulation of the horizontal semicircular canals. While Magliulo et al[23] described unilateral normal cVEMP responses in three of four patients with clinically defined Usher syndrome type I, the lack of genetic confirmation and the patients' past histories of vertigo raises doubt about the Usher syndrome diagnosis.[37] To our knowledge, ours is the first report of bilateral normal saccular function in a patient with genetically confirmed USH1.

Dysfunction in the saccular otolith pathway can exist in patients with *USH2A* pathogenic variants in the absence of current or past vertigo. We anticipated normal saccular function in patients with USH2 based on the classically described phenotype that includes normal vestibular function. We documented bilateral abnormal saccular function in six (17%) of 35 patients with mutations in *USH2A*. While there are known age-related changes in the cVEMP including absence of the P1-N1 response that increases from 7% in the fifth decade to 32% in the eighth decade,[35] age did not explain absence of a cVEMP in our *USH2A* group. Magliulo et al[24] reported bilateral saccular dysfunction in two of five patients with genetically confirmed USH2 (one with mutations in *USH2A*, one with mutations in *ADGRV1)* and histories of sporadic vertigo. Here, we extend the observation of saccular dysfunction in *USH2A* to those with no current or past history of vertigo.

Traditional phenotypes would dictate an absence of vestibular response for patients with USH1.Our study confirms residual

vestibular and balance function for patients with pathogenic variants in USH1 genes including *MYO7A*, *CDH23*, or *USH1G*, and extends this observation to patients with mutations in *PCDH15* or *USH1C*. There was evidence of residual horizontal semicircular canal function in 46.2% of our USH1 subgroup (Table 2), documented by the presence of a VOR to rotary or caloric stimulation. Conversely, a normal vestibular response for patients with USH2 would be classically expected, however both unilateral and bilateral reduced VOR gain for six patients in this group was observed during rotational and caloric stimulation. Others have reported atypical caloric responses in patients with USH1[11,16]; we extend this observation to patients with USH2.

Our platform posturography findings revealed that 19% (5 of 26) of patients with USH1 were unexpectedly able to maintain sufficient postural stability to prevent a fall during vestibular-dependent SOT conditions. This suggests that some individuals with USH1 are able to utilize alternative sensory input (eg, somatosensory) to maintain postural stability in vestibular dependent environments. Similarly, we hypothesized that patients with USH2 would have normal postural stability on vestibular-dependent conditions. While this was the case for the majority of our USH2 group, we observed reduced postural stability and falls for 9% (5 of 53) of our cohort.

Visual-field, but not visual acuity, correlates with postural stability in patients with Usher syndrome. Specifically, there was a reduction in postural stability in patients with Usher syndrome that not only resulted from reduced or absent vestibular function but also correlated with declining visual-field. This same relationship was not observed with visual acuity. We confirm previous reports identifying an overall aging effect on postural stability.[32] Our posturography data further support and expand upon observations by Caldani et al[17]that patients with Usher syndrome have reduced postural stability that results from deficits in visual and vestibular contributions to balance.

Platform posturography can be used to assess fall risk using the SOT composite score. Whitney et al,[38] found that a composite score ≤38 on SOT is associated with an increased risk for falls over the preceding six-months. Based on this criterion, we found that 13 (50%) of those with USH1 and one (2%) with USH2 would be identified as being at risk for falling (Figure 3). These findings have implications for personalized and targeted counseling and (re)habilitation of postural stability and balance in patients with Usher syndrome. This study also extends knowledge of postural stability in USH1 and USH2, which has been previously limited to one study in a genetically confirmed population,[17] and supplements the comprehensive report of variable SOT findings in USH3.[39]

As with most biological systems, age has a deleterious effect on visual and vestibular physiology. Age was a significant factor in the declining postural stability observed in the patients within our cohort. As such, age is likely a significant compound comorbidity in the declining balance function in patients with Usher syndrome, and may, create an added disadvantage for instability.

All four patients diagnosed with USH1 in family LMG353 (Figure 4) had an atypical finding of measurable VOR gain on SHA (rotational) testing. Four variants of *MYO7A*, a gene classically associated with USH1, were confirmed to segregate among these four patients (ID: 1999-2002) (Figure 4). Of the four variants, two were pathogenic (c.2904G>T, p.E968D; c.224dupA, p.Asp75Glufs*65) and were present in father, whereas the mother had two likely pathogenic variants (c.487G>C, p.G163R; c.1189G>A, p.A397T), while the

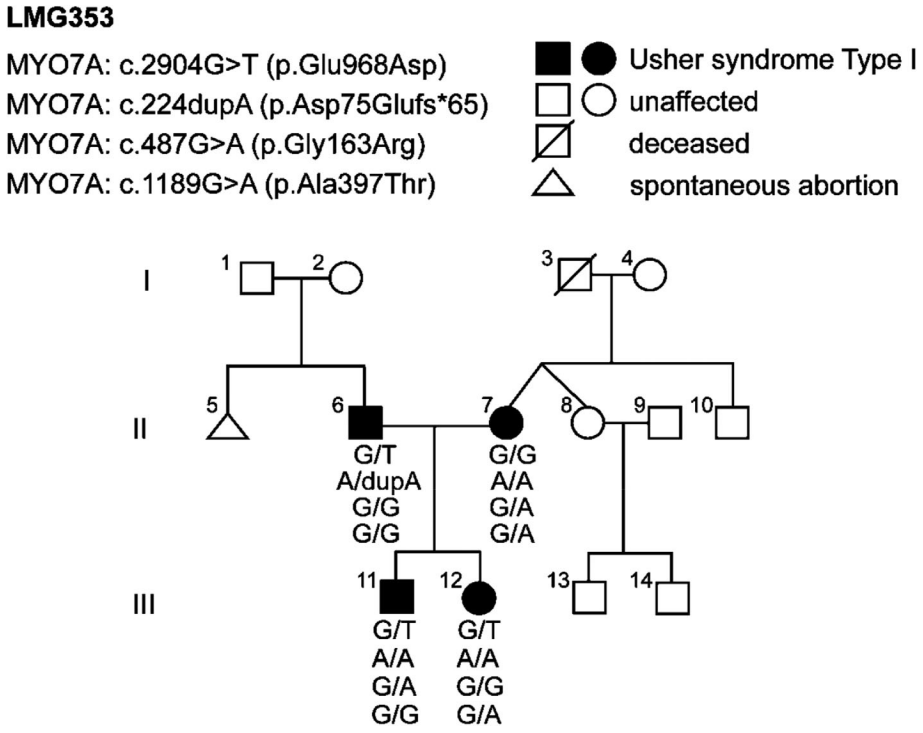

FIGURE 4 Pedigree of family LMG353 segregating atypical Usher syndrome. Squares and circles represent male and female patients. Four different variants of MYO7A are cosegregating with the phenotype in four patients (ID: 1999-2002) with atypical Usher syndrome. Genotypes are mentioned under each patient

offspring had one pathogenic and one likely pathogenic variant each. These variants are present in compound heterozygosity and are predicted to be pathogenic by various in silico tools.[5,40-42]

In our study of 90 patients with Usher syndrome, 105 likely pathogenic variants were identified in genes definitively known to underlie the disease[43] (Table 2, Table S5). We identified 13 novel variants in four of the Usher syndrome genes (Table 2 and Table S4). Previously reported pathogenic variants were also identified in our cohort in the *USH1C*, *MYO7A*, *CDH23*, and *USH1G* genes. In eight patients, only one variant of an Usher gene was identified after exome sequencing. However, using MLPA analyses, we identified a CNV as the second allele in the *USH2A* gene in two of these eight cases (Table S6) and confirmed by long range PCR of approximately 5Kb deletions (Figure S3). Whereas, for the remaining six individuals we could not rule out the possibility of noncoding regulatory variants disrupting transcription or splicing; five had a classical Usher syndrome phenotype and one (LMG2171) had clinical findings considered atypical due to low scores on vestibular dependent SOT conditions. Additionally, in our cohort of 90 USH subjects, we did not identify variants in *CIB2*, *PDZD7*, and *HARS* which are either debatable USH genes or are very rare contributors to Usher syndrome.[7,8]

Our findings have implications for the diagnosis and management of patients with Usher syndrome, as they fail to confirm uniform genotype-phenotype correlations. This is especially important in the neonatal population who may be screened for hearing loss at birth and subsequently identified as having congenital deafness and two pathogenic variants in genes associated with both Usher syndrome and nonsyndromic deafness.[44] In this case, demonstration of intact vestibular function is not sufficient to rule out Usher syndrome and continued surveillance for the onset of RP is warranted.

One limitation of this study was the small number of patients with mutations in genes other than *MYO7A* for USH1 and *USH2A* for USH2. Such limited sample sizes prevent meaningful statistical analyses for differences among USH1 genes and between *USH2A* and *ADGRV1* for USH2. Moreover, although we were able to identify biallelic pathogenic variants in the majority of patients, for some cases due to lack of parental gDNA samples we were unable to perform a segregation analysis to determine the phase of the two variants whether they are in the cis or trans. Another limitation was that a complete vestibular test battery could not be performed in some patients due to time limitations, combined with a lack of studies investigating the utricle and vertical semicircular canals. It is recommended that future studies expand the vestibular phenotype even further to include utricular function through ocular-VEMP and assessment of the anterior and posterior semicircular canals.

Our results provide comprehensive evidence of a lack of a definitive vestibular genotype-phenotype correlation for 29 of 90 (32%) patients in our cohort with Usher syndrome, 28 of whom had biallelic mutations in USH genes. Excluding the six patients with just one known pathogenic variant in an Usher syndrome gene does not alter this conclusion, as only one of these individuals presented with discordant clinical findings. The lack of definitive genotypic to vestibular phenotypic findings, and no clear vestibular patterns among atypical cases, indicates that vestibular results are not an infallible criterion for differentiating the USH types, nor is the Usher syndrome genotype sufficient to reliably predict vestibular function.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.13868.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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