The Laryngoscope

Familial Unilateral Vestibular Schwannoma Is Rarely Caused by Inherited Variants in the *NF2* Gene

D. Gareth Evans, MD; Andrew J. Wallace, PhD; Claire Hartley, BSc; Simon R. Freeman, FRCS; Simon K. Lloyd, FRCS; Owen Thomas, MD; Patrick Axon, FRCS; Charlotte L. Hammerbeck-Ward, FRCS; Omar Pathmanaban, FRCS; Scott A. Rutherford, FRCS; Mark Kellett, MD; Roger Laitt, MD; Andrew T. King, FRCS; Jemma Bischetsrieder, BSc; Jaishri Blakeley, MD; Miriam J. Smith, PhD

Objectives/Hypothesis: Unilateral vestibular schwannoma (VS) occurs with a lifetime risk of around 1 in 1,000 and is due to inactivation of the *NF2* gene, either somatically or from a constitutional mutation. It has been postulated that familial occurrence of unilateral VS occurs more frequently than by chance, but no causal mechanism has been confirmed.

Study Design: Retrospective database analysis.

Methods: The likelihood of chance occurrence of unilateral VS, or occurring in the context of neurofibromatosis type 2 (NF2), was assessed using national UK audit data and data from the national NF2 database. Families with familial unilateral VS (occurrence in first- and second-degree relatives) were assessed for constitutional *NF2* and *LZTR1* genetic variants, and where possible the tumor was also analyzed.

Results: Approximately 1,000 cases of unilateral VS occurred annually in the United Kingdom between 2013 and 2016. Of these, 2.5 may be expected to have a first-degree relative who had previously developed a unilateral VS. The likelihood of this occurring in NF2 was considered to be as low as 0.05 annually. None of 28 families with familial unilateral VS had a constitutional *NF2* intragenic variant, and in nine cases where the VS was analyzed, both mutational events in *NF2* were identified and excluded from the germline. Only three variants of uncertain significance were found in *LZTR1.*

Conclusions: Familial occurrence of unilateral VS is very unlikely to be due to a constitutional *NF2* or definitely pathogenic *LZTR1* variant. The occurrence of unilateral VS in two or more first-degree relatives is likely due to chance. This phenomenon may well increase in clinical practice with increasing use of cranial magnetic resonance imaging in older patients.

Key Words: Unilateral, vestibular schwannoma, *NF2*, *LZTR1*, familial.

Level of Evidence: 2b

***Laryngoscope*, 129:967–973, 2019**

INTRODUCTION

Vestibular schwannomas (VS) are benign nerve sheath tumors that occur in the cerebellopontine angle of the brain, usually commencing in the internal auditory meatus.[1] VSs are usually solitary tumors; however about 4% to 6% are associated with neurofibromatosis type 2 (NF2), an autosomal-dominant monogenic condition caused by pathogenic variants in the *NF2* gene on chromosome 22q.[2,3] Rarely, schwannomatosis caused by pathogenic variants in the *LZTR1*[4,5] gene can cause isolated VS, or VS that can be misdiagnosed as NF2. A previous report in this journal over 20 years ago suggested that familial occurrence of unilateral VS occurred more frequently than by chance, and this might be due to germline variants in the *NF2* gene.[6] We are not aware of any reports since to validate this theory.

Our laboratory increasingly receives samples from patients with unilateral isolated VS where a close family member also developed a unilateral VS. The natural question occurs as to whether this familial occurrence could be caused by hypomorphic (less disease causing) variants in the *NF2* gene. Although NF2 usually presents with bilateral VS at initial presentation, it may frequently present with a unilateral VS either with other NF2 features or as an isolated tumor.[3,5,7–9] Furthermore, *LZTR1* pathogenic variants can also present with apparently isolated VS at young ages.[5] We therefore reviewed

From the Department of Genomic Medicine (D.G.E., A.J.W., C.H., M.J.S.) and Department of Otolaryngology (S.R.F., S.K.L.), University of Manchester, Manchester Academic Health Science Centre, Division of Evolution and Genomic Sciences, Manchester Universities NHS Foundation Trust, Manchester, United Kingdom; Department of Neurosurgery (C.L.H.-W., O.P., S.A.R., A.T.K.) Department of NeuroRadiology (O.T., R.L.), Department of Neurology (M.K.), Salford Royal NHS Foundation Trust, Salford, Manchester, United Kingdom; Department of Otolaryngology (P.A.), Addenbrooke's Hospital Cambridge, United Kingdom Department of Genetics (J.BISCHETSRIEDER), Kaiser Permanente, Fontana, California, U.S.A.; and Johns Hopkins Hospital (J.BLAKELEY), Baltimore, Maryland, U.S.A.

Editor's Note: This Manuscript was accepted for publication on August 15, 2018.

This work was supported by the Manchester National Institute for Health Research Biomedical Research Centre (D.G.E, M.J.S.).

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to D. Gareth Evans, MD, Manchester Centre for Genomic Medicine, Manchester Academic Health Sciences Centre (MAHSC), St. Mary's Hospital, University of Manchester, Manchester M13 9WL United Kingdom. E-mail: gareth.evans@mft.nhs.uk

DOI: 10.1002/lary.27554

our laboratory testing of patients referred with an apparently isolated VS with a close relative diagnosed with the same problem.

MATERIALS AND METHODS

NF2 variant testing of lymphocyte DNA (and tumor when available) used sequencing of all exons and intron exon boundaries and multiple ligation-dependent probe amplification (MLPA). In addition, loss of heterozygosity (LOH) was assessed with an intragenic polymorphic marker as well as flanking markers on tumor specimens. Lymphocyte DNA was also screened for *LZTR1* and *SMARCB1* mutation. Assessment of whether LOH was due to loss of chromosome 22q material harboring *NF2* and *LZTR1* or copy-neutral mitotic recombination was also assessed by MLPA.

Sources for Calculation of Expected Rates of Unilateral VS

The national vestibular schwannoma audit for the British Skull Base Society, 2013–2017 (led by Dr. Patrick Axon) was used for current incidence of unilateral sporadic VS in the United Kingdom. All skull base centers in the United Kingdom provided data on all individuals diagnosed with VS in the audit period. The UK NF2 national database (D. Gareth Evans, Curator) was used to assess the proportion of NF2 presenting as a unilateral VS alone, both on a regional (population = 4.5 million) and national basis.

For assessment of unilateral VS in NF2, individuals initially presenting with an apparently isolated VS with no evidence of other NF2 tumors on magnetic resonance imaging (MRI) scan (meningioma, schwannoma, or ependymoma) and no previous history of an NF2-related tumor and no close relative with confirmed NF2 at diagnosis, were taken to have sporadic unilateral VS as a presenting feature of NF2. Ages have been classified by decade at diagnosis, and gender masked to preserve anonymity.

Likelihood of chance occurrence of familial unilateral VS or presentation within NF2 families were imputed from rates of unilateral VS in the United Kingdom, average number of first-degree relatives, and rates of presentation in NF2 of apparently isolated unilateral VS.

RESULTS

Twenty-eight families with two or more cases of apparently sporadic VS were identified, one from Baltimore, Maryland (Table I). The majority consisted of parent–child (n = 15) or sibling pairs (n = 11). There were four families with three cases of unilateral VS: a patient under age 10 years with a paternal aunt and grandfather affected (the proband still has no additional features of NF2 20 years later), an individual in their 60s with a twin and niece affected, and a three-generation vertical relationship to a grandchild in their 60s. These 28 index cases tested negative for pathogenic germline intragenic *NF2* variants and large rearrangements with VS diagnosed at age 4 to 76 years (median = 52 years). Seven relatives with VS also tested negative. All of the probands also tested negative for clearly pathogenic *LZTR1* variants. Two had a variant of uncertain significance (VUS) identified. One of these was rs778212001 (c.1230C>T p. Asn410Asn). This rare variant was seen only once in 72,432 alleles in ExAC (seen once in Gnomad but in only 30,966 alleles), and MutationTaster reported it to have the potential to affect splicing; however, the variant was not present in a dizygotic affected twin. The second VUS was rs178292 (c.1687G>C, p.Glu563Gln) seen once in 245,294 alleles in Gnomad. This is a missense change that also has the potential to affect splicing, but no DNA was available from relatives to determine whether the variant tracks with disease status. Screening of *SMARCB1* did not identify any pathogenic mutations. The final variant, c.2218 + 9A>G, was not predicted to affect splicing and is seen in 18/274,362 alleles on Gnomad and is likely benign.

The only *NF2* abnormality detected in germline DNA was a 5′ untranslated region (UTR) variant (c.-96T>C) in a woman in her 20s with a large VS. This variant was absent from the affected uncle with a VS aged 50 to 59 years. Furthermore, analysis of the index case's VS identified an *NF2* c.1515_1518deIGTCT p.(Phe507ThrfsTer7) pathogenic variant and LOH with the 5′UTR variant being on the retained allele. The uncle's VS had a pathogenic variant (c.415_447 + 23de156 p.[Val139_Lys149de1]) and LOH, neither of which were present in his blood DNA. Seven further VS cases had tumor DNA analyzed. All revealed pathogenic *NF2* variants (three nonsense, two splicing, two frameshift deletions), with five having LOH (two due to mitotic recombination) and two tumors harboring a second-point mutation. None of the variants detected in the tumors were present in germline DNA.

Likelihood of Familial VS by Chance

Incidence of VS in the United Kingdom was taken from two sources. An audit of skull base services in the United Kingdom for the British Skull Base Society identified 2,947 patients' newly diagnosed VS from April 2013 to March 2016. This represents an incidence in the UK population of approximately 65.6 million (2016) of one in 66,800 annually. This is an increase from around one in 71,000 in a regional survey from 1996 to 1999 and from around one in 100,000 in the years 1990 to 1995.[2] As there are around 1,000 cases annually (2,947 in 3 years) and the lifetime likelihood of a VS is approximately 1 in 1,000,[1,2] the average person with a VS aged 55 years will have had two parents who will have lived a full or near-full life expectancy and a sibling who will have lived through half of the risk period. Any children will have had little chance of developing a VS as they would mostly be <30 years old, where incidence is very low.[2] There were 24 families, with 30 affected first-degree relatives in the United Kingdom, who have been referred with a unilateral VS and a first-degree relative with a VS in an 18-year period from 2000 (there were three families with only second degree). This represents 1.64 per year, where 2.5 per year might have been expected with current VS incidence figures ($\chi^2 = 0.30$, $P = .59$). There were three cases from our regional population of 4.5 million over this period, whereas 2.2 might have been expected by chance ($\chi^2 = 0.29$, $P = .59$. The Manchester center has been referred an average of 205 cases of unilateral VS annually for the last 3 years, representing around 20% of the national total.

TABLE I.
Age of Onset Family History and Genetic Testing of Familial UVS Cases.

FAMNO	Age of VS by Decade	Family History	FDR	NF2 Variant	Family History	No. of Relatives With UVS	Tumor Analyzed	NF2 Mutation Hit 1	NF2 Mutation Hit 2	LZTR1 Negative
99913	1	SDR	No	Not identified	Grandparent and aunt affected	3	No			Yes
897654	3	Parent	Yes	Not identified	UVS	2	Yes	c.532C>T p.(Gln178Ter)	LOH	Yes
8989890	3	Uncle	No	c.-96T>C	UVS[1] does not carry variant	2	Yes	c.1515_1518delGTCT p.(Phe507ThrfsTer7)	LOH	Yes
99929	3	SDR	No	Not identified	UVS	2	Yes	c.523del2	c.448-1G>C	Yes
99948	4	Sibling	Yes	Not identified	UVS sibling + 2 others	3	Yes	c.676-3C>G	LOH[2]	Yes
99905	4	Parent	Yes	Not identified	UVS aged 60s	2	No			Yes
99906	4	Parent	Yes	Not identified	UVS	2	No			Yes
99930	5	Parent	Yes	Not identified	UVS	2	No			Yes
999602	5	Sibling	Yes	Not identified	UVS aged 40s	2	No			Yes
91268	5	Parent	Yes	Not identified	UVS	2	No			Yes
99911	5	Parent	Yes	Not identified	UVS aged 60s	2	No			Yes
UVS1	6	Child	Yes	Not identified	UVS[1]	2	No			Yes
99903	6	Parent	Yes	Not identified	UVS	2	No			Yes
107341	6	Parent	Yes	Not identified	UVS aged 70+	2	No			Yes
99912	6	Sibling	Yes	Not identified	UVS	2	No			Yes, but VUS rs178292 identified
99945	6	Parent	Yes	Not identified	UVS	2	No			Yes
99949	6	Parent	Yes	Not identified	UVS	2	No			Yes
99943	6	Sibling	Yes	Not identified	UVS	2	No			Yes
99936	6	Sibling	Yes	Not identified	UVS aged 50s	2	Yes	c.1575-2A>G	LOH	Yes, VUS c.1230C>T p.Asn410Asn identified, but not present in affected twin
99915	6	Parent	Yes	Not identified	UVS	2	No			Yes
100	6	Sibling	Yes	Not identified	UVS aged 50s[1]	2	No			Yes
76879	6	Sibling	Yes	Not identified	Sibling UVS[1]	2	Yes	c.551G>A	LOH	Yes
376890	7	Parent	Yes	Not identified	Parent and grandparent with UVS	3	No			Yes
999365	7	Sibling	Yes	Not identified	UVS aged 50s and offspring	3	Yes	c.383_398del16 p.(Asp128AlafsTer41)	LOH[2]	Yes
99960	7	Sibling	Yes	Not identified	UVS age 50s	2	No			Yes
99902	8	Sibling	Yes	Not identified	UVS age 60s	2	No			Yes
99940	8	Sibling	Yes	Not identified	UVS	2	No			Yes
BaUVS1	4	Parent	Yes	Not identified	UVS aged 30s	2	Yes	c.459C>A p.Tyr153[2]	c.176_213del36	Yes only VUS c.2218 + 9A>G

[1] Relative also tested for NF2.
[2] Mitotic recombination.
FAMNO = family number; FDR = first-degree relative; NF2 = neurofibromatosis type 2; SDR = second-degree relative; UVS = unilateral vestibular schwannoma; VUS = variant of uncertain significance.

Unilateral VS in Families With NF2

Initially, we assessed the likelihood of an individual later confirmed as having NF2 either by diagnostic criteria (Table II), or by the presence of a confirmed constitutional or mosaic *NF2* pathogenic variant presenting initially with a unilateral isolated VS. The UK national database currently holds information on 1,177 NF2-affected individuals who were residents in the United Kingdom, and 919 are still living (UK prevalence of one in 70,184). Of these 1,177 individuals, 80 (6.8%) initially presented with an apparently sporadic unilateral VS, with a median age of 34 years (range, 4–72 years). Of these, 12 (15%) were diagnosed with a contralateral VS within 2 years, and 56 in total (70%) have developed contralateral VS between 1 and 30 years (median = 6 years) after their first VS. The remainder developed further NF2 tumors to fulfill NF2 criteria, but had not developed a contralateral tumor 1 to 36 years later (median = 7 years). All 80 were de novo cases, and no familial NF2 patient presented with a unilateral VS without a parent affected with NF2 (none had a parent and themselves with unilateral VS). Of the 80 cases, 74 had undergone mutation analysis. In one family, a parent of an NF2 case presenting with bilateral VS and a deletion of exons 15 to 17 on MLPA was not available for blood analysis (died in 1976), but had presented initially with a unilateral VS and was diagnosed a year later with bilateral VS. Of those tested, 41/74 (55.4%) had no identifiable *NF2* variant on gene analysis. Eight of 74 (11%) aged 13 to 54 years (median = 28 years) had a full constitutional pathogenic variant identified. One patient was diagnosed with a constitutional *LZTR1* pathogenic variant,[4] and two further patients were shown to have different *NF2* mutational events in their schwannomas. One patient with bilateral VS only was presumed to have developed this by chance.[10] The remaining 22 cases had either confirmed mosaic NF2 from blood analysis (n = 10) or from eventual testing of two tumors finding identical nonsense variants (n = 2), or were presumed mosaics from finding both mutational events in the tumor, with neither found on blood analysis (n = 9).

We next assessed incidence in our regional population with very high ascertainment over a 30-year period (1988–2017). Over this time period, 115 patients have been diagnosed with NF2 (3.8 annually) within the regional boundaries. This represents an incidence rate of 0.84 per million, suggesting that 54.5 patients with NF2 would be diagnosed annually in the United Kingdom. In the last 10 years in the United Kingdom, we have records of 452 NF2 patients being diagnosed, consistent with 45.2 patients annually. Taking the mean of this, around 50 (5%) of the patients diagnosed with a VS annually will have NF2. Of the 115 regional cases, 13 initially presented with a unilateral VS; however, one described above had a VS by chance (ID-9131), and a second (ID-151) had bilateral VS at age 72 and 75 years, which is as likely to be a chance occurrence due to NF2 (Table III).[10] A third case (ID-98765432) had two additional schwannomas, but the second schwannoma did not carry the mutational events found in the first, also probably ruling out NF2 (Table III). As such, perhaps 9% to 10% (10–11/113) of NF2 patients may present with an apparently sporadic VS. This would suggest that possibly five of the 1,000 unilateral sporadic UK VS patients diagnosed annually actually have NF2. Theoretically, these individuals could have a child with NF2, and with usual autosomal dominant inheritance, one of the average two children would usually have the condition. This would mean that perhaps 10% of these individuals could have a child that presents with NF2, representing 0.5 people annually, or 20% of the rate of familial unilateral VS that occur by chance. However, the great majority of the 80 presenting with unilateral VS are likely to be mosaic for an *NF2* variant, with only 10% having a full constitutional variant. As such, it is unlikely that those 80 individuals would have more than 10 affected children.[11] Therefore, the theoretical rate of NF2 presenting with parent and offspring with unilateral VS would be nearer 0.05 annually or only 2% of the possibility of chance occurrence ($\chi^2 = 2.40$, $P = .12$) (Fig. 1).

We next assessed the cases of unilateral VS that had been referred with a relative with confirmed NF2. Offspring of patients with confirmed NF2 were excluded, as these are almost certain to have NF2[3] and did not meet eligibility criteria. Three parents with a unilateral VS at presentation with affected offspring were mosaic for the family pathogenic variant, and a fourth case described above presented with what was probably bilateral VS in 1976, but was noted initially only to have unilateral disease. All of their affected five children presented with bilateral VS at diagnosis, with two parents being diagnosed after their children. However, two siblings of affected NF2 patients with a unilateral VS aged in their 20s and 50s did not carry the family pathogenic variant.

TABLE II.
Diagnostic Criteria for NF2.

Bilateral vestibular schwannomas or family history of NF2 plus
1. Unilateral VS or
2. Any two of: meningioma, glioma,[1] neurofibroma, schwannoma, posterior subcapsular lenticular opacities
Additional criteria: Unilateral VS plus any two of: meningioma, glioma, neurofibroma, schwannoma, and posterior subcapsular opacities or
Multiple meningioma (two or more) plus unilateral VS or any two of: glioma, neurofibroma, schwannoma, and cataract

These criteria include the National Institutes of Health criteria with additional criteria. The phrase "any two of" refers to individual tumors or cataracts, not to tumor types.

[1] Usually spinal cord ependymoma.

NF2 = neurofibromatosis type 2; VS = vestibular schwannoma.

TABLE III.
Regional Patients Presenting With a Unilateral Sporadic VS Who Later Fulfilled NF2 Criteria

ID	Age of VS, yr	VS Side	Delay to Bilateral VS	Delay to Mening, yr	No Mening	Delay to Spinal, yr	No Spinal	Mutation Found in Blood	Type of Tumor Mutation	Sequence Change	First Tumor Analyzed	Hit 1	Hit 2	Tumor 2 Hit 1	Conclusion (Mechanism)
151	70s	Bilateral	2	No mening	0	NA (no spinals)	0	No	Not identified		No				Possible chance VS
22320	30s	Bilateral	6	No mening	0	11	2	Yes	Splice donor site	1446 + 1G>T	No				Constitutional NF2
22530	20s	Bilateral	1	11	5	NA (no spinals)	0	No	Splice donor site	810G>A	Yes	c.810G>A	c.735delC		Likely mosaic
3608	30s	Bilateral	2	2	2	6	2	No	Nonsense	c.169C>T p. (Arg57Ter)	Yes	c.169C>T	LOH	c.169C>T	Proven mosaic
5085	20s	Bilateral	2	No mening	0	NA (no spinals)	0	No	Nonsense	794 C>T	Yes	c.794C>T, p. R262X	c.599 + 1G>A		Likely mosaic
42907	40s	Bilateral	5	No mening	0	NA (no spinals)	0	No	Not identified		No				NF2 (unknown)
6056	40s	Bilateral	7	15	1	NA (no spinals)	0	No	Not identified		No				NF2 (unknown)
9131	50s	Bilateral	15	No mening	0	NA (no spinals)	0	No	Missense	c.647T>G p. Met216Arg	Yes	c.647T>G p. Met216Arg	LOH	LOH other allele	Chance VS
90781	60s	Bilateral	4	No mening	0	NA (no spinals)	0	No	Not identified		No				NF2 (unknown)
91319	30s	Bilateral	27	30	1	NA (no spinals)	0	No	Not Identified		No				NF2 (unknown)
129836	60s	Left	3	No mening	0	4	1	No	Not identified		No				NF2 (unknown)
98765432	40s	Right	13	No Mening	0	20	1	No	Nonsense	c.1021C>T p. (Arg341 Ter)	Yes	c.1021C>T p. (Arg341 Ter)	LOH	Not found	Not NF2[1]
987123	20s	Bilateral	4	No Mening	0	5	4	Yes, 2%	Splice donor site	c.675 + 1G>A	Yes	c.675 + 1G>A	Not found	NF2 c.675 + 1 G>A	Proven Mosaic

[1] Patient had a third biopsy-proven subcutaneous schwannoma.
ID = identification; LOH = loss of heterozygosity; mening = meningioma; NA = not applicable; NF2 = neurofibromatosis type 2; VS = vestibular schwannoma.

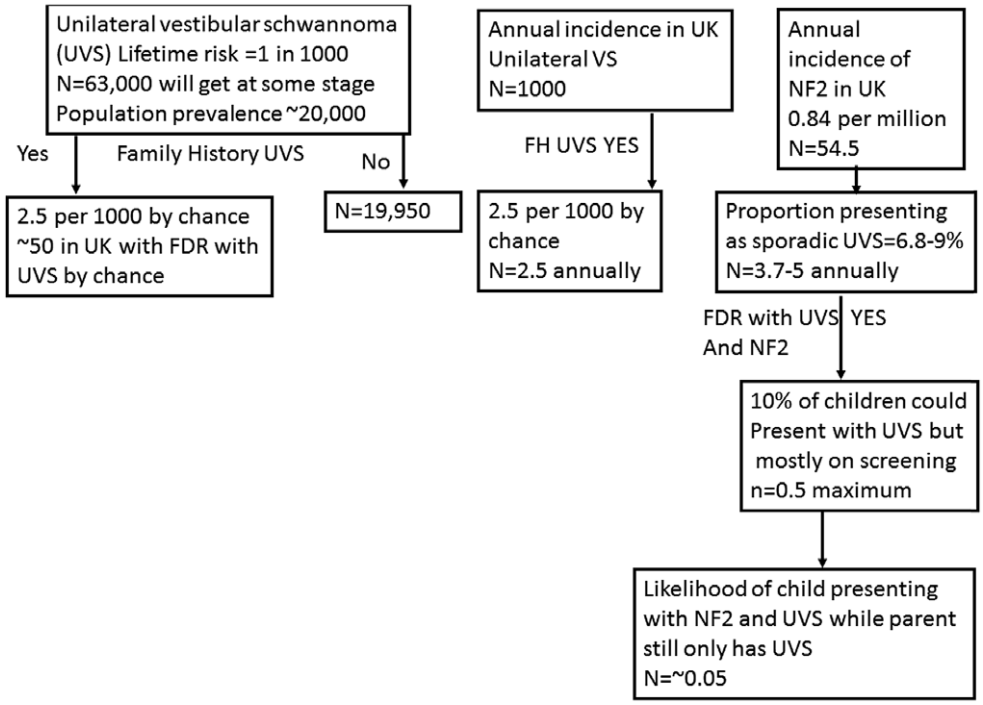

Fig. 1. Likelihood of familial unilateral vestibular schwannoma in the UK population of 63 million people. FDR = first-degree relative; FH = family history; NF2 = neurofibromatosis type 2; UVS = unilateral vestibular schwannoma.

Importantly neither had an affected parent. There were three further more distant relatives, a grandfather and two cousins of NF2 patients, who, less surprisingly, did not carry the pathogenic variant. No siblings with unilateral VS and an unaffected parent have so far been shown to have NF2 in more than 1,000 families referred to the Manchester service.

DISCUSSION

The present study has shown that familial occurrence of unilateral VS, even in multiple generations, is not due to inherited variants in the *NF2* gene. Our analysis for germline variants in *NF2* only identified a probable polymorphism in 1/28 families in the 5′ UTR region. As two mutational hits were identified in this patient's tumor and the variant was not present in the uncle, the variant is highly unlikely to be causative. Although it is possible that a germline variant would have been missed with our analysis, the current detection rate for familial *NF2* variants is 95% based on testing 147 families in the second generation. Furthermore, to demonstrate the sensitivity of testing, both mutational events were present in nine tumors that were tested, completely ruling out an inherited variant, as these were not present in the germline. Familial occurrence has been previously suggested to occur more often than by chance.[6] The previous report of nine families—four with first-degree relatives affected—did not undertake molecular testing. It is not possible with data from the present report to substantiate whether familial unilateral VS occurs more frequently than by chance, as a large epidemiological study with recording of all close relatives would be required. However, as the UK referral center for NF2 molecular testing, we have not been asked to test more families in the last 18 years as might have been expected based on ages at onset, incidence rates, and lifetime risk of VS. It is nonetheless quite possible that such patients were not referred. Nevertheless, based on our smaller regional rates, only a small nonsignificant excess of three cases (with 2.2 expected) was found. In addition to ruling out NF2 as a cause of familial unilateral VS, we have also excluded germline-definite pathogenic variants in *LZTR1* and *SMARCB1*. *LZTR1* is a plausible gene to cause familial unilateral VS, as we found germline pathogenic variants in 3/106 (2.7%) patients with young-onset isolated sporadic VS.[5] There has also been a further case report of a unilateral sporadic VS due to an *LZTR1* variant.[12] It can also be confused with NF2, as patients with a germline pathogenic *LZTR1* mutation usually develop multiple other non-intradermal schwannomas in addition to a unilateral VS.[4] As such, the likelihood of an *LZTR1* variant causing just unilateral VS and no other schwannomas in more than one family member appears unlikely. There has been no convincing evidence for VS with the other proven schwannomatosis gene, *SMARCB1*, although a case report has recorded a relative with unilateral VS in a single family with schwannomatosis and a *SMARCB1* variant.[13] The images provided by the authors are insufficient to confirm a vestibular nerve origin, as no meatal component is visible on the slices presented. The woman herself died from hemorrhage from the tumor during surgery, and no anatomical confirmation during surgery was described. We have previously reported a *SMARCB1*

mutation carrier with schwannomatosis who for many years was thought to have a VS, but closer examination of the scans showed a lower cranial nerve origin.[14]

Although the likelihood of familial unilateral VS due to NF2 is extremely low, presentation with a unilateral sporadic VS at a young age is not unusual, particularly for mosaic patients.[5,9,11,15] Recently, we identified that 9/106 (8.5%) of patients who presented at age <25 years with a unilateral sporadic VS had an *NF2* pathogenic variant.[5] However, most, even those presenting in the first decade of life, do not have a constitutional pathogenic variant.[5,9] The patient in the present report had 20 years of follow-up with no further features of NF2 developing.

The increasing incidence of VS from greater use of MRI and longer life expectancy means that more individuals are being diagnosed each year. The widely accepted diagnostic criteria for NF2[3] (Table II) include the diagnosis of NF2 in first-degree relatives of a proven case with a unilateral VS. We have presented two siblings of NF2 patients who had a unilateral VS but did not have the family mutation. As such, it should not be assumed that if a sibling has a VS identified on a scan with no affected parent that they have NF2. Reports of affected siblings without an affected parent are vanishingly rare. One report dates from some of the original work at the National Institutes of Health,[16] with parents who had died in advanced age (without scans) having two affected offspring. This could have been due to nonpaternity with the same male partner or due to gonadal mosaicism. Mosaicism in NF2 causes a milder phenotype as the variant is in a smaller proportion of cells. As such, affected parents may be diagnosed after, or at the same time as, the child(ren) they pass the pathogenic variant onto, as they have the variant in all cells.[11] Mosaicism should always be suspected if a parent of an NF2 patient is identified with a unilateral VS. This can usually be confirmed in blood DNA, although it may be necessary to confirm it in the tumor if available. However, mosaicism in NF2 appears to be nearly always gonosomal, if it is sufficient to have more than one affected child, and we are not aware of any sibling pairs affected with NF2 with an unaffected parent. As such, the sibling of a unilateral VS patient with NF2 should be tested to identify the germline variant to confirm or refute whether the unilateral VS patient has the tumor by chance. Overall, even in the situation of a mosaic parent presenting with a unilateral VS, the interval to them developing more NF-related tumors is usually small. Therefore, the likelihood of a more severely affected child with a full constitutional mutation presenting in that interval with just a unilateral VS would appear to be extremely small.

The present study has some potential weaknesses. Tumor DNA was not available on most patients referred, and a germline variant cannot be completely ruled out in the majority of families, although it is highly unlikely that more than one or two of the 23 families with no available tumor DNA carry an undetectable mutation given the 95% detection rate in familial NF2. Some strengths of the study are the large series of familial cases and full molecular analysis including *LZTR1.*

CONCLUSION

Individuals diagnosed with a unilateral VS who have a close relative also diagnosed, but no other NF2 features, can be reassured that it is unlikely they have NF2 or will pass on a high risk to their offspring.

Acknowledgments

D. Gareth Evans, MD, is a National Institute for Health Research Senior Investigator. The authors are grateful for statistical support from Dr. Elaine Harkness.

BIBLIOGRAPHY

1. Stivaros SM, Stemmer-Rachamimov AO, Alston R, et al. Multiple synchronous sites of origin of vestibular schwannomas in neurofibromatosis Type 2. *J Med Genet* 2015;52:557–562
2. Evans DGR, Moran A, King A, Saeed S, Gurusinghe N, Ramsden R. Incidence of vestibular schwannoma and neurofibromatosis 2 in the north west of England over a 10 year period: higher incidence than previously thought. *Otol Neurotol* 2005;26:93–97.
3. Evans DGR, Huson SM, Donnai D, et al. A clinical study of type 2 neurofibromatosis. *Q J Med* 1992;84:603–618.
4. Smith MJ, Bowers NL, Bulman M, et al. Revisiting neurofibromatosis type 2 diagnostic criteria to exclude LZTR1-related schwannomatosis. *Neurology* 2017;88:87–92.
5. Pathmanaban ON, Sadler KV, Kamaly-Asl ID, et al. Association of Genetic Predisposition With Solitary Schwannoma or Meningioma in Children and Young Adults. *JAMA Neurol* 2017;74:1123–1129.
6. Bikhazi NB, Slattery WH III, Lalwani AK, Jackler RK, Bikhazi PH, Brackmann DE. Familial occurrence of unilateral vestibular schwannoma. *Laryngoscope* 1997;107:1176–1180.
7. Evans DGR, Lye R, Neary W, et al. The probability of bilateral disease in individuals presenting with a unilateral vestibular schwannoma. *J Neurol Neurosurg Psychiatry* 1999;66:764–767.
8. Evans DGR, Ramsden RT, Shenton A, et al. What are the implications in individuals with unilateral vestibular schwannoma and other neurogenic tumors? *J Neurosurg* 2008;108:92–96.
9. Evans DG, Ramsden RT, Gokhale C, Bowers N, Huson SM, Wallace A. Should NF2 mutation screening be undertaken in patients with an apparently isolated vestibular schwannoma? *Clin Genet* 2007;71:354–358.
10. Evans DG, Freeman S, Gokhale C, et al.; on Behalf of the Manchester NF2 service. Bilateral vestibular schwannomas in older patients: NF2 or chance? *J Med Genet* 2015;52:422–425.
11. Evans DGR, Ramsden RT, Shenton A, et al. Mosaicism in NF2 an update of risk based on uni/bilaterality of vestibular schwannoma at presentation and sensitive mutation analysis including MLPA. *J Med Genet* 2007;44: 424–428.
12. Gripp KW, Baker L, Kandula V, et al. Constitutional LZTR1 mutation presenting with a unilateral vestibular schwannoma in a teenager. *Clin Genet* 2017;92:540–543.
13. Wu J, Kong M, Bi Q. Identification of a novel germline SMARCB1 nonsense mutation in a family manifesting both schwannomatosis and unilateral vestibular schwannoma. *J Neurooncol* 2015;125:439–441.
14. Smith MJ, Kulkarni A, Rustad C, et al. Vestibular schwannomas occur in schwannomatosis and should not be considered an exclusion criterion for clinical diagnosis. *Am J Med Genet A* 2012;158A:215–219.
15. Mohyuddin A, Neary WJ, Wallace AJ, et al. Molecular genetic exclusion of NF2 in young patients diagnosed with a unilateral vestibular schwannoma. *J Med Genet* 2002;39:315–322.
16. Parry DM, Eldridge R, Kaiser-Kupfer MI, Bouzas EA, Pikus A, Patronas N. Neurofibromatosis 2 (NF2): clinical characteristics of 63 affected individuals and clinical evidence for heterogeneity. *Am J Med Genet* 1994;52: 450–461.