SMARCB1 mutations are not a common cause of multiple meningiomas

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ABSTRACT

Schwannomas and meningiomas are both part of the tumour spectrum of neurofibromatosis type 2 (NF2) and are associated with somatic loss of chromosome 22. They are also found commonly within the general population, unrelated to NF2. Germline *SMARCB1* mutations have recently been identified as a pathogenic cause of a subset of familial schwannomatosis cases and is a candidate gene for causation of both schwannomas and meningiomas. Recently, Bacci et al reported a germline *SMARCB1* mutation associated with familial schwannomatosis and multiple meningiomas. They concluded that *SMARCB1* mutations can predispose to multiple meningiomas. We screened the *SMARCB1* gene in a panel of 47 patients with multiple meningioma unrelated to NF2 and found no germline mutations. We conclude that while meningiomas may be associated with the schwannomatosis phenotype, *SMARCB1* is not a major contributor to multiple meningioma disease.

INTRODUCTION

Bacci et al recently reported a germline *SMARCB1/INI1* mutation associated with familial schwannomatosis and multiple meningiomas. [1] They identified a missense p.Glu31Val variant in *SMARCB1* which segregated with schwannomatosis, multiple meningiomas or both conditions and concluded that *SMARCB1* mutations predispose to multiple meningiomas.

The relationship between schwannomas and meningiomas is well established. Both are key diagnostic features of neurofibromatosis type 2 (NF2) [2], associated with loss of heterozygosity of chromosome 22 and more specifically with mutations in the *NF2* gene. [3,4] Other genes on chromosome 22, including *SMARCB1,* have been identified as candidates for both schwannomatosis and meningiomas. Previously, we described germline mutations in *SMARCB1* in a subset of patients with familial and sporadic schwannomatosis (MIM 162091). [5] In this cohort of 43 individuals, a single patient in a family with schwannomatosis and a *SMARCB1* mutation was affected with a spinal meningioma. In a previous screen of 126 meningiomas, four (3%) carried an identical somatic missense mutation in exon 9 (p.Arg377His) of *SMARCB1*. [6] A subsequent study using targeted screening of exons 1, 4, 5 and 9 of *SMARCB1* in 80 meningiomas [7] revealed a single mutation (an insertion of a cytosine that resulted in removal of the normal stop codon and elongation of the *SMARCB1* protein by an additional 59 amino acids). Hence, *SMARCB1* mutation may be considered a rare contributor to the pathogenesis of meningioma formation.

Previously, we have shown that multiple meningiomas in adulthood are unlikely to be due to germline mutations in the *NF2* gene (no mutations found in 23 adults with multiple meningiomas, including eight familial samples) although mosaic involvement may be quite common [8]. We have now investigated the *SMARCB1* gene as a potential cause of multiple meningiomas.

METHODS

Patients and samples

Here we define multiple meningiomas as two or more separate meningiomas in the same individual, but include affected relatives if a first degree relative of an individual with more than one meningioma has developed a single meningioma. We have now extended our analysis of germline DNA samples from patients with multiple meningiomas to 47 individuals from unrelated families, including 8 families containing more than one affected individual. The *NF2* gene was analysed for all exons as previously described [8] but the additional 24 samples were all tested using direct sequencing and Multiple Ligation Dependant Probe Amplification: this combination has been shown by us to have 92% sensitivity [9]. In the current study, we have screened this select group of patients with multiple meningiomas without pathogenic germline *NF2* mutations and without additional evidence of NF2 (no vestibular schwannomas and no family history of NF2) or schwannomatosis, for mutations in the *SMARCB1* gene. Forty seven individuals with multiple meningiomas were included in the study. None of the patients have a family history of NF2 and were all are negative for pathogenic germline *NF2* mutations. Thirty nine patients were sporadic cases and eight were from families with more than one affected individual. Tumour samples were available from eight individuals (six with a matched blood sample {sporadic} and two with no blood sample available {familial}). Approval for this study was provided by the local ethics committee.

Mutational Analysis

Genomic DNA was extracted from blood lymphocytes of each patient and the *SMARCB1* gene was screened by direct sequencing of exons 1-9, using an ABI Prism 3100 sequence analyser (Applied Biosystems, Warrington, UK). Real time polymerase chain reaction (PCR) was used to establish exon copy number variations, using the ABI Prism 7900 sequence

detection system (Applied Biosystems). Loss of heterozygosity (LOH) at 22q was determined using the microsatellite markers: D22S303, D22S310, D22S446, D22S449, D22S1174, D22S275, NF2CA3, and D22S268. PCR products amplified by FAM labelled oligonucleotide primers were analysed on an ABI 3100 automated sequencer (Applied Biosystems). [4]

RESULTS

We screened blood DNA from 45 patients with non-NF2 related multiple meningiomas and tumour DNA was screened from six of these. A further two tumour samples with no matching blood were also screened. No *SMARCB1* mutations were identified in germline DNA. Two of the eight meningiomas tested positive for an *NF2* mutation (sporadic) and loss of heterozygosity at the *NF2* locus. A further sporadic tumour sample was identified with a heterozygous deletion of chromosome 22 encompassing *SMARCB1* and *NF2*. No other *SMARCB1* or *NF2* mutations were identified.

DISCUSSION

Our study shows that *SMARCB1* mutations are not commonly associated with the development of multiple meningiomas. Mutations in the *SMARCB1* gene are associated with a range of cancer syndromes, including the aggressive paediatric cancer syndrome malignant rhabdoid tumours (MRT) [10] and chronic myeloid leukaemia (CML) [11]. Recently, they have also been identified in individuals and families with the benign tumour syndrome schwannomatosis [4, 11,13,14].

We have now extended our schwannomatosis cohort to include 28 individuals from 11 families, with pathogenic *SMARCB1* mutations. Only the one patient we previously reported had a spinal meningioma resected aged 61 years, but none had cranial meningiomas. It is therefore possible that meningiomas are an inconstant feature of *SMARCB1* mutation and could be part of the schwannomatosis tumour phenotype. We conclude that *SMARCB1* is not a major contributor to multiple meningioma disease. Definition of the modifiers directing the phenotypic expression of meningiomas and schwannomas will have important implications for disease management.

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Competing interests

The authors have no competing interests to declare.

Ethics approval

Approval for the study was provided by the local ethics committee.

Patient consent

Informed consent was obtained from patients and their families for publication of their details in this report.

Licence Statement

REFERENCE LIST

1. Bacci C, Sestini R, Provenzano A, Paganini I, Mancini I, Porfirio B, Vivarelli R, Genuardi M, Papi L. Schwannomatosis associated with multiple meningiomas due to a familial SMARCB1 mutation. *Neurogenetics* 2009.
2. Neurofibromatosis. Conference statement. National Institutes of Health Consensus Development Conference. *Arch Neurol* 1988;**45**(5):575-8.
3. Mohyuddin A, Neary WJ, Wallace AJ, Purcell S, Wu C-L, Reid H, Ramsden RT, Read AP, Evans DGR. Molecular genetic exclusion of NF2 in young patients diagnosed with a unilateral vestibular schwannoma. J Med Genet 2002; 39:315-322.
4. Dumanski JP, Rouleau GA, Nordenskjold M, Collins VP. Molecular genetic analysis of chromosome 22 in 81 cases of meningioma. *Cancer Res* 1990;**50**(18):5863-7.
5. Hadfield KD, Newman WG, Bowers NL, Wallace A, Bolger C, Colley A, McCann E, Trump D, Prescott T, Evans, DG. Molecular characterisation of SMARCB1 and NF2 in familial and sporadic schwannomatosis. *J Med Genet* 2008;**45**(6):332-9.
6. Schmitz U, Mueller W, Weber M, Sevenet N, Delattre O, von Deimling A. INI1 mutations in meningiomas at a potential hotspot in exon 9. *Br J Cancer* 2001;**84**(2):199-201.
7. Rieske P, Zakrzewska M, Piaskowski S, Jaskolski D, Sikorska B, Papierz W, Zakrzewski K, Liberski PP. Molecular heterogeneity of meningioma with INI1 mutation. *Mol Pathol* 2003;**56**(5):299-301.
8. Evans DG, Watson C, King A, Wallace AJ, Baser ME. Multiple meningiomas: differential involvement of the NF2 gene in children and adults. *J Med Genet* 2005;**42**(1):45-8.
9. Evans DGR, Ramsden RT, Shenton A, Gokhale C, Bowers NL, Huson SM, Wallace A. 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(7):424-8.

10. Versteege I, Sevenet N, Lange J, Rousseau-Merck MF, Ambros P, Handgretinger R, Aurias A, Delattre O. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature* 1998;**394**(6689):203-6.

11. Grand F, Kulkarni S, Chase A, Goldman JM, Gordon M, Cross NC. Frequent deletion of hSNF5/INI1, a component of the SWI/SNF complex, in chronic myeloid leukemia. *Cancer Res* 1999;**59**(16):3870-4.

12. Hulsebos TJ, Plomp AS, Wolterman RA, Robanus-Maandag EC, Baas F, Wesseling P. Germline mutation of INI1/SMARCB1 in familial schwannomatosis. *Am J Hum Genet* 2007;**80**:805-10.

13. Sestini R, Bacci C, Provenzano A, Genuardi M, Papi L. Evidence of a four-hit mechanism involving SMARCB1 and NF2 in schwannomatosis-associated schwannomas. *Hum Mutat* 2008;**29**(2):227-31.

14. Boyd C, Smith MJ, Kluwe L, Balogh A, Maccollin M, Plotkin SR. Alterations in the SMARCB1 (INI1) tumor suppressor gene in familial schwannomatosis. *Clin Genet* 2008;**74**(4):358-66.