ORIGINAL ARTICLE

Multiple meningiomas: differential involvement of the *NF2* gene in children and adults

D G R Evans, C Watson, A King, A J Wallace, M E Baser

See end of article for authors' affiliations

Correspondence to: Dr D Gareth R Evans, Department of Medical Genetics, St Mary's Hospital, Hathersage Road, Manchester M13 0JH, UK; gareth.evans@cmmc.nhs.uk

Revised version received 12 August 2004
Accepted for publication 12 August 2004

J Med Genet 2005;**42**:45–48. doi: 10.1136/jmg.2004.023705

Objective: To screen for *NF2* mutations in people with meningiomas.
Methods: Lymphocyte or tumour DNA was analysed from 46 individuals from 36 families who presented with a meningioma at age ⩽15 years without vestibular schwannoma (VS), or who had multiple meningiomas in adulthood before the diagnosis of VS.
Results: Eight of 13 people with meningioma and other features of neurofibromatosis 2 (NF2) had an identified constitutional *NF2* mutation in blood DNA, but none of the other subjects had identified constitutional *NF2* mutations.
Conclusions: Constitutional *NF2* mutations are the most likely cause of meningioma in children and in people with a meningioma plus other non-VS features of NF2. Mosaic NF2 may be the cause of about 8% of multiple meningiomas in sporadic adult cases, but there are other causes in the majority of other such patients and in multiple meningioma in families.

Meningiomas are generally slow growing tumours that are derived from the arachnoid membrane surrounding the central nervous system. They are among the most common intracranial tumours, with an overall incidence of 2.3/100 000 (20% of all brain tumours) and a 2:1 female to male ratio.[1] About 90% of meningiomas occur in the cranial meninges and 10% in the spinal meninges. Asymptomatic meningiomas are often found on computed tomography and magnetic resonance studies, and at necropsy.[2]

Meningiomas commonly occur in neurofibromatosis 2 (NF2). NF2 is an autosomal dominant disorder caused by inactivating mutations of the *NF2* tumour suppressor gene. Meningiomas are found in about half the patients with NF2, and sporadic meningiomas often have somatic mutations in the *NF2* gene.[3–6]

Apart from *NF2*, other genes are probably involved in the multistep development of meningiomas. These include those that are presumably inactivated by deletion or mutation in 1p32, a region of frequent loss of heterozygosity (LOH), in sporadic and hereditary meningiomas.[5 7] *RAD54L* (OMIM 603615, Locus Link 8438) has been mapped to 1p32 and probably functions in mitotic and meiotic recombination. *RAD54L* is a potential candidate gene for meningioma progression but appears to be unrelated to inherited predisposition to meningioma. The *DAL-1* gene on chromosome 18 is also frequently deleted or mutated in meningiomas.[8] In one study of multiple meningioma, five of seven patients had non-truncating *NF2* mutations in blood–tumour pairs, but these mutations were not considered to be pathogenic.[9] It is thought that *DAL-1* is inactivated mainly in those tumours in which *NF2* function is already abrogated.[10]

NF2 is often the underlying disease in young people who present with meningioma,[11 12] but adults with multiple meningiomas and no other signs of NF2 are usually not considered to be at high risk for NF2. In this study, we screened for *NF2* mutations in children with meningioma and other features of NF2 except vestibular schwannoma (VS), and in adults with multiple meningiomas but without VS.

METHODS

Individuals were eligible for the study if they presented with a meningioma at age ⩽15 years but without VS, or had at least two meningiomas in adulthood but without VS on cranial imaging or at necropsy. In addition, the United Kingdom NF2 registry was searched for individuals who were diagnosed with meningioma at least one year before their diagnosis of VS. Blood samples were obtained with appropriate consent for diagnostic purposes.

Samples were tested for the presence of point mutations by direct sequencing of meta-PCR (polymerase chain reaction) products.[13] The entire cDNA of the *NF2* gene was covered in four meta-PCR reactions. The meta-PCR products were then sequenced in both orientations using BigDye v2 chemistry according to the manufacturer's instructions. Sequencing data were scanned for mutations using the trace subtraction algorithm which is available as a component of the Staden package (www.mrc-lmb.cam.ac.uk/pubseq/). We confirmed identified mutations by repeating the analysis. Dosage analysis was carried out using a quantitative PCR based assay (developed in Manchester) in 10 ml volumes with 100 ng genomic DNA. Gene dosage was measured for four exons of the *NF2* gene (1, 4, 8, and 15).

RESULTS

The patients in tables 1 and 2 are organised into three groups. Patients 1–13 have meningiomas plus other disease features of NF2. Patients 14–17 have multiple meningiomas without other disease features of NF2, but have family members who were diagnosed with NF2 after their own diagnosis of multiple meningioma. Patients 18–50 have multiple meningiomas without other disease features of NF2. Five of these families had more than one affected family member (families 17 and 22–25). In families 22–24, each affected member had spinal meningiomas, while in families 17 and 25, meningiomas were predominantly cranial.

Abbreviations: LOH, loss of heterozygosity; NF2, neurofibromatosis 2; SSCP, single strand conformation polymorphism; VS, vestibular schwannoma

DNA from blood lymphocytes was available from an affected individual in 33 of the 36 families, but pathogenic constitutional *NF2* mutations were not identified in any of these samples. A 26 year old man with multiple meningiomas and his 65 year old clinically unaffected father each had a constitutional 613 G→A missense mutation. A tumour specimen showed LOH for the other allele, but there was an SSCP shift in tumour that was not present in blood DNA. We were not able to sequence this change owing to insufficient tumour DNA.

Seventeen people had meningiomas plus other disease features of NF2, or a family member with NF2 (patients 1–17). Four of these had a family member who was diagnosed with NF2 after their own diagnosis with multiple meningioma (patients 14–17), and three met the clinical diagnostic criteria for NF2 retrospectively after the diagnosis of NF2 in a child (patients 14–16). In a fourth family (patient 17), a man with three meningiomas had a brother with a single meningioma and a high grade glioma at necropsy. These brothers had a nephew, through an unaffected brother, who had severe NF2 with bilateral VS.

Tumour samples were analysed from patients 9, 16, 17, 23, and 26. *NF2* mutations were not identified in patient 16 or in one of the three sisters with spinal meningiomas (patient 23). It was possible to test blood from the children of the two other patients who subsequently met the clinical NF2 diagnostic criteria by having an affected child (patients 14 and 15). A constitutional splice acceptor site mutation (448-1 G→A) was identified in the two affected children of patient 15, but a mutation was not identified in the affected child of patient 14. It was possible to test meningioma tissue from patient 17 for the *NF2* mutation that was found in his affected nephew. There was no evidence of this mutation in DNA extracted from the paraffin block.

In contrast to the lack of identified constitutional *NF2* mutations in these four patients, eight of the 13 individuals with meningioma and other NF2 features had an identifiable constitutional *NF2* mutation in blood DNA. Patient 9 had identical 1228 C→T mutations in each of two tumours that were analysed (meningioma and peripheral nerve schwannoma). This suggests that she was mosaic for this mutation, although further analysis of blood DNA did not reveal evidence of the mutation. A constitutional *NF2* mutation was not found in a parent–child pair (patients 3 and 4) who had peripheral nerve tumours as well as multiple meningiomas.

In addition to the patients mentioned above, the United Kingdom NF2 registry was searched for NF2 patients who had meningiomas that were identified before the diagnosis of VS. Of the 529 NF2 patients with VS, 34 (6%) were diagnosed with meningioma at least one year before the diagnosis of VS. If the 16 patients in table 1 who met the clinical diagnostic criteria for NF2 or who had identified constitutional *NF2* mutations (patients 1–16) are added to these 34 patients, about 8% of NF2 patients present with meningioma before developing VS.

DISCUSSION

In published reports, multiple meningiomas are most often described in association with NF2. An epidemiological study in Finland suggested that as few as 20% of patients with multiple meningiomas had NF2.[14] This is probably an underestimate because the estimated birth incidence of NF2 was only 1 in 87 410, compared with 1 in 33–40 000 in a larger study in the United Kingdom.[15] Also, it is well known that some people who present only with meningiomas develop classical NF2.

In this study, we found that about 8% of NF2 patients present with meningioma before a VS. We previously reported that at least 20% of children who present with a meningioma developed NF2.[11] In the present study, seven of nine children with meningioma and other features of NF2, but no family history of NF2, had an identifiable constitutional *NF2* mutation. However, three children who presented with multiple meningiomas alone did not have an identifiable constitutional *NF2* mutation. Nonetheless, all children with a meningioma should be considered to be at risk for NF2 and have follow up imaging of the cranium and constitutional *NF2* mutation analysis.

The situation in adults with multiple meningiomas is more complex. In this study, constitutional *NF2* mutations were not identified in any adults who had multiple meningiomas but no other features of NF2. However, three of 36 adults with multiple meningiomas had a child who developed NF2 after their parent's diagnosis of meningioma.

Only one of these three adults was alive, and a pathogenic *NF2* mutation in lymphocyte DNA could not be identified, but he must have been at least mosaic in the gonads and neural crest because both of his daughters had identified constitutional *NF2* mutations. Another patient was a proven *NF2* mosaic. Taken together, these results indicate that at

Table 1 Children with multiple meningiomas and other NF2 features or a family history of NF2

Pt	Sex	Age at Dx/last exam (years)	Number and location of meningiomas	Presenting symptom	Other NF2 features	Family history	Mutation
1	M	9/9	1 cranial	Visual loss	Trigeminal schwannoma	None	Not found
2	M	7/9	1 cranial	Visual loss	4 cutaneous tumours and hypoglossal schwannoma	None	Not found
4*	M	9/16†	4 cranial	Seizures	4 cutaneous tumours	Mother (patient 3)	Not found
6	M	2/6†	3 cranial	Visual loss	Trigeminal schwannoma (bilateral)	None	Not found
7	M	5/8	2 cranial	Visual loss	2 spinal and 3 cutaneous schwannomas	None	887 del T
8	M	9/10	1 cranial	Seizures	3 spinal tumours	None	58 ins A
10	M	7/9	2 cranial	Seizures	Cataract	None	592 C>T
12	M	4/4	1 cranial	Visual loss	3rd nerve palsy and 3 cutaneous schwannomas	None	241–2 G>A
13	M	7/8	1 cranial	Facial palsy	Facial palsy	None	443 del C
19	M	10	2 cranial	NA	NA	None	Not found
20	M	15	2: optic sheath and falx	NA	NA	None	Not found
21	M	13/33	2 cranial	NA	None	None	Not found

*Deceased.
†Family history in first degree relative of multiple meningiomas.
Patients 1–13 have meningioma(s) plus other disease features of NF2. Patients 19–21 have multiple meningiomas without other disease features of NF2.
Dx, diagnosis; exam, examination; F, female; M, male; NA, data not available; NF2, neurofibromatosis 2; pt, patient.

Table 2 Adults with multiple meningiomas and other NF2 features or a family history of NF2

Pt	Sex	Age at Dx/last exam (years)	Number and location of meningiomas	Presenting symptom	Other NF2 features	Family history	Mutation
3*	F	20/30†	5 cranial	Headaches	9 cutaneous tumours	Son (patient 4)	Not found
5	M	42/56†	10 cranial	Weakness/wasting	Ependymoma	Son with BVS	169 C>T in son
9	F	51/55	10 cranial	Seizures	2 cutaneous and 1 spinal schwannoma	None	Mosaic for 1228 C>T‡
11	M	30/30	3 cranial	Seizures	4 cutaneous and 1 spinal schwannoma	None	Not found
14	M	36/36†	2 cranial	Weakness/wasting	None	Son with BVS	Not found in son
15	M	26/26†	9 cranial	Headache/seizures	None	Two daughters with BVS	448-1 G>A
16	F	52/70†	5 cranial	Seizures	None	Daughter with BVS	None found in tumour‡
17*	M	50/59†	3 cranial	Headaches	None	Brother with meningioma/ astrocytoma, nephew with BVS	Does not carry nephew's 1048 C>T mutation‡
18	F	27	Multiple cranial	NA	None	None	Not found
22*	F	31/31	1 spinal	Pain	None	Daughter with spinal meningioma at age 15	Not found
23*	F	22	2 spinal	Pain	None	Two sisters with spinal Meningiomas	Nil on tumour analysis‡
24*	F	41	2 spinal	NA	None	Mother with multiple spinal Tumours	Not found
25*	F	29/54	2 cranial	NA	None	Two sisters with meningiomas at ages 47 and 56, niece with meningiomas at age 10	Not found
26	F	50	Multiple cranial	NA	NA	None	Not found‡
27	M	24/33	5 in posterior fossa and lower cranial nerves	Swallowing difficulties	12th nerve palsy	None	613 G>A and LOH in tumour‡
28	M	28	Multiple cranial	NA	None	None	Not found
29	M	62	3 cranial	NA	None	None	Not found
30	F	55	2 cranial	NA	None	None	Not found
31	M	36	4 cranial	NA	None	None	Not found
32	F	34/37	3 cranial	NA	None	None	Not found
33	M	72	3 cranial	NA	None	None	Not found
34	F	26	3: skull base, orbita1, C2	Visual loss	None	None	Not found
35	F	26	3 cranial	NA	None	None	Not found
36	F	54	2: orbital and sphenoid wing	Visual loss	None	None	Not tested
37	F	43/56	4 in left-sided cerebral hemisphere	Headaches	None	None	Not found
38	F	49/51	3 cranial and 2 spinal	NA	None	None	Not found
39	F	44	4 cranial	Pain	Cervical ependymoma	None	Not found
40	F	59/62	5 in vault and falx	Seizure	None	None	Not found
41	M	35/45	4 cranial	Ptosis	None	None	Not found
42	M	28	Multiple cranial	NA	None	None	Not found
43	M	47	Multiple cranial	NA	None	None	Not found
44	F	39	Multiple cranial	NA	None	None	Not found
45	F	50	Multiple cranial	NA	None	None	Not found
46	M	57	5: bilateral optic sheath and fossa, parasagittal and temporal	Visual loss at age 19, CVA at age 57	None	None	Not found
47	F	39	Multiple cranial	NA	None	None	Not found
48	F	49	Multiple cranial	NA	None	None	Not found
49	F	53	Multiple cranial	NA	None	None	Not found
50	F	47	Multiple cranial	NA	None	None	Not found

*Family history in first degree relative of multiple meningiomas.
†Deceased.
‡Tumour analysed.
Patients 3–11 have meningioma(s) plus other disease features of NF2. Patients 14–17 have multiple meningiomas without other disease features of NF2, but have family members who were diagnosed with NF2 after their own diagnosis of multiple meningioma. Patients 18–50 have multiple meningiomas without other disease features of NF2.
BVS, bilateral vestibular schwannoma; CVA, cerebrovascular accident; Dx, diagnosis; exam, examination; F, female; M, male; NA, data not available; NF2, neurofibromatosis 2; pt, patient.

least some adults with multiple meningiomas but without VS are mosaic for an *NF2* mutation.[16 17] The occurrence of meningioma in two uncles of a person with NF2 is almost certainly a coincidence because the intervening male relative died at age 68 without features of NF2 (which is very unlikely if he had the same *NF2* truncating mutation) and the two uncles did not have VS at necropsy.

Analysis of lymphocyte DNA often misses mosaicism.[16 18] Consideration should be given to analysing tumour specimens, particularly if more than one tumour is being removed. The presence of an identical *NF2* mutation in more than one tumour is indicative of mosaicism. Absence of the family *NF2* mutation in blood samples from children would rule out NF2, although there may still be a small risk of meningioma. This risk is due to the known monoclonal pattern of involvement in some multiple meningioma patients[18] and the occurrence of true meningioma families. A study of seven families with multiple meningiomas found two isolated patients who had the same *NF2* mutation in multiply sampled meningiomas, and it was unclear if this represented mosaicism or clonal spread across the meninges.[9] Patient 9 in the present study is clearly mosaic because the second tumour analysed was a schwannoma.

Somatic *NF2* mosaicism is the probable cause of multiple meningiomas in some individual adults, but there are other genetic causes in the majority of such adults and in probably all the reported multiple meningioma families. The *NF2* locus has essentially been excluded by protein analysis in some multiple meningioma families [19]; no such families have been reported with constitutional *NF2* mutations,[20] and linkage analysis excluded the *NF2* locus in one family with multiple meningiomas and ependymomas.[21] Similarly, the results of tumour mutation analysis do not implicate the *NF2* locus in most individual multiple meningioma patients.[9 20] Some isolated meningiomas can be caused by radiotherapy,[22] and a previous history of radiation treatment should always be elicited.

A polymorphic marker in the *RAD54L* gene on 1p has been implicated as a risk factor for meningioma, but it is probably not sufficient to cause multiple tumours.[23] Tumour pathology is useful because meningiomatous meningiomas are almost never *NF2* related[24] and skull base tumours are also rare.[25] Unfortunately, details on the pathology of the meningiomas in our study were not complete.

This is the largest series of multiple meningioma patients reported to date. Heritable and non-heritable meningiomas differ with respect to haploinsufficiency in surrounding tissues and mutational spectra. Constitutional *NF2* mutations are the most likely cause of meningioma in children and in individuals with a meningioma plus other non-VS features of NF2. Mosaic NF2 may be the cause of about 8% of multiple meningiomas in individual adult patients, but there are other causes in the majority of such adults and in multiple meningioma families.

Authors' affiliations

D G R Evans, C Watson, A J Wallace, Academic Unit of Department of Medical Genetics, National Genetics Reference Laboratory and Regional Genetics Service, St Mary's Hospital, Manchester, UK
A King, Department of Neurosurgery, Hope Hospital, Manchester
M E Baser, Los Angeles, California, USA

Competing interests: none declared

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