Journal of Pathology
J Pathol 2014; **234:** 436–440
Published online 6 October 2014 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.4427

BRIEF DEFINITIVE REPORT

Germline *SMARCE1* mutations predispose to both spinal and cranial clear cell meningiomas

Miriam J Smith,[1] Andrew J Wallace,[1] Chris Bennett,[2] Martin Hasselblatt,[3] Ewelina Elert-Dobkowska,[4,5] Linton T Evans,[6] William F Hickey,[7] Jack van Hoff,[8] David Bauer,[8] Amy Lee,[9] Robert F Hevner,[10] Christian Beetz,[4] Daniel du Plessis,[11] John-Paul Kilday,[12] William G Newman[1] and D Gareth Evans[1]*

[1] *Manchester Centre for Genomic Medicine, University of Manchester, Manchester Academic Health Sciences Centre (MAHSC), UK*
[2] *Department of Clinical Genetics, Chapel Allerton Hospital, Leeds Teaching Hospitals NHS Trust, UK*
[3] *Institute of Neuropathology, University Hospital Münster, Germany*
[4] *Institut für Klinische Chemie und Laboratoriumsdiagnostik Universitätsklinikum Jena, Germany*
[5] *Institute of Psychiatry and Neurology, Department of Genetics, Warsaw, Poland*
[6] *Section of Neurosurgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA*
[7] *Department of Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA*
[8] *Pediatric Hematology/Oncology, Dartmouth–Hitchcock Medical Center, Lebanon, NH, USA*
[9] *Division of Neurosurgery, Seattle Children's Hospital, WA, USA*
[10] *Center for Integrative Brain Research, Seattle Children's Research Institute, WA, USA*
[11] *Department of Cellular Pathology and Greater Manchester Neurosciences Centre, Salford Royal Hospitals NHS Foundation Trust, UK*
[12] *Children's Brain Tumour Research Network, Department of Haematology/Oncology, Royal Manchester Children's Hospital, UK*

**Correspondence to: DG Evans, Manchester Centre for Genomic Medicine, University of Manchester, Manchester Academic Health Sciences Centre (MAHSC), Central Manchester University Hospitals NHS Foundation Trust, Manchester M13 9WL, UK. E-mail: gareth.evans@cmft.nhs.uk*

Abstract

We recently reported *SMARCE1* mutations as a cause of spinal clear cell meningiomas. Here, we have identified five further cases with non-NF2 spinal meningiomas and six with non-NF2 cranial meningiomas. Three of the spinal cases and three of the cranial cases were clear cell tumours. We screened them for *SMARCE1* mutations and investigated copy number changes in all point mutation-negative samples. We identified two novel mutations in individuals with spinal clear cell meningiomas and three mutations in individuals with cranial clear cell meningiomas. Copy number analysis identified a large deletion of the 5′ end of *SMARCE1* in two unrelated probands with spinal clear cell meningiomas. Testing of affected and unaffected relatives of one of these individuals identified the same deletion in two affected female siblings and their unaffected father, providing further evidence of incomplete penetrance of meningioma disease in males. In addition, we found loss of SMARCE1 protein in three of 10 paraffin-embedded cranial clear cell meningiomas. Together, these results demonstrate that loss of SMARCE1 is relevant to cranial as well as spinal meningiomas. Our study broadens the spectrum of mutations in the *SMARCE1* gene and expands the phenotype to include cranial clear cell meningiomas.

Keywords: *SMARCE1*; meningioma; clear cell

Received 17 June 2014; Revised 29 July 2014; Accepted 14 August 2014

No conflicts of interest were declared.

Introduction

Germline *NF2* mutations predispose individuals to meningiomas in neurofibromatosis type 2 (NF2) disease [1]. Somatic *NF2* mutations are also detectable in up to 60% of sporadic meningiomas [2,3]. Germline schwannomatosis-associated *SMARCB1* mutations confer a lower risk (~5%) and are rarely found in sporadic [4] or multiple [5] meningiomas.

We recently described *SMARCE1* mutations in spinal clear cell meningioma disease [6]. The phenotype of this novel syndrome is distinct from meningiomas caused by mutation of the *NF2* or *SMARCB1* genes.

SMARCE1, *SMARCB1* and *SUFU* have all been implicated in meningioma disease [6–8] and are all Sonic Hedgehog–Gli pathway protein, Gli1 interactors [9]. Two large studies, defining somatic mutations in meningiomas, identified a role for the hedgehog–Gli pathway gene, *SMO*, and the mTor pathway gene, *Akt1* [10,11].

Mutations in different meningioma genes seem to lead to differences in tumour subtype. *SMARCB1*-associated meningiomas occur predominantly at the falx cerebri [8], while *NF2*-associated meningiomas are more likely to be located in the lateral and posterior skull base [10]. Acquired non-*NF2* mutations tend to result in tumours

located more medially than tumours with *NF2* mutations. *NF2* mutations are also more likely to be found in fibroblastic and transitional histological subtypes, while meningothelial meningiomas tend to have *SMO* or *Akt1* mutations [11].

The *SMARCE1* mutations identified previously were present only in spinal clear cell meningiomas [6], although it was unclear whether the phenotype is specific to location or the histological subtype, since none of the cranial meningiomas examined were clear cell. The ability to genetically categorize meningiomas more specifically would be useful for determining a more specific prognosis. Therefore, we investigated further cases of spinal and cranial meningiomas for *SMARCE1* mutations to characterize the phenotype further.

Materials and methods

Patient material

Paraffin-embedded tumour tissue and genomic DNA from lymphocytes or tumours were obtained from a retrospectively collected historical archive. Ethical approval was obtained from the North West 7–Greater Manchester Central Research Ethics Committee (Reference No. 10/H1008/74).

Sanger sequencing

SMARCE1 exons were amplified from genomic DNA samples. Sequencing PCR was performed using a BigDye® Terminator v 3.1 Cycle Sequencing Kit (ABI, Life Technologies, Paisley, UK) and analysed on a 3730*xl* DNA Analyser (ABI).

SMARCE1 copy number analysis

The genomic sequence of the human *SMARCE1* gene was downloaded from the USCS genome browser (www.genome.ucsc.edu). Seven *SMARCE1* MLPA probes were designed according to criteria provided by MRC-Holland (The Netherlands) at www.mlpa.com (Figure 2A). The exon 5-specific MLPA probe was designed to overlap the known alteration, c.237 + 2T>C. Six reference probes targeting physically distinct genomic regions were derived from previously established probe sets [12]. Oligonucleotides for MLPA probes were from MWG Eurofins (Ebersberg, Germany). MLPA reagents were from MRC-Holland. Products were visualized on a LICOR4200 (LICOR Biosciences, Lincoln, NE, USA). Relative MLPA signals were calculated as described previously [13].

Immunohistochemistry

Paraffin-embedded meningiomas were immunostained for SMARCE1 protein. Briefly, 4 µm paraffin sections were dried for 4 h at 37 °C, followed by 1 h at 60 °C. The sections were then stained with 1:100 anti-SMARCE1 HPA003916 antibody (Sigma-Aldrich, St. Louis, MO, USA), using an indirect peroxidase method on the Roche Ultra IHC autostainer. The cells were counterstained with haematoxylin II and blueing reagent. The sections were then dehydrated with 100% methylated spirits and cleared with xylene before adding Pertex mountant.

Results and discussion

We screened five individuals with spinal meningiomas and six individuals with cranial meningiomas for point mutations in the *SMARCE1* gene (Table 1). Three individuals with spinal meningiomas and three individuals with cranial meningiomas were known to have clear cell tumours. Two novel point mutations were identified in individuals with spinal meningiomas. A heterozygous frameshift mutation, c.275_276insA, p.(Leu93Valfs*17), was identified in a boy who had developed a spinal clear cell meningioma at 2 years of age. Sequencing of his tumour DNA indicated loss of heterozygosity (LOH) as the second, somatic mutation. A frameshift mutation, c.624_627delTGAG, p.(Ser208Argfs*26), was identified, in conjunction with LOH, in the tumour of another individual with a clear cell meningioma. No point mutations were found in the three remaining spinal cases, including one clear cell case.

A germline inversion, c.374_395inv22, p.(Glu125_Ala132delinsGlyLeuHisArgPheIleValLeu), was identified in one individual with a cranial clear cell meningioma and a second, somatic point mutation c.267delT, p.(Asp90Thrfs*2) was identified in a matched tumour. Two other cranial clear cell meningiomas carried the nonsense mutation, c.357C > G, p.(Tyr119*), and the nonsense mutation, c.688C > T, p.(Gln230*), respectively. For both of these mutations, LOH was indicated as the second hit; however, no lymphocyte DNA was available to confirm the mutations in the germline. No mutations were seen in the three remaining individuals.

Immunohistochemical analyses of tissue from four of five mutation-positive meningiomas, and the point mutation-negative spinal meningioma showed loss of SMARCE1 protein in all tumours (Figure 1A–E). To determine whether loss of protein in the point mutation-negative tumour could be due to a larger mutation, we developed a copy number assay (Figure 2A) for *SMARCE1*. Negative control samples were normal and an exon 5 mutation-positive control showed a reduced signal in exon 5 (Figure 2B). The point mutation-negative tumour DNA showed signal reduction, indicating the presence of a heterozygous deletion which removes the 5′ half of *SMARCE1* on one allele in conjunction with a whole gene deletion on the other allele (Figure 2C).

Due to this result, we analysed 10 additional samples, previously found to be *SMARCE1* mutation-negative. We found the same deletion heterozygously in a second, unrelated individual

Table 1. Details of proven *SMARCE1* mutation carriers; all tumour tissue available from these individuals had clear cell histology

Patient	Gender	Age of onset (years)	Tumour location(s)	Germline mutation	Predicted protein change	Tumour *SMARCE1* sequence
1	Male	7	Spinal	Not tested	Not tested	Hit 1: c.624_627delTGAG, p.(Ser208Argfs*26) Hit 2: LOH
2	Female	22	Cranial	Not tested	Not tested	Hit 1: c.357C > G, p.(Tyr119*) Hit 2: LOH
3	Male	10	Cranial	Not tested	Not tested	Hit 1: c.688C > T, p.(Gln230*) Hit 2: LOH
4	Male	8	Spinal	Not tested	Not tested	Hit 1: Del promoter-E5/6 Hit 2: LOH
5	Male	2	Spinal	c.275_276insA	p.Leu93Valfs*17	Hit 1: c.275_276insA, p.(Leu93Valfs*17) Hit 2: LOH
6:I:1	Female	Asymptomatic*	Spinal/cranial	c.374_395inv22	p.(Glu125_Ala132 delinsGlyLeuHis ArgPhelleValLeu)	N/A
6:II:1	Male	Unaffected at age 17	N/A	c.374_395inv22	p.(Glu125_Ala132 delinsGlyLeuHis ArgPhelleValLeu)	N/A
6:II:2	Female	14	Cranial	c.374_395inv22	p.(Glu125_Ala132 delinsGlyLeuHis ArgPhelleValLeu)	Hit 1: c.374_395inv22, p.(Glu125_Ala132delins GlyLeuHisArgPhelle ValLeu) Hit 2: c.267delT, p.(Asp90Thrfs*2)
7:I:1	Male	Unaffected at age 71	N/A	Del promoter-E5/6	No protein product	N/A
7:II:1	Female	30	Spinal	Del promoter-E5/6	No protein product	Not tested
7:II:2	Female	25	Spinal/Cranial	Del promoter-E5/6	No protein product	Not tested
7:II:3	Female	17	Spinal	Del promoter-E5/6	No protein product	Hit 1: del promoter to E5/6 Hit 2:c.757C > T, p.(Gln253*)

*Reflexive screening after family diagnosis identified a cranial and a spinal meningioma on MRI in this asymptomatic individual.

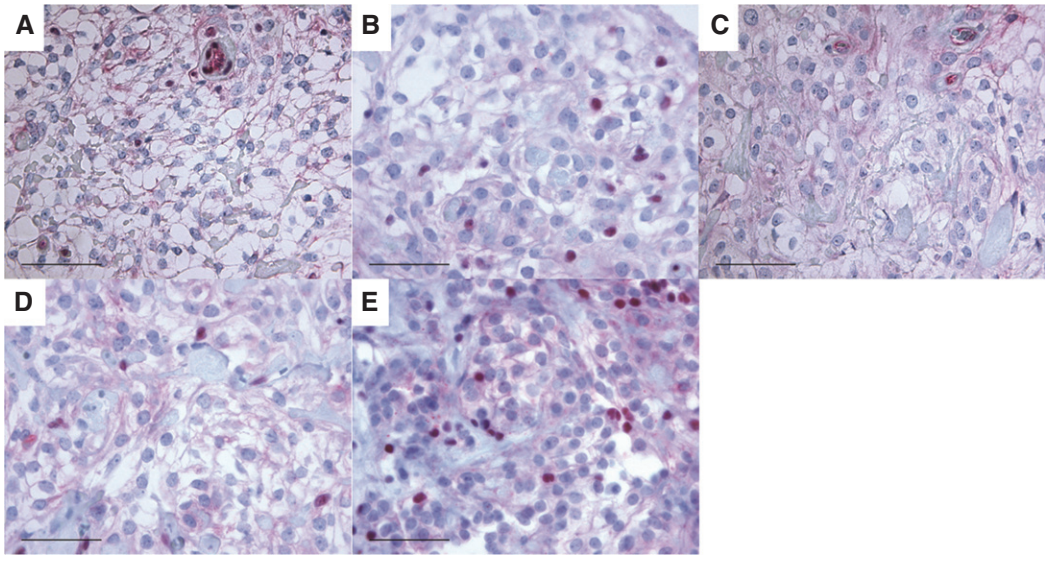

Figure 1. Immunohistochemical staining of *SMARCE1* mutation-positive clear cell meningiomas, showing loss of nuclear staining with retained staining in vascular endothelium. (A–C) Spinal clear cell meningiomas: (A) c.275_276insA; (B) c.624_627delTGAG; (C) large deletion. (D, E) Cranial clear cell meningiomas: (D) c.357C > G; (E) c.688C > T. Scale bar = 30 μm.

with spinal clear cell meningiomas (Figure 2D). Samples from four relatives of this individual identified the deletion in her two affected female siblings and their unaffected father, but not in their unaffected mother. This result provides further evidence of incomplete penetrance of meningioma disease in males. Tumour DNA from one of the affected siblings showed a heterozygous deletion in conjunction with a somatic nonsense mutation, c.757C > T, p.(Gln253*). Pedigrees of confirmed germline *SMARCE1* mutation carriers are shown in Figure 3.

Immunohistochemical analysis of an additional panel of 10 clear cell cranial meningiomas found loss of SMARCE1 protein in three tumours. One tumour

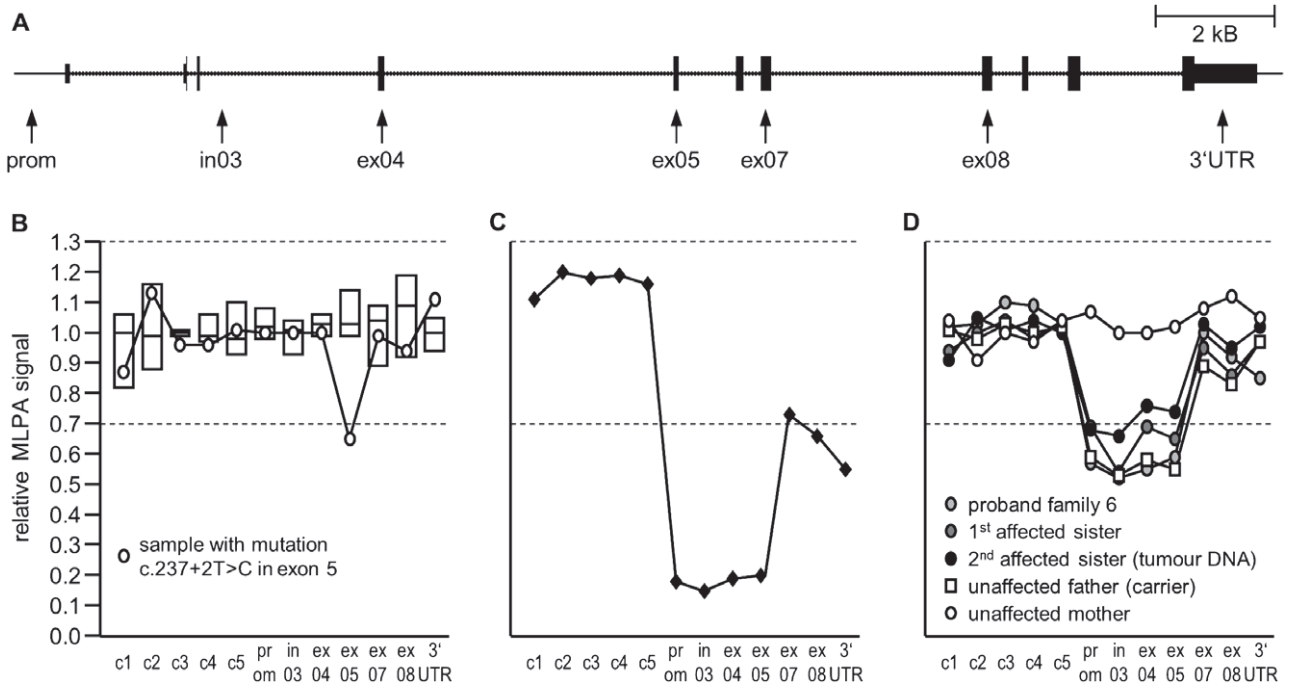

Figure 2. MLPA-based determination of *SMARCE1* copy number. (A) Schematic diagram of the human *SMARCE1* gene (NM_003979): exons are depicted as vertical bars; arrows denote the target sites of MLPA probes; ex, exon; in, intron; prom, promoter; 3′ UTR, 3′ untranslated region. (B) Exemplary negative MLPA findings and finding on a sample carrying a known nucleotide substitution: boxes visualize maximum, median and minimum values for eight samples found to not have altered *SMARCE1* copy number; values for a positive control sample, which carries a single nucleotide substitution at the ligation site for the exon 5 MLPA probes, are depicted by open circles; stippled horizontal lines mark the 0.7–1.3 range, outside of which signals are considered aberrant. (C) Large deletion with loss of the wild-type allele in a tumour sample for which no corresponding germline DNA was available. (D) Large deletion in a family, including germline DNA from the proband and one affected sister, tumour DNA from a second affected sister and germline DNA from her unaffected parents.

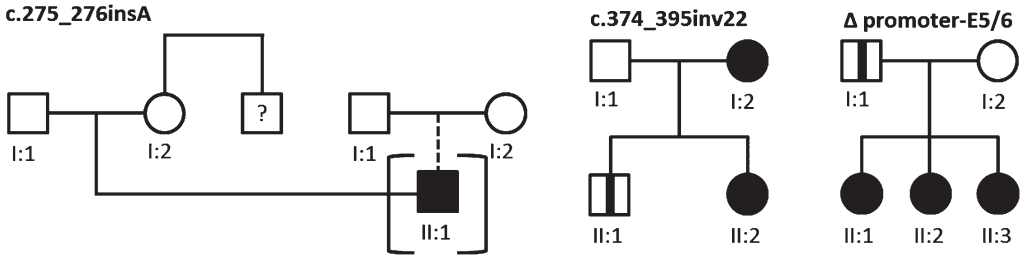

Figure 3. Pedigrees of confirmed germline *SMARCE1* mutation carriers: black stripe, unaffected mutation carrier; ?, uncertain whether affected.

with SMARCE1 loss was from a patient with a clinical diagnosis of NF2, with multiple schwannomas and meningiomas but no germline *NF2* mutation. No *SMARCE1* mutation was identified in germline DNA, indicating that the observed protein loss in tumour is probably due to a somatic mutation, although analysis of tumour DNA was not possible. No other tumours from this patient were available for analysis. The two remaining SMARCE1 protein-negative meningiomas were single tumours and no DNA was available for mutation testing.

Overall, we identified seven novel *SMARCE1* mutations in clear cell meningioma patients, three of which were confirmed in the germline (Table 1). Four of these were identified in individuals with spinal menigniomas. However, the other three individuals had cranial meningiomas.

One of three female siblings with spinal clear cell meningiomas, who inherited a large deletion from their unaffected father, also developed a cranial meningioma. An unrelated female developed a cranial meningioma at the age of 14 years. Her mother is now also known to have asymptomatic cranial meningiomas on MRI scan. The proband's brother also carries the mutation, although he remains clinically unaffected at 17 years of age. One mutation was found in the cranial tumour of a 10 year-old boy with an affected uncle, presumed to have NF2 disease, although no germline *NF2* mutation was identified. No lymphocyte DNA was available for *SMARCE1* analysis; therefore the germline status of this mutation remains uncertain.

All *SMARCE1* mutation-positive males in this study developed tumours between the ages of 2 and 10 years, with the exception of the two clinically unaffected

males, who remain free of symptoms at 17 and 70 years of age, respectively (Table 1). The mutation-positive females developed tumours between ages 14 and 30 years. This supports our previous observation that there is incomplete penetrance of meningioma disease in males, but that those who do develop menigiomas tend to develop them at an earlier age than females. The reason for this is still unclear, but may reflect a hormonal stimulus to meningioma growth. In summary, we have shown that SMARCE1 protein loss occurs in cranial as well as spinal meningiomas, demonstrating that it is the histological subtype, rather than tumour location, that is determined by *SMARCE1* mutations.

Mutation identification can be used to define risks in relatives and improve classification of risk categories in terms of likely location and histology of meningiomas. Clear cell meningiomas are classed as grade II, due to an apparent high recurrence rate, in comparison to grade I tumours. However, natural history studies are required to define definitive recurrence risks so that appropriate surveillance schedules can be established. Initial data in our small series of *SMARCE1*-positive cases indicate that the recurrence risk for these tumours is low. It is also uncertain whether clear cell meningiomas with somatic SMARCE1 loss occurring in the context of NF2 follow a different natural history to those due to germline *SMARCE1* mutations. Further work is required to delineate the mechanism of *SMARCE1*-associated meningioma development.

Acknowledgements

This study was supported by a Young Investigator Award from the Children's Tumor Foundation (to MJS) and a project grant from the Association for International Cancer Research (to DGE, WGN and MJS). The Centre for Genomic Medicine is supported by the Manchester Biomedical Research Centre. We would also like to thank all the patients who provided samples for the study.

Author contributions

MJS, WGN and DGE conceived the study design and wrote the first draft, which was edited by all authors; and MJS, AJW, CBennet, MH, EED, LTE, WFH, JvH, DB, AL, RFH CBeetz, DdP, JPK, WGN and DGE collected and analysed the data. All authors approved the final manuscript.

References

1. Mulvihill JJ, Parry DM, Sherman JL, *et al.* NIH conference. Neurofibromatosis 1 (Recklinghausen disease) and neurofibromatosis 2 (bilateral acoustic neurofibromatosis). An update. *Ann Intern Med* 1990; **113:** 39–52.
2. Wellenreuther R, Kraus JA, Lenartz D, *et al.* Analysis of the neurofibromatosis 2 gene reveals molecular variants of meningioma. *Am J Pathol* 1995; **146:** 827–832.
3. Lekanne Deprez RH, Bianchi AB, Groen NA, *et al.* Frequent *NF2* gene transcript mutations in sporadic meningiomas and vestibular schwannomas. *Am J Hum Genet* 1994; **54:** 1022–1029.
4. Schmitz U, Mueller W, Weber M, *et al. INI1* mutations in meningiomas at a potential hotspot in exon 9. *Br J Cancer* 2001; **84:** 199–201.
5. Hadfield KD, Smith MJ, Trump D, *et al. SMARCB1* mutations are not a common cause of multiple meningiomas. *J Med Genet* 2010; **47:** 567–568.
6. Smith MJ, O'Sullivan J, Bhaskar SS, *et al.* Loss-of-function mutations in *SMARCE1* cause an inherited disorder of multiple spinal meningiomas. *Nat Genet* 2013; **45:** 295–298.
7. Aavikko M, Li SP, Saarinen S, *et al.* Loss of *SUFU* function in familial multiple meningioma. *Am J Hum Genet* 2012; **91:** 520–526.
8. van den Munckhof P, Christiaans I, Kenter SB, *et al.* Germline *SMARCB1* mutation predisposes to multiple meningiomas and schwannomas with preferential location of cranial meningiomas at the falx cerebri. *Neurogenetics* 2012; **13**: 1–7.
9. Jagani Z, Mora-Blanco EL, Sansam CG, *et al.* Loss of the tumor suppressor *Snf5* leads to aberrant activation of the Hedgehog–Gli pathway. *Nat Med* 2010; **16:** 1429–1433.
10. Clark VE, Erson-Omay EZ, Serin A, *et al.* Genomic analysis of non-*NF2* meningiomas reveals mutations in *TRAF7*, *KLF4*, *AKT1*, and *SMO*. *Science* 2013; **339:** 1077–1080.
11. Brastianos PK, Horowitz PM, Santagata S, *et al.* Genomic sequencing of meningiomas identifies oncogenic *SMO* and *AKT1* mutations. *Nat Genet* 2013; **45:** 285–289.
12. Bauer P, Stevanin G, Beetz C, *et al.* Spinocerebellar ataxia type 11 (SCA11) is an uncommon cause of dominant ataxia among French and German kindreds. *J Neurol Neurosurg Psychiat* 2010; **81:** 1229–1232.
13. Beetz C, Nygren AO, Schickel J, *et al.* High frequency of partial *SPAST* deletions in autosomal dominant hereditary spastic paraplegia. *Neurology* 2006; **67:** 1926–1930.