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CORRECTION

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There were two errors in a paper published in the June issue of the journal (Hadfield KD, Newman WG, Bowers NL, Wallace A, Bolger C, Colley A, McCann E, Trump D, Prescott T, Evans DGR**.** Molecular characterisation of *SMARCB1* and *NF2* in familial and sporadic schwannomatosis. *J Med Genet* 2008;**45**:332–9). ''c.1032-12C>G'' should be ''c.1119-12C>G'' and ''p.G29_530del'' should be ''p.G29_S30del'' both in table 2 and elsewhere in the text. A corrected PDF is available at http://jmg.bmj.com/supplemental.

Molecular characterisation of *SMARCB1* and *NF2* in familial and sporadic schwannomatosis

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ABSTRACT

Background: Schwannomatosis is a rare condition characterised by multiple schwannomas and lack of involvement of the vestibular nerve. A recent report identified bi-allelic mutations in the *SMARCB1/INI1* gene in a single family with schwannomatosis. We aimed to establish the contribution of the *SMARCB1* and the *NF2* genes to sporadic and familial schwannomatosis in our cohort.

Methods: We performed DNA sequence and dosage analysis of *SMARCB1* and *NF2* in 28 sporadic cases and 15 families with schwannomatosis.

Results: We identified germline mutations in *SMARCB1* in 5 of 15 (33.3%) families with schwannomatosis and 2 of 28 (7.1%) individuals with sporadic schwannomatosis. In all individuals with a germline mutation in *SMARCB1* in whom tumour tissue was available, we detected a second hit with loss of *SMARCB1*. In addition, in all affected individuals with *SMARCB1* mutations and available tumour tissue, we detected bi-allelic somatic inactivation of the *NF2* gene. *SMARCB1* mutations were associated with a higher number of spinal tumours in patients with a positive family history ($p = 0.004$).

Conclusion: In contrast to the recent report where no *NF2* mutations were identified in a schwannomatosis family with *SMARCB1* mutations, in our cohort, a four hit model with mutations in both *SMARCB1* and *NF2* define a subset of patients with schwannomatosis.

Schwannomatosis, the occurrence of multiple benign tumours of Schwann cells (schwannomas) (MIM 162091), was first described by a Japanese group in 1973.[1] Subsequent studies delineated schwannomatosis as an entity distinct from neurofibromatosis type 2 (NF2, MIM 101000). NF2 is characterised by multiple schwannomas including vestibular schwannomas of the eighth cranial nerve and germline *NF2* mutations.[2] Vestibular schwannomas are considered an exclusion criterion for schwannomatosis.[3] Schwannomas can arise wherever Schwann cells occur, in the spinal cord and along peripheral and cranial nerves. The tumours manifest most commonly with pain and/or neurological deficit.

Some patients with multiple non-vestibular nerve schwannomas (schwannomatosis) and a negative family history are mosaic for a *NF2* mutation.[3–6] In contrast, a subgroup of patients, in whom tumours are largely confined to the peripheral nerves, do not have an underlying *NF2* mutation.[7] These individuals may pass the condition on to their children, and in families where this occurs there is tight linkage to the NF2 locus.[7 8] However, in two large families with schwannomatosis the locus has been shown to be located in a 4cM region on chromosome 22q11 between the DiGeorge locus and the *NF2* gene.[9] Analysis of this region has identified copy number variants in the *GSTT1* gene and missense variants in the *CABIN* gene in patients with schwannomatosis, but a definitive causal relationship has not been established.[10] Recent candidate gene screening of the region identified a germline nonsense mutation p.Q12X in exon 1 of the *SMARCB1 (INI1)* gene in a father and daughter with schwannomatosis.[11] In addition to the germline mutation, a nonsense mutation (p.Q182X) in exon 5 of *SMARCB1* on the putative normal allele was detected in one tumour from the father.[11] The loss of the second allele correlated with a lack of SMARCB1 protein measured by immunohistochemical staining. This suggested that *SMARCB1* acts as a tumour suppressor gene, and that loss of both functional alleles is required for the schwannomatosis phenotype to manifest. Furthermore, no mutations were identified in *NF2* in the germline or in tumours of the affected individuals in the original report,[11] suggesting that mutations in *SMARCB1* can effect the development of schwannomas independently of *NF2*.

SMARCB1 encodes a member of the chromatin remodelling SWI/SNF multiprotein complexes and had previously been excluded as a candidate for schwannomatosis.[12] However, in that previous study only exons two to eight of the nine exons of the *SMARCB1* gene were analysed in 23 schwannomas. Moreover, these schwannomas may not have been from patients with schwannomatosis.[11]

Somatic mutations in *SMARCB1* have also been identified in rhabdoid tumours (MIM 609322), atypical teratoid tumours, choroid plexus carcinomas, medulloblastomas, central primitive neuroectodermal tumours, and meningiomas.[13–15] Constitutional *SMARCB1* mutations are also the cause of inherited predisposition to rhabdoid tumours.[14] In contrast to schwannomatosis, rhabdoid tumours are highly malignant and usually occur in children younger than 2 years of age. However, both tumour types are characterised by bi-allelic, somatic alterations leading to complete loss of function of *SMARCB1*. Therefore, we were keen to establish the contribution of *SMARCB1* and *NF2* mutations to the phenotype in our cohort with sporadic and familial schwannomatosis.

PATIENTS AND METHODS

Patients

The diagnosis of schwannomatosis was made in accordance with published clinical criteria.[16 17]

Table 1 Clinical characteristics of familial and sporadic schwannomatosis cases

Schwannomatosis patients	Median age (years) at disease onset (range)	Median number of spinal tumours (range)	Median number of subcutaneous tumours (range)	Median number of cranial nerve tumours (range)
Familial (n = 29)	30.5 (9–54)	1 (0–7)	1 (0–8)	1* (0–1)
Sporadic (n = 28)	29.5 (14–62)	5 (0–8)	3 (0–30)	1* (0–1)

*Four familial cases had single cranial nerve schwannomas (2 trigeminal, 2 IX nerve); 5 sporadic cases had single cranial nerve tumours (2 trigeminal, 3 facial nerve).

Fifteen families with multiple affected family members were screened negative for *NF2* germline mutations in at least one family member by sequencing and multiple ligation dependent probe amplification (MLPA). Additionally, 28 sporadic cases with at least three schwannomas (at least one histologically proven) in more than one body segment, were also screened negative for germline *NF2* mutations. Lymphocyte or tumour DNA from the 28 individuals with sporadic schwannomatosis (that is, no family history, and unaffected parents) and affected individuals from the youngest generation of 15 families with multiple affected family members (see table 1 for clinical details) was screened for mutations. Approval for the study was provided by the local ethics committee.

Mutation analysis

Mutation analysis was performed on genomic DNA extracted from blood lymphocytes and/or tumour tissue. Polymerase chain reaction (PCR) amplification of *SMARCB1* exons 1–9 was performed using the oligonucleotide primers listed in supplementary table 1, designed from the gene sequence (Genbank Accession NC_000022.9) to include the whole exon and flanking intronic sequence. PCR products were analysed by direct bi-directional sequencing using the ABI Prism 3100 sequence analyser (Applied Biosystems, Warrington, UK). Nucleotide positions of reported mutations are numbered according to the mRNA coding sequence U04847. *NF2* mutation and exonic deletion screening was performed as previously described.[18 19]

cDNA preparation and cloning

Total RNA was isolated from tumour tissue or blood lymphocytes using Trizol reagent according to the manufacturer's protocol (Invitrogen Life Technologies, Paisley, UK). cDNA was prepared by reverse transcription of 1 μg RNA using random hexamers (Promega, Southampton, UK) and the *SMARCB1* coding sequence was amplified using primers *SMARCB1*exon1cF and *SMARCB1*exon9cR listed in supplementary table 2. *SMARCB1* cDNA products were cloned into the pCR2.1 TOPO vector (Invitrogen) and resulting plasmid DNA was sequenced using M13 and *SMARCB1* specific primers designed to span exon–exon boundaries to obtain full length sequences (supplementary table 2). *SMARCB1*ex2/3cR2 and *SMARCB1*ex2/3cR were designed against the *SMARCB1* transcript variants type 1 (NM_003073) and 2 (NM_001007468), respectively.

Exon copy number analysis

SMARCB1 exon deletions or amplifications were identified using a quantitative real-time PCR (TaqMan) assay, modified from Kohashi *et al*.[20] Real-time PCR was performed using the ABI Prism 7900 sequence detection system (Applied Biosystems). Amplification reactions were performed in duplicate and in a final reaction volume of 25 μl, containing 50 ng genomic DNA, 1xSYBR Green PCR Master Mix (Applied Biosystems) and 3.5 pmol specific forward and reverse primers. Cycling conditions for all exons and the housekeeping gene GAPDH were: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Quantification was performed using the standard curve method. For each assay a standard curve was generated using 1:2 serial dilutions of a standard quantity of genomic DNA (calibrator). Assuming that test samples and the calibrator have two copies of GAPDH, the exon dosage ratio was calculated as the average copy number of target exon/average copy number reference gene (GAPDH). All exon dosage ratios were normalised against the normal diploid control DNA (male genomic DNA) to give the exon dosage ratio.

Determination of loss of heterozygosity

Loss of heterozygosity (LOH) at 22q was investigated using microsatellite markers D22S303, D22S310, D22S446, D22S449, D22S1174, D22S275, NF2CA3, and D22S268. PCR reactions were performed using FAM labelled oligonucleotide primers and products were analysed on an ABI 3100 automated sequencer (Applied Biosystems).

RESULTS

We screened blood and, where possible, tumour DNA from 15 familial and 28 sporadic cases for the presence of *SMARCB1* mutations, including copy number changes. Where available, additional family members of patients with identifiable mutations were screened, to ensure segregation of the mutation with the disease phenotype. All *SMARCB1* and *NF2* mutations are summarised in table 2.

SMARCB1 analysis

Novel germline *SMARCB1* mutations were identified in five of the 15 (33.3%) families and in two individuals from the 28 (7.1%) sporadic cases. The putative mutations were not present in databases of genomic variation or in a panel of at least 50 healthy control individuals (100 for exon 1). The five familial cases included a nonsense mutation (c.46A>T, p.K16X) in exon 1; a 7bp deletion (c.233-2_237delagATCAC) involving the splice acceptor site of exon 3; and three missense mutations in exons 1 (c.41C>A, p.P14H), 7 (c.864C>G, p.N288K) and 8 (c.1106A>T, p.D369V). In the three familial cases for whom segregation analysis could be done (families 1–3), the mutation segregated with the disease implicating these mutations as causative. Where missense mutations were observed, the predicted wild type amino acid residue was highly conserved between the human, chimp, mouse, bovine, chick, Xenopus, zebra fish, and Drosophila species (table 3). In-silico analysis of the missense variants, using the Polyphen (*Poly*morphism *Phen*otyping) prediction tool (www.genetics.bwh.harvard.edu/pph),[21] showed that each substitution was likely to be damaging to the function of *SMARCB1*. Importantly, dosage analysis revealed no germline exonic deletions in *SMARCB1* in any germline sample, familial or sporadic.

Table 2 *SMARCB1* and *NF2* mutations identified in familial and sporadic schwannomatosis cases

Family	Patient	*SMARCB1* in germline	Location	Putative effect of mutation	*SMARCB1* in tumour ("second hit")	*NF2* in tumour
1	I-1	c.233-2_237delagATCAC	Exon 3	SP	NA	NA
	II-1	c.233-2_237delagATCAC	Exon 3	SP	Loss *SMARCB1*	c.1148del35bp, deletion of *NF2*
	II-2	NA	Exon 3	NA	Loss *SMARCB1*	c.305del, deletion of *NF2*
	II-3	c.233-2_237delagATCAC	Exon 3	SP	Loss *SMARCB1*	c.1531G>A;1536_1563del, deletion of *NF2*
2	II-1	c.864C>G p.N288K	Exon 7	Missense	Evidence for loss *SMARCB1*	c.600-2A>G, evidence for *NF2* deletion
		c.1119-12C>G	Intron 8	SP		
	I-1	c.864C>G p.N288K	Exon 7	Missense	NA	NA
		c.1119-12C>G	Intron 8	SP		
	II-2	c.864C>G, p.N288K	Exon 7	Missense	Loss SMARCB1	c.1038G>T, deletion of *NF2*
		c.1119-12C>G	Intron 8	SP		
3	1,2,3,4	c.41C>A, p.P14H	Exon 1	Missense	NA	NA
4	5	c.46A>T, p.K16X	Exon 1	Nonsense	NA	NA
5	6	c.1106A>T, p.D369V	Exon 8	Missense	Loss *SMARCB1*	Exon2del, deletion of *NF2*
6	7	–			Loss *SMARCB1*	c.1571_72delAA, deletion of *NF2*
	8	–			Loss *SMARCB1*	c.531T>A, deletion of *NF2*
Sporadic	9	c.86_91delGCTCCG, p.G29_S30del	Exon 1	In-frame deletion	NA	NA
Sporadic	10	c.86_91delGCTCCG, p.G29_S30del	Exon 1	In-frame deletion	NA	NA

SP (splicing) represents mutation predicted to disrupt transcript splicing. NA indicates blood/tumour tissue was not available. – indicates no mutations were identified in these samples. No germline *NF2* mutations were detected.

Table 3 Multiple species alignments of *SMARCB1/INI1* sequence

(A)	*
Human	TFGQKPVKFQ
Mouse	TFGQKPVKFQ
Chick	TFGQKPVKFQ
Xenopus	TFGQKPVKFQ
Drosophila	TYGDKPVAFQ
Bovine	TFGQKPVKFQ
Chimp	TFGQKPVKFQ
Zebrafish	----------
(B)	*
Human	DMSEKENSPEKF
Mouse	DMSEKENSPEKF
Chick	DMSEKENSPEKF
Xenopus	DMSEKENSPEKF
Drosophila	DMSEKNNNPEEF
Bovine	DMSEKENSPEKF
Chimp	DMSEKENSPEKF
Zebrafish	DMSEKENSPESF
(C)	*
Human	PLLETLTDAEMEKK
Mouse	PLLETLTDAEMEKK
Chick	PLLETLTDAEMEKK
Xenopus	PLLETLTDAEMEKK
Drosophila	PFLETLTDAEMEKK
Bovine	PLLETLTDAEMEKK
Chimp	PLLETLTDAEMEKK
Zebrafish	PLLETLTDAEMEKK

Segments shown include the amino acid residues at which missense mutations occurred (*) (A) P14H (B) N288K (C) D369V.

In family 1 (pedigree shown in fig 1A), sequencing the constitutional DNA of the proband (II-1) revealed a 7bp deletion (c.233-2_237delagATCAC) at the start of exon 3, which deleted the splice acceptor site (fig 1Bi, ii). This mutation was also identified in the germline of the proband's affected father (I-1) and brother (II-3) and in a tumour from the proband's sister (II-2). Analysis of cDNA from the tumour of patient II-2 revealed the creation of a new splice site, inserting 83bp of intronic sequence (fig 1C) and creating a frameshift mutation with a premature termination stop codon at amino acid 71 (p.69DfsX71) (fig 1D). Dosage analysis confirmed that exons 1-8 of the normal allele were deleted in the tumours of patients II-1, II-2 and II-3. Microsatellite analysis suggestive of LOH and MLPA analysis indicating a deletion of *NF2* were compatible with a loss of the long arm of chromosome 22.

Molecular analysis of *SMARCB1* in family 2 (pedigree shown in fig 2A) identified two possible causative mutations occurring on the same allele in the paired blood and tumour samples of the proband (II-1), in his brother (II-2), and in the blood of their father (I-1). A heterozygous point mutation in exon 7 (c.864C>G) was identified in the blood and tumour of affected family members and is predicted to substitute an asparagine with a lysine residue (p.N288K). In addition, in all three affected members of this family, a heterozygous C>G base change was observed 12bp upstream of the start of exon 9 in germline DNA (c.1119-12C>G) (fig 2Bi, ii). This change is predicted by the BDGP:splice site prediction by Neural Network (www.fruitfly.org/cgi-bin/seq_tools/splice.pl) to introduce an alternative splice acceptor site. The insertion of 11 bases of intronic sequence in the alternative transcript would introduce a frameshift mutation resulting in a novel stop codon (p. R373fsX379) (fig 2Biii). Although we were unable to confirm the transcribed sequence, due to lack of tumour material for RNA extraction and analysis, it is more likely that c.1119-12C>G is the causative mutation

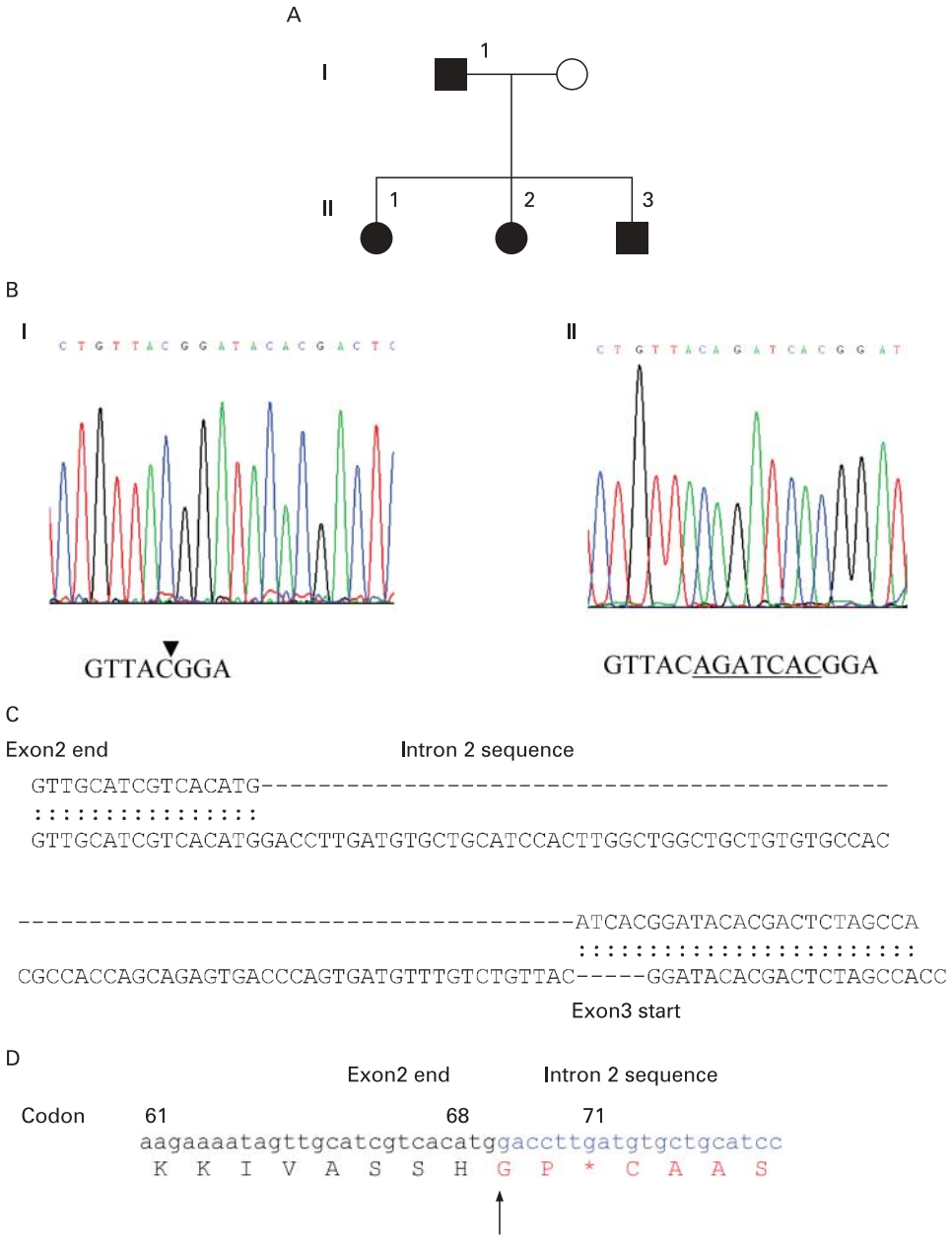

Figure 1 *SMARCB1* mutation identified in family 1. (A) Family 1 pedigree. (B) Sequence analysis of constitutional and tumour DNA of family 1 revealed a heterozygous deletion in exon 3. Sequence of cloned mutant (i) and normal (ii) alleles in blood DNA of patient II-1 confirmed a 7bp deletion (c.233-2_237delagATCAC) (ii). Arrow and underlining indicate position and sequence of deletion, respectively. (C) Alignment of cDNA sequences from tumour of patient II-2 with normal control. Deletion of the splice acceptor site creates an alternative splice site resulting in insertion of intronic sequence. (D) Inserted intronic sequence (arrow) introduces a premature termination signal at codon 71 (*).

with c.864C>G present as a rare polymorphism. In the schwannoma of patient II-2, there is complete loss of *SMARCB1* by dosage analysis, which is supported by the observed LOH for the intron 8 mutation and variant rs2070457 in the tumour (fig 2Bii, Cii). This contrasts with the dosage and microsatellite analysis for the tumour of patient II-1, which showed only a tendency towards LOH for markers around both the schwannomatosis and NF2 loci. This may be explained by admixture of non-tumour tissue in the sample from which the DNA was extracted.

In another familial case (family 5, patient 6) a germline missense mutation in exon 8 of *SMARCB1* was identified (c.1106A>T, p.D369V) and tumour tissue was available to establish a somatic loss of the normal allele on dosage and by LOH. The missense change removes an exonic splice enhancer (fig 3) (rulai.cshl.edu/tools/ESE/).[22] If intron 8 is not removed by splicing, a frameshift mutation is introduced (p.374RfsX417).

Two of the 28 unrelated patients with sporadic schwannomatosis had the same germline mutation in *SMARCB1*, a deletion of 6bp at the end of exon 1 (c.86_91delGCTCCG). This deletion is predicted to result in an in-frame deletion of two amino acid residues (p.G29_S30del). Unfortunately, tumour tissue was unavailable for analysis in the two sporadic cases with germline mutations. No *SMARCB1* mutations were detected in tumour DNA from four of the other 26 sporadic cases without germline mutations.

NF2 analysis

In all the individuals where adequate tumour DNA was available for analysis, screening of the *NF2* gene was undertaken. In each of the patients with a germline mutation and somatic involvement of *SMARCB1*, both alleles of *NF2* were also affected by somatic mutation and loss of the *NF2* locus in the tumour. In the tumour available from patient II-1 in family 1 a 35bp deletion in exon 12 was detected (c.1148del35bp) which has not been previously described in schwannomas. A single base pair deletion (c.305delC) in exon 3, previously reported as a somatic mutation in vestibular schwannoma,[23]

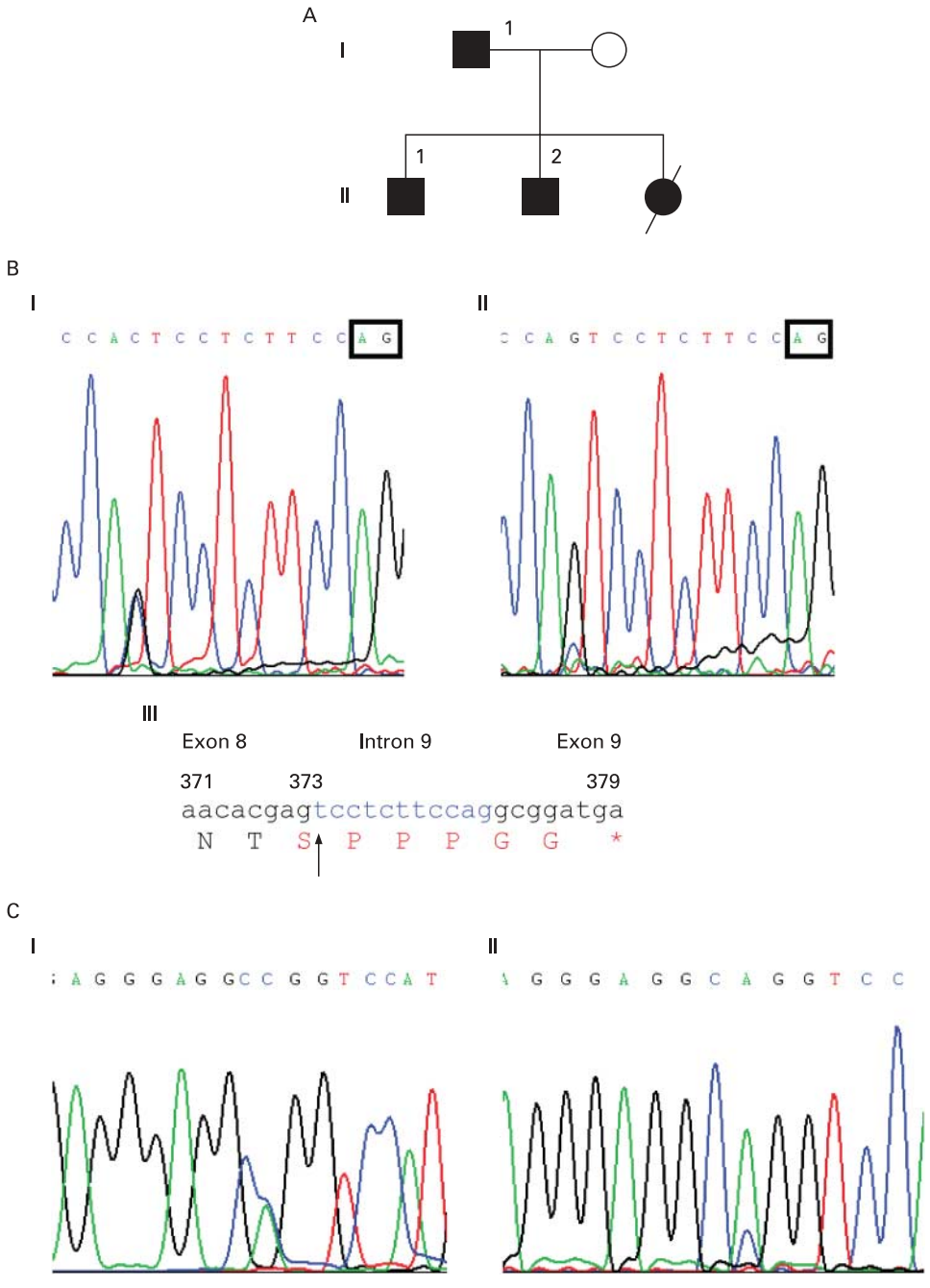

Figure 2 *SMARCB1* mutations in family 2. (A) Family pedigree. (B) *SMARCB1* heterozygous mutation 12bp upstream of exon 9 (c.1119-12C>G) identified in constitutional (i) and tumour (ii) DNA of family 2 members. The mutation potentially introduces a new splice acceptor site thereby inserting 11bp of intronic sequence. Box indicates normal splice site at start of exon 9. (iii) Inserted intronic sequence (arrow) introduces a premature termination signal at codon 379 (*) (C) Heterozygous single nucleotide polymorphism (SNP rs2070457 (C>A) within intron 5 of *SMARCB1* identified in blood (i) and tumour (ii) DNA of family 2 members. In both Bii and Cii sequence data of tumour DNA from patient II-2 indicates almost complete loss of the wild-type allele in each case.

was identified in the tumour from patient II-2 in family 1. In the tumour from patient II-3, a novel complex missense/frameshift deletion, in exon 14, was observed (c.1531G>A;1536_1563del). Other mutations within the same region of exon 14 have been described in schwannomatosis[7] and vestibular schwannoma.[24 25] The other allele was lost in tumour tissue from all three siblings in family 1 (II-1, -2,-3). Similar findings were noted in tumour tissue from patients II-1 and II-2 in family 2, with a splice variant in intron 6 (c.600-2A>G) and a single point mutation (c.1038G>T) in exon 11, respectively. Both showed loss of the other *NF2* allele. Loss of exon 2 and the normal allele was observed in tumour from patient 6. Microsatellite analysis indicated LOH for the long arm of chromosome 22 in each of the six tumours.

NF2 analysis in tumours from a family without a *SMARCB1* mutation

NF2 sequence analysis of tumour tissue from family 6 revealed a 2bp deletion in exon 14 in patient 7 (c.1571_72delAA) and an exon 6 point mutation in patient 8 (c.531T>A), with loss of the normal allele in both. It is of note that these patients did not, however, have germline *SMARCB1* mutations, although dosage analysis of *SMARCB1* showed these tumours to have somatic loss of one allele.

Phenotype of patients with *SMARCB1* and *NF2* mutations

We compared the clinical characteristics of patients with sporadic and familial schwannomatosis in our cohort with: *SMARCB1* and *NF2* mutations (the latter only in tumours) and

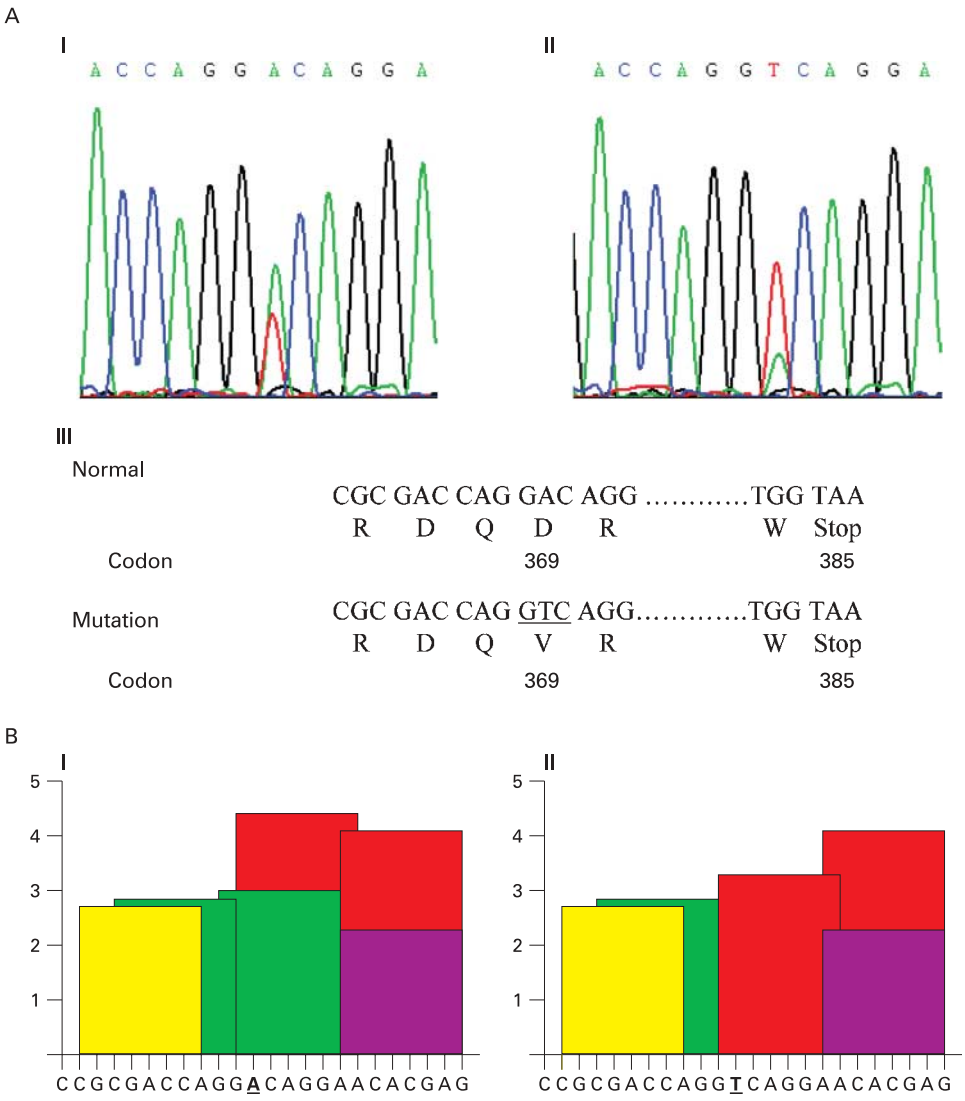

Figure 3 *SMARCB1* mutation identified in family 5. (A) Sequence analysis of *SMARCB1* exon 8 in (i) blood and (ii) tumour DNA of patient 6. Evidence of loss of heterozygosity in the tumour DNA is shown with significant loss of the wild-type A allele. (iii) A single base change (A>T) is predicted to result in a missense mutation at codon 369 (pD369V). (B) Graphical representations of putative exonic splicing enhancer (ESE) motifs within the sequence near to and including the missense mutation in exon 8. Four different serine/arginine rich (SR) proteins that recognise specific ESEs are represented; SF2/ASF (red), SF2/ASF (IgM-BRCA1) (pink), SRp40 (green), SRp55 (yellow). Mutation of the wild-type A allele (i) to a T (ii) removes the putative binding sites for one SRp40 protein and one SF2/ASF (IgM-BRCA1) protein.

with no identifiable mutation in *SMARCB1*. Interestingly, there was a significantly greater number of spinal schwannomas in the familial group with *SMARCB1* and *NF2* mutations compared to the patients without mutations (median 2.5 vs 0.5, $p = 0.004$, Mann–Whitney U test). There were no differences in age of onset or in distribution or number of subcutaneous schwannomas between the groups.

Of the two large schwannomatosis families in our previous report of linkage to *NF2* in 1997,[8] family C (family 3 in this report) had eight affected family members. One individual with an unbiopsied subcutaneous lump aged 35 years had been ascribed affected status on the basis of *NF2* linkage. However, he did not have the family *SMARCB1* mutation and craniospinal MRI was normal. He therefore represents a phenocopy with a recombination between *NF2* and *SMARCB1*. One female family member had a biopsy proven spinal meningioma aged 53 years and another, a ninth cranial nerve schwannoma, that required craniotomy aged 37 years. Family M that contained 1/5 affected individuals with a unilateral vestibular schwannoma (the family, but not the individual, fulfilled schwannomatosis criteria) did not have a *SMARCB1* mutation. Cranial imaging in the other four affected members revealed no vestibular schwannomas at 50–75 years, despite multiple spinal and peripheral nerve schwannomas.

Family 2, reported here with *SMARCB1* and *NF2* mutations, had an additional affected family member (see Pedigree in fig 2A) who died at the age of 17 from a malignant peripheral nerve sheath tumour, which has not previously been described in schwannomatosis. We were unable to test whether this patient had the family *SMARCB1* mutation, although she did have seven spinal schwannomas. No other family affected with a *SMARCB1* mutation had a vestibular schwannoma, meningioma or ependymoma, which are the other typical tumours in NF2.

DISCUSSION

Our study confirms the role of *SMARCB1* in the pathogenesis of sporadic and familial schwannomatosis. In the original study, which identified germline and somatic *SMARCB1* mutations in a single family with schwannomatosis, there were no identifiable *NF2* mutations in tumour tissue.[11] In contrast, in our patients with germline *SMARCB1* mutations, where tumour tissue was available, we also identified bi-allelic somatic involvement of *NF2* suggesting a "four-hit" mechanism. The germline *SMARCB1* mutations, and the somatic loss of the normal allele in the tumour, identified in our study, support the original report that a loss of function of *SMARCB1* is the

pathogenic mechanism.[11] However, the involvement of *NF2* in our patient cohort may explain the development of schwannomatosis rather than the more aggressive malignant rhabdoid tumour phenotype, which has previously been associated with bi-allelic somatic loss of *SMARCB1*.[13 14] It is still intriguing that families with rhabdoid tumours due to germline *SMARCB1* mutations have not been described with non-vestibular schwannomas and that schwannomatosis families have not had rhabdoid tumours. Involvement of exon 1 in a number of our families and missense mutations in others may to some extent explain the difference in tumour disposition. An early truncating mutation would give rise to little if any protein product and a missense mutation could be partially functional as in NF2.[19] Later truncating mutations may have a dominant negative effect leading to a more severe phenotype as also seen in NF2 and APC.[19 26] However, further studies are required to establish the reasons for such pronounced phenotypic discordance. In addition, our study is the first to report a germline mutation in two sporadic cases of schwannomatosis. The fact that these two unrelated cases share the same mutation is interesting and future studies will determine if this is a hotspot for mutation in schwannomatosis. The finding that only a third of familial cases and approximately 7% of sporadic schwannomatosis cases have *SMARCB1* mutations indicates that other genes are involved in the pathogenesis of this condition. Further studies are required to establish whether other mechanisms, including somatic hypermethylation with loss of function of *SMARCB1* or mutation of genes, which encode proteins that interact with the chromatin-remodelling SWI/SNF multiprotein complex, contribute to the pathogenesis of schwannomatosis.

A possible mechanism for the *SMARCB1* related subset of schwannomatosis is that loss of the normal copy of the gene by loss of chromosome 22 or at least loss of the long arm that includes the *NF2* locus, leads to some degree of Schwann cell proliferation. This increases the likelihood of a somatic mutation in the remaining *NF2* allele, which would lead to schwannoma development. Why this does not target the vestibular nerve, as in NF2, remains to be determined. It is nonetheless notable that this was the mechanism in all six available tumours and none demonstrated either mitotic recombination (a common mechanism in schwannomatosis)[27] or somatic mutation in both NF2 alleles (common in NF2).[28]

Tumour tissue for *NF2* mutation analysis was unavailable from families 3 and 4 and from the two sporadic schwannomatosis patients. We could therefore not establish if germline *SMARCB1* mutations consistently lead to schwannomatosis by this mechanism. The failure of the original report to identify the mechanism could in part be explained by the mosaic appearance of chromosome loss in the tumours for SMARCB1 staining.[11] This would suggest that there was contamination with normal material perhaps masking loss of the *NF2* locus. However, identification of a somatic truncating mutation in one tumour from their report suggests that at least some *SMARCB1* related schwannomas could be caused by other mechanisms than loss of the second *SMARCB1* allele. Nonetheless, a further recent report of a single patient with the same four hit mechanism in two tumours does support this as the usual mechanism of schwannoma development via *SMARCB1*.[29] This adds to the debate on development of the two hit model.[26] The present report shows that four hits are usually necessary to develop schwannomas in schwannomatosis, adding to the three hits that sometimes occur in APC and TP53.[26]

Importantly, patients with familial disease were more likely to have a greater number of spinal tumours; however, this correlation does not allow for targeting of mutation analysis towards a subset of individuals with schwannomatosis in whom *SMARCB1* mutations are more likely. The tumour spectrum in *SMARCB1* does include predisposition to cranial nerve schwannomas and potentially meningioma as demonstrated by family 3 and malignant peripheral nerve sheath tumour. However, more reports are required before reliable estimates can be made of the risk of other tumour types that occur as part of NF2.

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