Identifying the deficiencies of current diagnostic criteria for neurofibromatosis 2 using databases of 2777 individuals with molecular testing

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Abstract (200 max)

Purpose: We have evaluated deficiencies in existing diagnostic criteria for neurofibromatosis 2 (NF2).

Methods: Two large databases of individuals fulfilling NF2 criteria (n=1361) and those tested for *NF2* variants with criteria short of diagnosis (n=1416) were interrogated. We assessed the proportions meeting each diagnostic criterion with constitutional or mosaic *NF2* variants and the specificity with regard to refuted diagnosis.

Results: There was no evidence for usefulness of old criteria 'glioma' or 'neurofibroma'. 'Ependymoma' had 100% specificity and high levels of confirmed NF2 diagnosis (67.7%). Those with bilateral vestibular schwannoma (VS) alone aged ≥60 years had the lowest confirmation rate (6.6%) and reduced specificity (80%). Siblings as a first-degree-relative, without an affected parent, had 0% specificity. All three individuals with a unilateral VS and an affected sibling were proven not to have NF2. The biggest overlap was with *LZTR1*-associated schwannomatosis. In this category, seven individuals with a unilateral VS plus ≥2 non-dermal schwannomas reduced specificity to 67%.

Conclusion: The present study has confirmed important deficiencies in NF2 diagnostic criteria. The term 'glioma' should be dropped and replaced by 'ependymoma'. Similarly 'neurofibroma' should be removed. Dropping 'sibling' from first-degree-relatives should be considered and testing of *LZTR1* should be recommended for unilateral VS.

Key words: Neurofibromatosis type 2, schwannoma, diagnostic criteria, NF2, LZTR1

INTRODUCTION (words 3904 max 4000)

The two main sets of diagnostic criteria for neurofibromatosis 2 (NF2) date back to 1987 [1] and 1992, [2] although a points based system was devised in 2011 [3]. The Manchester criteria devised in 1992 [2] still appear to be the most widely used and were shown to be superior to the original criteria in 2002 [4]. Deficiencies were still noted, in that individuals with *de novo* NF2 often had a prolonged period with signs attributable to the disease, but without meeting diagnostic criteria [4]. More recently a number of the individual criteria have received more scrutiny. The term 'glioma' in the original sets of criteria has increasingly been seen as an incorrect descriptor. There is no convincing evidence that high grade glioma is part of NF2 [5], with the great majority of intrinsic NF2 associated spinal cord lesions being histologically proven to be ependymomas [6], and low grade gliomas being relatively uncommon [5]. The discovery of the involvement of the *LZTR1* gene in the development of schwannomas in 2014 [7] led to the identification of substantial diagnostic overlap between schwannomatosis and NF2 in particular in those with a unilateral vestibular schwannoma (VS) and multiple other non-dermal schwannomas [8,9]. A proposed update to the Manchester NF2 criteria was made to particularly address the overlap with schwannomatosis (table 1) [9]. Even the hallmark of NF2, bilateral vestibular schwannoma, has recently been shown to occur by chance, unrelated to a common *NF2* pathogenic variant. Indeed, calculations showed that in almost 50% of those with symptomatic bilateral tumours alone over the age of 70, co-occurrence may have happened by chance [10]. These deficiencies prompted us to re-examine the criteria using two large clinical and molecular databases in the UK.

METHODS

Two clinical databases curated since 1994 were utilised. A database of 1460 patients (1210-83% from the UK) meeting existing NF2 diagnostic criteria, or harbouring a constitutional pathogenic variant in *NF2*, or a mosaic pathogenic variant classified as occurring at lower than 50% allele frequency or identified as common between two anatomically distinct NF2 related tumours. A second database containing 1416 individuals who had undergone molecular analysis with one or more NF2 diagnostic criterion without fulfilling full NF2 criteria was also interrogated.

Each main diagnostic feature was taken as a major criterion. Thus bilateral vestibular schwannoma, unilateral vestibular schwannoma, multiple meningiomas and an affected first degree relative with NF2 were taken as the 'major' criteria leading to diagnosis. Whichever of the major criteria was met first, ensuring a confirmed diagnosis (if required with sufficient minor criteria), was taken as the main route to diagnosis. If for instance an individual presented with a unilateral vestibular schwannoma (VS) aged 25 years and a single meningioma aged 27 years before developing a contralateral VS aged 30 years, they only met diagnostic criteria at 30 years by virtue of bilateral VS. If, however, two meningiomas were present aged 27 years, the unilateral VS plus 2 items from another category would have fulfilled diagnostic NF2 criteria at age 27 years. If the diagnosis was made based on unilateral VS plus 2+ meningiomas they were included in the Unilateral VS plus 2 other category rather than in the 2+ meningiomas + unilateral VS category.

Separate analysis was carried out on those who had an ependymoma without bilateral VS at diagnosis and on all those who met the 2+ meningioma category without bilateral VS at diagnosis (to include the unilateral VS criterion). Finally late onset (age ≥60 years) of bilateral VS alone was assessed separately.

Molecular analysis

All individuals underwent lymphocyte DNA analysis for *NF2*, with additional analysis in *LZTR1* and *SMARCB1* in cases meeting the unilateral VS category as well as those with multiple schwannomas. *NF2* pathogenic variant testing of lymphocyte DNA (and tumour when available) used sequencing of all exons and intron exon boundaries and multiple ligation-dependant probe amplification (MLPA). In addition loss of heterozygosity was assessed with intragenic polymorphic markers as well as flanking markers on tumour specimens. Similar analysis was performed for *LZTR1* and *SMARCB1*. Since 2013, all clinical genetic testing has been by next generation sequencing (NGS). Individuals with *de novo* NF2 and learning problems also had chromosome analysis for ring 22 and those with unfound familial NF2 were tested for translocations. Mosaicism was defined as definite when: 1. A pathogenic variant was detectable in blood (often only after NGS guided by tumour analysis) or 2. An identical pathogenic variant was found in two anatomically distinct tumours. A third category of 'probable' mosaicism was when an individual, fulfilling NF2 diagnostic criteria, but with only one tumour available for analysis, had both mutational events found in a single tumour only. An NF2 diagnosis was refuted when molecular events were not consistent between two tumours or when *NF2* testing did not identify a constitutional or mosaic variant and/or a pathogenic variant was found in another gene (e.g. *LZTR1*).

Specificity was calculated for those with either definite confirmed or refuted NF2. Apart from identification of an *LZTR1* pathogenic variant in the absence of a germline *NF2* variant the absence of a common genetic variant in two NF2 tumours was considered evidence of exclusion of NF2.

RESULTS

In total there were 1029 *de novo* individuals (three without an affected parent but with a sibling with NF2), and 332 with an affected parent that had sufficient information to assess diagnostic category. The 1029 *de novo* cases were diagnosed at a median age of 34 years (range 0.5-86) whereas the inherited cases were diagnosed at a median age of 22 years (range

0.2-82). The summary data of molecular analysis in those meeting existing criteria are presented in table 2. The population was divided into *de novo* cases or those without an affected parent and those with an affected parent. Median age at diagnosis in each molecular category: constitutional pathogenic variant, mosaic/presumed mosaic variant, and no pathogenic variant found, is presented for *de novo* cases in table 3.

De novo or with unaffected parent

There were high levels of specificity for a definite confirmed diagnosis in all diagnostic categories (last column table 2) except for individuals with a unilateral VS plus a sibling with NF2 and individuals with a unilateral VS plus two or more schwannomas. However, across all categories, there were low levels of NF2 confirmation (19.6-31.9%) in those initially presenting with a unilateral VS. This is a particular concern in the unilateral VS plus ≥2 schwannomas category where only 27.6% had a definite NF2 diagnosis and 7 cases were ultimately found to have a pathogenic *LZTR1* variant. Of four cases without an *NF2* or *LZTR1* variant identifiable in blood, but for whom two tumours were available for analysis, three carried an identical *NF2* variant in both tumours, while one did not have an *NF2* variant, or an *LZTR1* or *SMARCB1* variant in common in both tumours. This excludes NF2 as the diagnosis in this last case and, along with the *LZTR1* cases, means that, of those who had been given a definite diagnosis on clinical grounds, 8/24 (33%) did not have NF2. All *NF2* negative cases had *LZTR1* and *SMARCB1* analysis. This case with refuted NF2 had received brain and mantle radiotherapy for lymphoreticular malignancy in late teenage (case 157731-table 4) and subsequently developed a unilateral VS aged 40 with a C8 spinal lesion and axillary schwannoma. He subsequently developed a Malignant Peripheral Nerve Sheath Tumour (MPNST) aged 51 in the C8 lesion, and thyroid cancer. Neither the C8 nor axillary lesion had an identifiable *NF2* variant, nor chromosomal loss, ruling out the c.1574+1G>A variant found in the VS as the causative variant. Five further cases with childhood radiotherapy are shown in table 4. Case 9116 was identified as having a c.241-9A>G splicing variant confirmed after fractionated radiotherapy for bilateral optic pathway meningioma. Bilateral VS were identified 2 years later. The remainder all had NF2 tumours 12-25 years later consistent with radiation induced tumours.

The lowest detection rate was found in individuals with bilateral VS diagnosed aged ≥60 years. Overall, only four of 61 (6.6%) had a full constitutional pathogenic variant (c.600-447_600-445delins8; c.19delT, p.(Ser7ProfsTer3); c.15delC, p.(Ile5MetfsTer5); c.1737+3A>T). There was no confirmed case of mosaicism in this category. Although three individuals in this category had two pathogenic variants identified in a single tumour, neither was detectable in lymphocyte DNA. In a fourth case, two tumours were analysed and different unrelated *NF2* pathogenic variants were identified in each tumour, excluding a diagnosis of NF2 [10].

The median age at onset was significantly lower for each main diagnostic category in individuals with an identified constitutional *NF2* variant versus those with no variant identified, while mosaic cases were usually intermediate.

<u>UVS in sibling or parent of NF2 case</u>

Two cases of individuals with unilateral VS were diagnosed after having a child diagnosed with NF2. Low level *NF2* mosaicism (allele frequency 10%), was diagnosed in one parent after a daughter was diagnosed with bilateral VS. A second parent had been diagnosed with a unilateral VS aged 22 and was only diagnosed with NF2 after his child developed bilateral VS in childhood. Cutaneous examination revealed likely schwannomas but DNA confirmation was never undertaken. Three unrelated individuals with a unilateral VS and a sibling with NF2 (parents unaffected) did not carry the pathogenic variant identified in their sibling. The VS were diagnosed aged 29, 39 and 49 years. Full *NF2, LZTR1* and *SMARCB1* variant analysis also proved negative in lymphocyte DNA. For the 29 year old the variant identified in her tumour was not seen in lymphocyte DNA. There were no situations in 1361 NF2 cases in which an individual with a unilateral VS, an affected sibling and unaffected parents would have been diagnosed with NF2.

Multiple meningiomas as a criterion

Although a single meningioma is in the 'other' category, 2+ meningiomas can count as a 'major' criterion. When analysing 2+ meningiomas separately in all the categories including unilateral VS + 2 meningiomas the detection rates were higher and more specific than the Unilateral VS category. Overall, 52/137 (38%) with 2 or more meningiomas, had confirmed NF2 with none where the diagnosis was refuted, compared to only 45/207 (21.7%) with a unilateral VS as major criterion (p=0.001). In particular, there were 32 individuals with 2+ meningiomas in whom the NF2 diagnosis was made with two additional NF2 features, but no VS, and 17/32 (53%) had molecularly confirmed NF2. This was particularly useful in childhood with 11/12 of those diagnosed aged <15 years having a full constitutional pathogenic variant. There were 4 mosaic cases. A 60 year old with 7 meningiomas and 4 spinal schwannomas had a 4% allele frequency of the c.169C>T p.(Arg57Ter) variant. Three further cases aged 47, 48 and 51 had an identical pathogenic variant found in two anatomically distinct meningiomas.

There were 5 unrelated parents with an affected child with NF2 and bilateral VS who had died with multiple meningiomas and no known VS. Unfortunately, no material was available to confirm the proven constitutional pathogenic variant in the child in these cases. A sixth parent with 6 meningiomas (died aged 68 years) who had a deceased child with NF2, had no pathogenic variant identified in blood and no material was available from the daughter. Four of the six parents were males.

None of the *SMARCB1* variant positive patients in the second Manchester database met NF2 criteria with 2 or more meningiomas (3/70 had a single meningioma). Fifty individuals with multiple meningiomas have been tested, including 20 who meet NF2 criteria, and no *SMARCB1* variant has been found. Eight unrelated individuals with multiple meningiomas, but no other features of NF2, had germline pathogenic variants in *SMARCE1* [11,12]. All of these individuals had clear cell meningiomas, rather than the fibroblastic or transitional meningiomas, which are more common in NF2.

Intrinsic brain and spinal cord tumours

The great majority of intrinsic tumours in NF2 were presumed ependymomas with very few undergoing resection. Of those intrinsic tumours with pathological confirmation, four were low grade gliomas and only one was a high grade glioma occurring after previous irradiation [5]. None of these tumours would have aided an earlier diagnosis of NF2. There were 157 (12%) confirmed (n=10) or presumed (n=147) spinal cord ependymomas. The presence of an ependymoma increased the likelihood of identifying a pathogenic variant in those without bilateral VS to 68% (21/31), which was significantly higher than all other categories ($p<0.0001$). The addition of ependymoma to the criteria would have advanced diagnosis by 1-23 years in 18 individuals who would not otherwise have met criteria. In 3 cases, an apparently sporadic ependymoma aged 13, 14 and 24 years would have led to an even earlier diagnosis by 2-4 years if genetic analysis of *NF2* was initiated at time of ependymoma diagnosis.

Ocular features

The database did not hold extensive ocular features on NF2 patients. Nonetheless, undertaking molecular analysis on children with visual symptoms revealing retinal hamartoma or epiretinal membranes would have led to an earlier diagnosis before VS were diagnosed in at least 15 de *novo* affected children. In one child, the presence of amblyopia and epiretinal membranes led to mutational analysis that identified a pathogenic *NF2* variant when the child was one year old (Table 2).

Neurofibroma

At least 67 (5%) NF2 patients had a pathology report stating ‘neurofibroma’. The great majority of these, that had undergone secondary pathology review, were reclassified as schwannoma. Even assessing those without pathology review, none would have led to an earlier diagnosis of NF2 using existing criteria.

Offspring of NF2 affected individuals

There were no particular issues identified to suggest deficiencies in the diagnostic criteria in this category, although a single case of unilateral VS aged 52 years in the daughter of a late onset case with bilateral VS aged 75 years may reflect an inaccurate diagnosis. No pathogenic variant was identified in either the woman or her mother. Overall, detection rates were all >95% in keeping with overall detection rates for familial NF2.

DISCUSSION

The present study has confirmed a number of deficiencies with the 1987 National Institutes of Health and [1] 1992 Manchester criteria [2]. Perhaps the most pressing need for a change in the criteria is from the term ‘glioma’ to ‘ependymoma’. It is clear that the radiological and pathological features of the predominant CNS intrinsic tumour seen in NF2 is a spinal cord ependymoma [5,6]. These have generally been treated conservatively as they are indolent in the great majority of cases, but timely surgery clearly has a place in those with tumours over 15mm in length [13]. Ependymoma is clearly a very useful tumour in classifying NF2 in those with a unilateral VS [9] or multiple meningiomas. The high pathogenic variant detection rate of 68% is the highest of all the *de novo* categories.

The main diagnostic overlap is with schwannomatosis. In this report, we document two further *de novo* cases with a unilateral VS and two or more non-dermal schwannomas (without other NF2 features), who harbour *LZTR1* pathogenic variants, bringing the total number to seven [9]. This means that of those with a clinically confirmed diagnosis, only 67% have NF2, and the majority of these are mosaic. It is likely that the great remainder of those in this category without confirmed diagnosis have NF2 as they do not have an *LZTR1* variant in blood or tumour. However, at least one further case without a *SMARCB1* or *LZTR1* variant had two tumours with divergent *NF2* variants, excluding NF2 and potentially confirming a missed *LZTR1* variant or another chromosome 22 schwannomatosis gene. It should be mandatory to undertake molecular analysis to confirm whether individuals with unilateral VS and other schwannomas have NF2 or *LZTR1*-related schwannomatosis, as the consequences of these two disorders and differences in transmission risk will be substantial, particularly as those with *NF2* variants only detectable in tumour will have a very low transmission risk to children [14]. Similarly, those with multiple non-vestibular schwannomas, without other NF2 features, may well have mosaic NF2. In the current report, three of 13 cases in this category have developed a VS and 10 further cases have not [15]. About 50% of apparent schwannomatosis cases who do not have an *LZTR1* or *SMARCB1* variant have mosaic NF2 [15].

The present report also confirms that the presence of multiple meningiomas is as useful, or better than, a unilateral VS as a ‘major’ criterion. The *NF2* pathogenic variant detection rate is significantly higher in *de novo* cases meeting criteria without bilateral VS in those with two or more meningiomas than in those with a unilateral VS. A number of parents of NF2 cases had multiple meningiomas and it is likely that these were mosaic for the pathogenic *NF2* variant (the fact that 4/6 were male is unusual). Multiple meningiomas account for only 5%

of patients with meningioma and at least 20% of these have NF2 [16]. The population lifetime risk for multiple meningioma without NF2 is likely to be no more than 1 in 10-20,000 [16] and so a chance association with NF2 appears less likely than a unilateral VS, which occurs in 1 in 1000 people in their lifetime [10]. The main diagnostic overlap concern for multiple meningiomas would be with *SMARCB1*-associated schwannomatosis. Although we have not found *SMARCB1* pathogenic variants in any case with more than one meningioma, occasional families with multiple meningioma and a *SMARCB1* pathogenic variant have been described [17].

The present report also calls into question the use of a 'sibling' with an unaffected parent as a diagnostic criterion. There are only two reported instances in the literature of siblings affected with NF2 and no affected parent [18,19]. In neither of these cases did one present with a unilateral VS only. The likelihood of a sibling presenting only with a unilateral VS is small as only around 5% of *de novo* NF2 patients present with an apparently sporadic unilateral VS [20]. Nearly all of these are mosaic for the pathogenic variant [20] whereas a sibling would have a full constitutional change. This scenario also depends on the parent only having confined gonadal mosaicism which appears extremely rare in NF2 as nearly all cases of parental mosaicism involve at least some level of detection in other tissues [14]. All three cases in the present report who have a unilateral VS and an NF2 affected sibling had not inherited the pathogenic variant identified in the sibling. Thus, the term first degree relative in the diagnostic criteria should probably exclude siblings with clearly unaffected parents, although molecular testing should clarify the situation in most instances.

Ophthalmic features consistent with NF2 in childhood should prompt molecular analysis [21,22]. A number of children could have had an earlier diagnosis with timely genetic assessment.

Retinal hamartoma and childhood epiretinal membranes should be considered as potential 'minor' criteria for NF2 in addition to juvenile subcapsular and cortical cataracts [21-23]. The nerve sheath tumour 'neurofibroma' is what has given neurofibromatosis its name. However, true pathological neurofibromas are rare in NF2 [24]. Nonetheless, about 26% of nerve sheath tumours in NF2 have 'features' of schwannoma and neurofibroma and are more correctly designated as 'hybrid' tumours [24]. It is likely that until this pathological term becomes universal that NF2 patients will continue to get an 'inaccurate' diagnosis of neurofibroma. Such a diagnosis in a patient with NF2 features should prompt secondary pathology review. The continued use of 'neurofibroma' within the NF2 criteria is highly questionable.

The final criterion that needs addressing is the hallmark of NF2 itself, bilateral VS. Both of the early criteria [1,2] make bilateral VS sufficient for diagnosis although the points system from 2011 includes an age cut off of 30 years such that bilateral VS >30 years alone did not meet criteria for definite NF2. We have previously calculated that 1 in 2 million people will develop bilateral VS by chance [10] and that close to 50% of those with symptomatic bilateral VS ≥70 would be due to chance alone. In reality, with increasing use of magnetic resonance imaging, many people with bilateral VS identified in older life are not even symptomatic on one side. The very low detection rate for pathogenic variants of 6.6% (4/61) in isolated bilateral VS aged ≥60 is highly significantly less than in other diagnostic categories: 52/137 (38%) for 2+ meningiomas ($p<0.0001$) and 45/209 (21.5%) $p=0.0075$ for unilateral VS plus two other. Nonetheless, the four identified with pathogenic variants had hypomorphic *de novo* variants (two exon 1 frameshift variants and two splicing variants) that would still have important implications to children [25]. If an age limit for definite NF2 were introduced for bilateral VS it would be vital that offspring risks were still addressed.

A final consideration is that schwannomas and meningiomas are radiation inducible tumours, especially with therapeutic radiation in childhood [26]. In one study, amongst 3013 patients treated with radiotherapy before the age of 16, mostly for enlarged tonsils [27], seventy (2.3%) of the patients developed neural tumours, with seven developing multiple schwannomas or meningiomas. This is far higher than the birth incidence of NF2 and schwannomatosis combined [15]. More recently, three of 33 sporadic adults meeting NF2 criteria in Israel had received cranial radiotherapy in childhood and none had an identifiable *NF2* variant on blood analysis [28]. We have presented five further cases with childhood radiotherapy who also had no *NF2* variant on blood analysis. In one of these cases the diagnosis of NF2 could be refuted. As such, individuals who meet NF2 criteria only due to tumours arising >8 years post radiotherapy in childhood, it should be considered that their tumour are more likely to be caused by radiation than NF2 [29].

The current study has some limitations. In an ideal situation, to evaluate the true specificity of each criterion, two tumours from all those without confirmed NF2 should be analysed. In those with late onset bilateral VS it is extremely rare for more than one to be removed and a high proportion of those that are treated receive radiation therapy. In the 30 patients without *LZTR1* variants that had two tumours analysed, three (10%) refuted the diagnosis of NF2. As such, the specificity values reported in this paper are likely to be overestimates, particularly in the categories with a low overall *NF2* detection rate. Nonetheless, the present study represents by far the largest assessment of diagnostic criteria based on close to 3000 patients with molecular analysis. It includes potentially all of the identified NF2 cases in England through the four designated highly specialised commissioned centres [15] and includes referrals for molecular testing of all those with a >1% chance of harbouring an NF2 pathogenic variant in the last 8 years [19]. Although the molecular confirmation of NF2 appears very low, this is most likely to be attributable to mosaicism, as most evaluable cases (90%) with two tumours had an identical *NF2* variant detected in each tumour. The overall detection rate of 95% (sensitivity) for the second generation is reflected in table 2 and our previous research [15]. We did not evaluate 'cerebral calcification' as the use of CT scans has been limited since 1992. As we would not recommend use of CT solely to identify if calcification were present particularly in childhood we would not recommend this criterion is retained due to concerns about specificity.

In conclusion, the present report has identified a number of clear deficiencies in the current diagnostic criteria for NF2. There is a pressing need to develop new consensus criteria for NF2 that differentiate NF2 from schwannomatosis and remove criteria with poor specificity.

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Table 1: Current and proposed revised (2017) Manchester Criteria for NF2

1. Bilateral vestibular schwannomas ***<70+*** OR
2. Family history AND unilateral VS OR
3. Family history OR unilateral VS AND two of*:

meningioma, cataract, glioma, neurofibroma, schwannoma, cerebral calcification

(***if UVS + ≥2 schwannomas only need Negative LZTR1 test***)+, OR

4. Multiple meningioma (2 or more) AND two of:

unilateral VS, cataract, glioma, neurofibroma, schwannoma, cerebral calcification, OR

5. ***Constitutional pathogenic NF2 gene variant in blood or identical in two tumours+***

*Any two of includes two of any tumour type such as schwannoma

+2017 suggested revisions (Smith et al 2017[9])

Table 2: Molecular assessment of 1361 individuals meeting NF2 diagnostic criteria or harbouring a pathogenic variant

Category	Number	% of all NF2	Full constitutional path_variant		Presumed mosaic	Mosaic blood	Mosaic two tumours	Two hits one tumour not seen blood	Not found	NF2 pathogenic variant different in two tumours	*LZTR1*	NF2 excluded	Proportion definitely NF2	Proportion of those with definite diagnosis
de novo/no affected parent														
Bilateral VS no FH*	680	65.7%	303	44.6%	122	84	5	33	255	0	0	0	57.6%	100.0%
BVS UVS first#	69	6.7%	18	26.1%	12	4	0	8	38	1	0	1	31.9%	95.7%
UVS & 2+ schwannomas#	58	5.6%	5	8.5%	17	8	3	6	28	1	7	8	27.6%	66.7%
UVS & 2 other#	148	14.3%	18	12.2%	39	9	2	29	90	1	0	1	19.6%	96.7%
2+ meningioma & 2 other	32	3.1%	13	40.6%	4	1	3	0	15	0	0	0	53.1%	100.0%
Pathogenic variant & 1 tumour	13	1.3%	11	84.6%	2	2	0	0	0	0	0	0	100.0%	100.0%
Pathogenic variant & 2 tumours	8	0.8%	3	37.5%	5	0	5	0	0	0	0	0	100.0%	100.0%
Schwannomatosis NF2 mosaic	9	0.9%	0	0.0%	9	0	9	0	0	0	0	0	100.0%	100.0%
UVS & sibling NF2	3	0.3%	0	0.0%	0	0	0	0	0	0	0	3	0.0%	0.0%
UVS & child NF2	2	0.2%	0	0.0%	1	1	0	0	1	0	0	0	100.0%	100.0%
2+ Meningioma child NF2	6	0.6%	0	0.0%	0	0	0	0	0	0	0	0	unknown	unknown
Ocular & pathogenic variant	1	0.1%	1	100.0 %	0	0	0	0	0	0	0	0	100.0%	100.0%

Total	1029	99.4%	372	36.2%	211	109	27	76	427	3	7	13	49.4%	98.1%
Subanalysis														
BVS only 60+	61	5.9%	4	6.6%		0	0	3	53	1	0	1	6.6%	80.0%
Ependymoma, no BVS	31	3.0%	15	48.4%	8	6	0	2	8	0	0	0	67.7%	100.0%
2+ meningioma no BVS	137	13.2%	31	22.6%	32	17	4	11	74	0	0	0	38.0%	100.0%
parent affected														
UVS + parent NF2	46	13.6%	42	91.3%	0	0	0	0	4*	0	0	0	95.7%	100.0%
2 meningiomas	3	0.9%	3	100.0%	0	0	0	0	0	0	0	0	100.0%	100.0%
2 schwannomas	40	11.8%	39	97.5%	0	0	0	0	1+	0	0	0	100.0%	100.0%
Bilateral VS	203	60.1%	196	96.6%	0	0	0	0	7	0	0	0	96.6%	100.0%
Asymptomatic gene test	40	11.8%	40	100.0%	0	0	0	0	0	0	0	0	100.0%	100.0%
Total	332	98.2%	320	96.4%	0	0	0	0	12	0	0	0	96.4%	100.0%
Full total	**1361**		**692**	**50.8%**	**211**	**109**	**27**	**76**	**439**	**3**	**7**	**13**	**60.7%**	**98.8%**

*****7 cases presenting with bilateral VS and learning problems had ring 22; # 4 cases developed Unilateral VS + 2 additional features 22-25 years post childhood radiotherapy and one a contralateral VS 12 years after radiotherapy aged 17 (table 4).

Table 3: Median age at diagnosis and Inter Quartile Range (IQR) in *de novo* patients in each diagnostic category by constitutional, mosaic/presumed mosaic and no pathogenic variant found

	Full constitutional path_variant			Mosaic/presumed mosaic			Pathogenic variant not found			
de novo category	n	median age at diagnosis	IQR	n	median age at diagnosis	IQR	n	median age at diagnosis	IQR	p value
Bilateral VS no FH	303	21	15.0-34.0	122	31	22.0-42.25	255	48	30-62	<0.001
BVS UVS first	18	29.5	18.5-38.5	12	32	19.25-55.25	39	48	30-61	0.003
UVS + 2 schwannomas	5	13	8.0-34.0	18	36	20.75-47.0	29	41	33.5-52.0	0.016
UVS + 2 other	18	37	23.75-60.25	39	42	32.0-49.0	91	47	36.0-58.0	0.044
2+ meningioma + 2 other	13	16	8.5-20.75	4	49.5	47.25-57.75	15	35	26.0-48.0	<0.001
Pathogenic variant + 1 tumour	11	8	4.0-15.0	2	2.5	2.0-2.5	0			0.231
Pathogenic variant + 2 tumours	3	9	2.0-9.0	5	32	11.0-40.5	0			0.143
Schwannomatosis NF2 mosaic	0			9	44	25.0-51.5	0			n/a
UVS + sibling NF2	0						0			
UVS + child NF2	0			1	50	n/a	1	44		n/a
2+ Meningioma child NF2	0			0			6	35.5	25.75-44.5	n/a
Ocular + pathogenic variant	0			1	1	n/a	0			n/a
Total	371	21	15.0-34.0	213	35	23.0-47.0	435	32	32.0-60.0	<0.001

UVS-Unilateral VS

Table 4: Patients meeting NF2 criteria after therapeutic radiotherapy aged <20 years

Patient ID	Age radiotherapy (in 5-year age group)	indication	sites	NF2 criteria	Delay to criteria being met in years	Tumour analysis	Lymphocyte
9803462	15-19	VS	Brain	Bilateral VS	12	-	Nil found
937087	0-4	neuroblastoma	Brain & spine	Unilateral VS + 2 spinal schwannoma	25	Nil found	Nil found
157731	15-19	Lymphoreticular malignancy	Brain & spine	Unilateral VS + 2 schwannoma	22	c.1574+1G>A + LOH	Nil found
9869906	5-9	Neurogenic tumour unrelated to NF2	Brain	Left VS, trigeminal schwannoma, C4 schwannoma	22	-	Nil found
9000765	0-4	Lymphoreticular malignancy	Brain & spine	Unilateral VS +2 meningiomas C3/4 schwannoma	25	-	Nil found
9116	5-9	meningioma	Brain	Bilateral VS	2	-	c.241-9A>G