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Familial occurrence of schwannomas and malignant rhabdoid tumour associated with a duplication in *SMARCB1*

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Abstract

Background—The role of germline and somatic *SMARCB1* gene mutations in malignant rhabdoid tumour (MRT) predisposition is well known. Germline *SMARCB1* mutations have also recently been identified in a subset of individuals with schwannomatosis. Surprisingly, MRT predisposition and schwannomatosis have never been reported to co-occur in a family. The correlation between genotype and phenotype for mutations in *SMARCB1* has not been determined.

Results—We have identified a germline 2631 bp duplication that includes exon 6 of *SMARCB1* in a unique family with a four generation history of MRT predisposition and schwannomatosis. This duplication segregates with disease in individuals affected with both conditions, linking MRT predisposition and schwannomatosis as components of the same syndrome in this family.

Conclusion—The unique combination of tumours that result from the duplication described in this report may provide important clues about the mechanisms that influence the phenotype associated with a given *SMARCB1* mutation.

Malignant rhabdoid tumour (MRT) predisposition (OMIM 609322) and schwannomatosis (OMIM 162091) are unique conditions that predispose to distinct tumour types. Autosomal dominant inheritance of each condition has been reported, although both most frequently occur sporadically. 1–5 MRT is an aggressive paediatric malignancy with a poor prognosis that commonly occurs in kidney, brain and soft tissue. In the central nervous system, it is termed atypical teratoid/rhabdoid tumour (AT/RT) reflecting the mixed cellular elements present in the tumours.^{6,7} MRT predisposition results from germline loss of function mutations in the

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SMARCB1 (also known as *INI1*, *hSNF5* and *BAF47*) tumour suppressor gene at 22q11.23.⁸ The Smarcb1 protein is a component of the SWI/SNF complex, which regulates gene transcription through remodelling chromatin.⁹ Somatic inactivation of *SMARCB1* also occurs in the majority of sporadic MRTs.^{10,11} In contrast, individuals with schwannomatosis have multiple schwannomas, but lack the vestibular schwannomas characteristic of neurofibromatosis type 2 (NF2).¹² Significantly, germline loss of function *SMARCB1* mutations have also recently been identified in a subset of familial and sporadic cases of schwannomatosis.^{13–16} Based on these initial studies of mutations as well as studies of genetic linkage and tumours from schwannomatosis families that implicate the chromosomal region encompassing *SMARCB1*,^{3,17} such mutations seem to be a frequent cause of schwannomatosis. Since seemingly similar germline *SMARCB1* mutations can result in either schwannomatosis or MRT, it is puzzling that there have been no reports of families in which both conditions have occurred.

CLINICAL DETAILS

The family we describe (fig 1) was referred following the death of IV.2 from AT/RT (see below) and the resection of a tumour from the hand of her father (III.1) that proved to be an epithelioid schwannoma. Within the extended family, individuals I.2 (by report), II.1 and III.4 also had multiple skin lumps consistent with schwannomas. The lesions of II.1 first appeared at age 15 years, are tender to palpation, but they have not been biopsied. III.4 first developed skin lesions, described as “painful,” at age 16–17; one resected scalp tumour was confirmed to be an epithelioid schwannoma. III.1 developed painful lumps at age 16. Examination of individuals II.1, III.1, and III.4 by one of the authors (MSW) revealed multiple (at least 10 in each) subcutaneous lesions. The number increased with age, so II.1 had the most lesions. The growths were firm to palpation and some of them were painful when palpated. The majority were located on the back or extremities with the exception of the scalp tumour that had been removed in III.4. None were associated with any local neurologic deficits. The age of onset, pattern of distribution and pain is consistent with other reported individuals.¹² No other cutaneous abnormalities, including café-au-lait macules, axillary or inguinal freckling, or other pigmentary changes were detected. No Lisch nodules were evident.

No imaging was performed looking for visceral lesions, nor did any of the evaluated patients have deep or referred pain that suggested a symptomatic lesion. III.4 underwent magnetic resonance imaging (MRI) of the brain and cranial base with gadolinium enhancement, specifically looking for vestibular schwannoma, that was normal at age 23, with the exception of the incidental finding of a Chiari I malformation. Vestibular schwannoma was not clinically diagnosed in any other individual although none were evaluated by MRI. None had symptoms of hearing loss or vestibular dysfunction, and neurologic evaluation by the author targeting cranial nerve VIII was unremarkable in all individuals. All of the children of II.1 had different fathers. III.4 meets the proposed research criteria¹² for definite schwannomatosis while III.1 meets the criteria for presumptive schwannomatosis. Other family members have not had imaging or biopsy; however, given the autosomal dominant inheritance pattern in this family, the phenotype in these affected individuals is consistent with hereditary schwannomatosis. Interestingly, the epithelioid variant of schwannoma has not previously been reported in schwannomatosis; however, the two cases with pathology were reviewed by one of the authors (CMC) who concurred with the diagnoses (fig 2).

In contrast, the phenotype in three other affected family members was consistent with hereditary MRT predisposition. III.3 died before 2 years of age from a malignant posterior fossa tumour (consistent with AT/RT, but pathology was unavailable), while IV.1 and IV.2 each died before 2 years of age from tumours confirmed to be AT/RTs (fig 2). Finally, two family members have no evidence of symptoms. IV.3 was examined clinically at the age of 7

years. MRI of the brain and spine (for evidence of AT/RT) were normal, and no cutaneous abnormalities were apparent. However, schwannomatosis commonly presents at a later age in this family and in other reported families.¹² Individual III.5 has not been examined, but she has no reported cutaneous abnormalities.

This is the first family in which schwannomatosis and MRT predisposition have been reported to co-occur, a significant finding given that *SMARCB1* mutations are causal for both conditions. As such, analysis of the germline mutation in this family might provide important clues about the molecular mechanisms that underlie both disorders. Following informed consent, the family, K18853, was enrolled in the University of Utah Clinical Genetics Research Program for study.

MOLECULAR FINDINGS

We initially evaluated genomic DNA samples from three family members affected with schwannomatosis (II.1, III.1 and III.4) for a germline point mutation in *SMARCB1*. Polymerase chain reaction (PCR) products amplified from each exon (primer sequences available upon request) were bidirectionally sequenced; however, no variants were detected in the coding sequence or splice sites of the gene. Two heterozygous single nucleotide polymorphisms (SNPs) in intron 5 (rs2070456 and rs2070457), detected in III.1 and III.4, allowed us to exclude a complete deletion of the gene. Next, a panel of eight microsatellite markers that spanned the region from 22q11.1 to 22q13.1 was used to confirm that III.1 and III.4 likely shared a maternal haplotype in the region encompassing *SMARCB1*, consistent with the presence of an undetected mutation in the gene. We subsequently submitted DNA from II.1 to NimbleGen for high resolution array comparative genomic hybridisation (array CGH) using a chromosome 22 specific array with 65 bp median probe spacing. The results, interpreted using NimbleGen SignalMap software and the University of California Santa Cruz (UCSC) Genome Browser (<http://genome.ucsc.edu>), revealed that a segment within *SMARCB1* was increased in copy number (fig 3A). Independent sets of PCR primers designed to amplify the junction of a direct duplication in this region (primer sequences available upon request) each specifically amplified products from all three family members that did not amplify from control individuals (fig 4). Upon sequencing the products, family members proved to carry a direct duplication of 2,631 bp (chr22:g.22,488,596_22,491,226 dup2631 (NCBI Build 36.1)) that included exon 6 of *SMARCB1* (fig 3B–D). Exon 6 contains 167 bp, so its direct duplication in the mRNA is predicted to result in a frameshift and protein truncation (p.Leu266fs). Short imperfect direct repeats were present at the duplication breakpoints, consistent with occurrence through slipped strand mispairing¹⁸ (fig 3B). We amplified and sequenced exon 6 independently from the centromeric and telomeric duplicated segments and confirmed that it was present and unaltered in both segments (fig 3D,E).

Based on the diagnosis of AT/RT in IV.1 and IV.2, molecular genetic analysis of the tumours had previously been performed, as reported.¹⁹ An inactivating somatic mutation of *SMARCB1* was detected in each tumour (a full gene deletion resulting from monosomy 22 in IV.1, and c.578_585dup8 in IV.2). To confirm that the duplication of exon 6 was present and represented the germline mutation in these individuals, we extracted DNA from paraffin embedded tissue blocks and successfully amplified a duplication specific PCR product from each sample (fig 4). Thus, the same germline mutation in *SMARCB1* is associated with both schwannomatosis and MRT predisposition in the family. IV.3 was subsequently also found to carry the duplication; although the risk of MRT is low by his age (7 years), he will be followed clinically for MRT predisposition and schwannomatosis.

Immunohistochemical staining (BAF47 antibody, BD Transduction Laboratories, BD Biosciences, San Jose, California, USA) of the two AT/RTs as well as the resected

schwannomas from III.1 and III.4 confirmed that *Smarchb1* was absent from the neoplastic cells of all four tumours (fig 2). Positive staining non-neoplastic cells provided good internal controls in each specimen; normal tissue from tonsil was used as an external positive control. Although the schwannomas contained immunopositive stromal cells and inflammatory cells, the mosaic staining pattern previously reported in schwannomas associated with schwannomatosis^{13,20} was not observed in neoplastic cells from either tumour.

We next extracted DNA from the paraffin embedded schwannomas of III.1 and III.4 to evaluate them for evidence of somatic loss of the wild-type (WT) *SMARCB1* allele. A PCR product that contained one of the heterozygous SNPs (rs2070457), located within the duplicated region, was amplified from each tumour and sequenced. The peak heights of the SNP alleles were compared between sequence of products from tumour and products from leucocyte DNA of the same individual. We did not anticipate a complete loss of the WT allele in the tumour DNA due to the intermixed stromal and inflammatory cells (fig 2). In the schwannoma from III.1, we observed a 47% reduction (averaged from sequence of both strands of the PCR product) in the height of the allele on the WT chromosome with respect to the height of the allele on the mutant chromosome. (Since the SNP was located within the duplication, the peak height of the allele on the mutant chromosome was derived from two copies of the same allele.) This result is consistent with loss of the WT allele of *SMARCB1* in a population of cells within the tumour. No loss of the WT allele was detected in the schwannoma from III.4 (results not shown).

DISCUSSION

There is some knowledge of the pathways through which *Smarchb1* suppresses neoplasia. As a core component of the chromatin remodelling SWI/SNF complex, it is involved in regulating gene transcription. *Smarchb1* suppression of MRT involves increasing the expression of p16INK4a and repressing the expression of cyclin D1.^{21–24} The pathways involved in schwannomatosis remain to be defined, although somatic biallelic inactivation of the *NF2* gene (mutated in *NF2*) at 22q12.2 is a frequent finding in schwannomas from *SMARCB1* mutation carriers.^{14–16}

The mechanisms by which *SMARCB1* germline mutations predispose to schwannomatosis versus MRT are unknown. In MRT, *SMARCB1* acts as a classic tumour suppressor gene that is subject to biallelic inactivation in tumours.^{8,11} Interestingly, *SMARCB1* appears to function in a similar fashion in schwannomatosis.^{13–16} It is, therefore, significant that MRT has not previously been reported in families with schwannomatosis, and unaffected *SMARCB1* mutation carriers in the three previously reported families with MRT predisposition have not been diagnosed with schwannomatosis.^{2,4,5} Accordingly, MRT predisposition and schwannomatosis could result from different types of mutations in *SMARCB1*. Although no clear genotype–phenotype correlation has yet emerged, schwannomatosis cases may have an increased frequency of missense mutations and mutations in exon 1.^{13,15} Additional factors must be involved, however, because a number of reported mutations associated with schwannomatosis, not all of which lie in exon 1, are predicted to result in complete loss of gene function.^{14–16} It has been proposed that an early developmental window exists during which an *SMARCB1* mutation carrier is predisposed to MRT, and survivors might subsequently develop schwannomatosis.^{4,13} Thus, mutant *SMARCB1* alleles that retain partial function might have low penetrance for MRT, yet still cause schwannomatosis. The fact that schwannomatosis was not detected in unaffected mutation carriers from previously reported MRT families could reflect the incomplete penetrance and variable expressivity that is a feature of hereditary schwannomatosis.³ It is also possible that common co-inherited variants associated with nearby genes, such as *NF2*, could play some role in the phenotype conferred by a mutation. In fact, the MRT from IV.1 was also deleted for *NF2* as a result of monosomy 22. Finally, since some sporadic cases of apparent schwannomatosis are known to result from mosaicism for *NF2*

mutations,¹⁷ mosaicism for *SMARCB1* mutations may be responsible for other cases. In such a circumstance, the tissue distribution of a mutation will, no doubt, influence the associated tumour spectrum.

How the germline duplication described in this report results in predisposition to both MRT and schwannomatosis in the family is still unknown. The duplicated *SMARCB1* exon could be variably spliced into the mRNA transcript, allowing some residual or altered gene function. Alternatively, a unique co-inherited variant in a neighbouring gene could be influencing the phenotype. We are currently examining the splicing of mutant *SMARCB1* mRNA in members of the family, and we plan to study *NF2* and other nearby genes as possible modifiers of the phenotype. It is also intriguing that the schwannomas studied in this report differ from those in other reported schwannomatosis families in two aspects: first, they are of the epithelioid variant; second, they are fully negative, rather than mosaic, for Smarcb1 immunostaining in neoplastic cells. We will evaluate other tumours from this family, as they are resected, to determine if these individuals have a unique subtype of schwannomatosis or if the unusual characteristics are confined to a subset of tumours.

Conclusion

This is the first report of a family with inherited predisposition to both MRT and schwannomatosis. Analysis of the germline duplication in *SMARCB1* that is responsible for this unique combination of conditions may allow for a greater understanding of the molecular bases of the disorders associated with this gene.

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Figure 1.

Pedigree of family K18853. Individuals with malignant rhabdoid tumour (MRT), confirmed schwannomatosis (schw) and clinically suspected schwannomatosis (skin lumps) are noted. Confirmed duplication carriers (*dup*⁺) and non-carriers (*dup*⁻) are marked. Individual IV.3 is unaffected at age 7 years.

Figure 2.

(A) Haematoxylin and eosin (H&E) stained section of the AT/RT from IV.1. (B) Immunohistochemical Smarcb1 (BAF47/INI1) stain of the atypical teratoid/rhabdoid tumour (AT/RT) from IV.1. Endothelial cell nuclei show positive staining. (C) H&E stained section of the epithelioid schwannoma from III.1. Note rounded epithelioid schwann cells arranged singly and in small aggregates. (D) Immunohistochemical Smarcb1 stain of the epithelioid schwannoma from III.1. Neoplastic cell nuclei are immunonegative. Original magnification $\times 500$.

Figure 3.

(A) Array comparative genomic hybridisation (CGH) results in a 50 kb window encompassing *SMARCB1*. The top plot shows all probes. Each point in the bottom plot represents the average of the probes in a 700 bp window; the raised horizontal bar marks the duplication. Nucleotides are numbered at top (from NCBI Build 36.1). (B) Sequences from the telomeric (Tel) and centromeric (Cen) ends of the duplication are aligned with the sequence of the duplication junction. The region of similarity is boxed. (C) Diagram of *SMARCB1*. Exons are represented by vertical bars, and exon 1 is at left. The arrow represents the duplicated region. (D) Locations of polymerase chain reaction (PCR) primers, represented by small arrows (labelled A–E). Each large arrow represents one segment of the duplication. (E) Confirmation of the duplication through analysis of the peak height ratios of an intronic *SMARCB1* SNP (rs2070457) in DNA sequences of three different PCR products amplified from III.4. The "C" allele is present on both segments of the duplication; the wild-type (WT) chromosome carries the "A" allele. All three copies (WT and mutant) of the duplicated region amplified in the product at top; the WT chromosome and the centromeric duplication segment amplified in the product at centre; the telomeric duplication segment amplified in the product at bottom.

Figure 4.

Agarose gel electrophoresis of a duplication specific polymerase chain reaction (PCR) product (primers C + E) amplified from the DNA of affected individuals in K18853. DNA samples from random unaffected individuals (R) were included as negative controls. Samples prepared from blood (bl) and paraffin embedded tumour (tu) are noted.