nature genetics

Loss-of-function mutations in *SMARCE1* cause an inherited disorder of multiple spinal meningiomas

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One-third of all primary central nervous system tumors in adults are meningiomas[1]. Rarely, meningiomas occur at multiple sites, usually occurring in individuals with type 2 neurofibromatosis (NF2). We sequenced the exomes of three unrelated individuals with familial multiple spinal meningiomas without *NF2* mutations. We identified two individuals with heterozygous loss-of-function mutations in the SWI/SNF chromatin-remodeling complex subunit gene *SMARCE1*. Sequencing of *SMARCE1* in six further individuals with spinal meningiomas identified two additional heterozygous loss-of-function mutations. Tumors from individuals with *SMARCE1* mutations were of clear-cell histological subtype, and all had loss of SMARCE1 protein, consistent with a tumor suppressor mechanism. Our findings identify multiple-spinal-meningioma disease as a new discrete entity and establish a key role for the SWI/SNF complex in the pathogenesis of both meningiomas and tumors with clear-cell histology.

Meningiomas are generally slow-growing tumors derived from the arachnoid membrane surrounding the central nervous system (CNS). They are the most common CNS tumors, with an overall incidence of 2.3 in 100,000 and a 2:1 female-male ratio[1,2]. About 90% of meningiomas occur in the cranial meninges, with the remaining 10% developing in the spinal meninges.

Meningiomas usually occur sporadically but are also closely associated with the tumor suppressor syndrome NF2, with 50–75% of individuals with NF2 developing a meningioma during their lifetime[3]. Loss of the wild-type *NF2* allele is detected in almost all NF2-associated meningiomas, and somatic *NF2* mutations are found in the majority of sporadic meningiomas[4]. In approximately 4% of cases, meningiomas occur at multiple locations in the CNS[5], but germline *NF2* mutations are rarely found in isolated adult cases without other features of NF2 (ref. 6).

Germline mutations in the SWI/SNF chromatin-remodeling complex gene *SMARCB1*, which also cause malignant rhabdoid tumors and schwannomatosis, have been identified in three families with both multiple schwannomas and meningiomas[7–9]. Somatic mutations in *SMARCB1* have been described in meningiomas[10,11]; however, screening of *SMARCB1* in the germline of individuals and families with multiple and isolated meningiomas suggests that mutations in this gene do not represent a major contributor to meningioma disease[11,12]. It has also been suggested that familial cases of *SMARCB1* mutation–positive meningiomas have tumors preferentially localized to the falx cerebri of the cranium[9].

In a subset of individuals, multiple-meningioma disease is a discrete entity, inherited in an autosomal dominant manner (MIM 607174) with no evidence of schwannomas or other clinical symptoms of NF2 or schwannomatosis. Further, the meningiomas in these individuals are not associated with loss of chromosome 22q, which harbors the *NF2* and *SMARCB1* loci[13]. Individuals with a first-degree relative with a meningioma have a threefold higher risk of developing a tumor[14], and a recent genome-wide association study of sporadic meningiomas defined a new susceptibility locus at 10p12.31 (near *MLLT10*), which confers modest risk[15].

To explore the genetic basis of meningiomas further and identify new targets for therapeutic intervention, we performed exome sequencing of three unrelated individuals with a positive family history of multiple spinal meningiomas consistent with dominant inheritance. All had previously tested negative for germline mutations in *NF2* and *SMARCB1* and had no other clinical evidence of NF2 or schwannoma disease.

Between 73% and 77% of the targeted exome was covered at least 20-fold for each sample. We excluded variants found in the dbSNP136 database. Initial filtering of the data identified no compelling candidates for causative loss-of-function mutations in genes common to all three individuals. Because of the role of *SMARCB1*

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Received 6 October 2012; accepted 11 January 2013; published online 3 February 2013; doi:10.1038/ng.2552

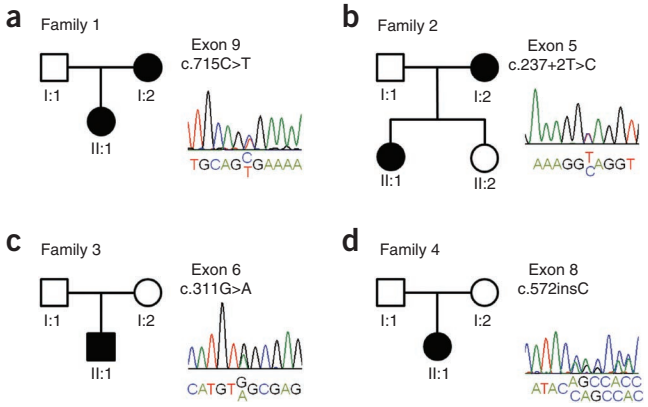

Figure 1 Pedigrees of families with spinal meningioma with *SMARCE1* mutations. (**a**–**d**) Pedigrees and identified *SMARCE1* mutations for family 1 (**a**), family 2 (**b**), family 3 (**c**) and family 4 (**d**). Filled symbols indicate individuals affected by spinal meningiomas. Chromatograms of the germline *SMARCE1* point mutation identified in each affected individual are shown.

in CNS tumors, we focused the screen of sequence data to prioritize the identification of loss-of-function, previously unreported variants in subunits of the SWI/SNF complex. Previously unreported heterozygous variants in *SMARCE1* were noted and verified by Sanger sequencing in two of the three affected individuals. The first was a mutation at the first base of exon 9, c.715C>T (p.Arg239*) (family 1; **Fig. 1a**), which introduces a stop codon and is predicted to result in nonsense-mediated mRNA decay (NMD) of the resultant transcript. This variant was also present in an affected daughter. The second variant was an exon 5 splice-site mutation, c.237+2T>C (family 2; **Fig. 1b**), also present in the affected mother but not in an unaffected sister. Analysis of cDNA derived from lymphocyte RNA from this individual identified two alternatively spliced *SMARCE1* transcripts that were not present in controls. The first contained an insertion of the first 18 bases of intron 5, which introduced an in-frame premature stop codon after three codons and is predicted to lead to decay (**Supplementary Fig. 1**). The second transcript was less abundant and contained an in-frame deletion of exon 5 (**Supplementary Fig. 1**). Skipping of exon 5 is predicted to remove the start of the high-mobility-group (HMG) domain. Previous studies have shown that human T cells lacking the SMARCE1 HMG domain are defective in the normal regulation of CD4/CD8 receptor expression[16].

Sanger sequencing of *SMARCE1* in lymphocyte DNA from six further individuals with spinal meningiomas identified two additional previously unreported loss-of-function mutations (families 3 and 4; **Fig. 1c**,**d** and **Table 1**). None of the mutations were present in the Exome Variant Server (EVS6500) of over 6,500 exomes, our in-house database of >150 exomes or a panel of 100 south Asian control samples (for family 1). Only one potential loss-of-function variant in *SMARCE1* was present in the EVS data, with a nonsense mutation in the penultimate codon of the last exon, which is likely to escape NMD. Sequencing *SMARCE1* in lymphocyte DNA from 34 individuals with multiple cranial meningiomas did not identify any mutations, suggesting that the mutations are specific for spinal tumors.

The mutations segregated with a spinal meningioma phenotype in families from which samples from additional family members were available. Notably, in family 4, the frameshift mutation present in the proband (**Fig. 1d** and **Supplementary Fig. 2**) was not detected in buccal or lymphocyte DNA from her mother, who had an olfactory groove (cranial) meningioma, but was present in her clinically unaffected father. It is notable that within our collection of 13 individuals with spinal meningiomas from 9 families, 12 are female. This bias may reflect incomplete penetrance in males and is analogous to the difference between the sexes in the penetrance of the genes responsible for hereditary non-polyposis colorectal cancer (HNPCC). Mutations in *MLH1* and *MLH2* have 80% penetrance for colorectal cancer in males and 40% in females[17]. A hormonal influence on penetrance is suggested by the presentation of tumors in both affected women in family 2 and the proband in family 4 during pregnancy.

We sequenced *SMARCE1* in DNA extracted from tumor tissue, where available, in individuals with germline mutations to determine whether there was evidence of loss of heterozygosity (LOH), consistent with a tumor suppressor mode of action. In family 3, the exon 6 mutation (c.311G>A) was heterozygous in one tumor, whereas a second tumor showed retention of the mutant allele with loss of the wild-type allele. In family 4, the exon 8 mutation present in the germline of the affected daughter was heterozygous in her tumor.

All spinal tumors from *SMARCE1* mutation–positive individuals were consistent with clear-cell histology (**Fig. 2**). Immunohistochemistry on 3 tumors from affected individuals from family 2 showed that SMARCE1 protein was absent, whereas mutation-negative tissue from 2 cranial meningiomas and a panel of 12 sporadic spinal meningiomas (4 transitional, 4 psammomatous and 4 atypical) showed diffuse positive staining (representative examples shown in **Supplementary Fig. 3**). Staining of a spinal (clear-cell) tumor from the mutation-positive proband (II-1) of family 4 showed loss of SMARCE1 staining (**Fig. 2c**), whereas the cranial (transitional) tumor from her mutation-negative mother showed diffuse positive staining (**Fig. 2d**), confirming that the mother's tumor is coincidental to the diagnosis of spinal meningioma in her daughter. Occasional nuclear staining observed within SMARCE1-negative tumor tissue seems to be attributable to the presence of macrophages and small T lymphocytes (**Supplementary Fig. 4**) and might account for the inconsistent LOH results, although other mechanisms, including DNA methylation, may be responsible for inactivation of the second allele.

Table 1 ***SMARCE1*** **sequence alterations identified in individuals with spinal meningiomas**

Family	Subject	Sex	Age of onset (years)	Location(s)	Mutation	Predicted protein alteration	Tumor *SMARCE1* sequence
1	I:2	Female	27	T11–S1 (×2)	c.715C>T	p.Arg239*	NA
	II:1	Female	15	T12/L1			
2	II:1	Female	30	T11 and L4/L5	c.237+2T>C	p.Lys79_Val80ins3* p.Ala53_Lys79del	NA
	I:2	Female	26	S1, L2 and L4			
3	II:1	Male	26	L4 and S1	c.311G>A	p.Trp104*	Tumor 1: c.311G>A heterozygous Tumor 2: c.311G>A + LOH
4	II:1	Female	17	Foramen magnum	c.572insC	p.Thr191Thrfs*14	c.572insC heterozygous
	I:2	Female	54	Olfactory groove	No *SMARCE1* mutation	None	NA

L, lumbar; S, sacral; T, thoracic; NA, not available.

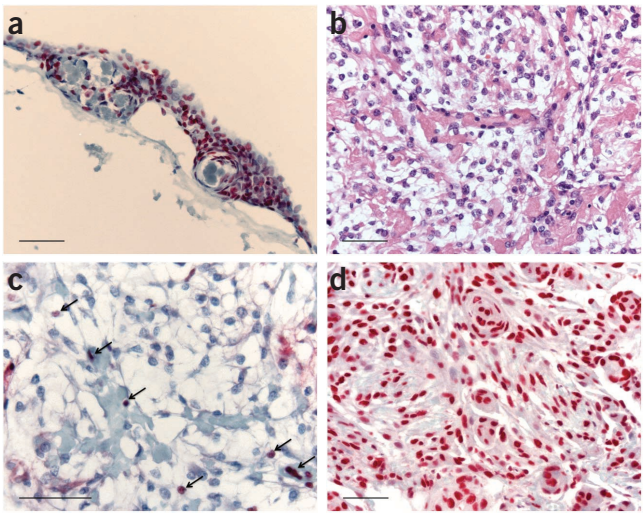

Figure 2 SMARCE1 immunohistochemistry. (**a**) Normal leptomeningeal tissue was used as a positive control for SMARCE1 immunoreactivity. (**b**,**c**) Spinal meningioma from individual II-1 in family 4. (**b**) Hematoxylin and eosin staining is consistent with clear-cell histology. (**c**) SMARCE1 immunohistochemistry showing no staining of tumor cell nuclei, focal nonspecific cytoplasmic staining of tumor cells and nuclear staining in occasional interstitial lymphocytes and endothelial nuclei (arrows). (**d**) SMARCE1 immunostaining of the cranial meningioma from the mother of II-1 (negative for the *SMARCE1* mutation) showing diffuse positive staining. Scale bars, 40 µm.

SMARCE1 is an 11-exon gene on chromosome 17q21 that encodes the 57-kDa subunit of the SWI/SNF complex, also termed BAF57. It is present only in higher eukaryotes[18] and is found in all mammalian SWI/SNF complexes. SMARCE1 isoforms lacking exons 3 and 4 are confined to neurons[19]. SWI/SNF multisubunit complexes regulate chromatin structure by nucleosome remodeling. Each complex contains one of two mutually exclusive catalytic ATPase subunits (either SMARCA2 or SMARCA4), a set of highly conserved core subunits (for example, SMARCB1, SMARCC1 and SMARCC2) and variant subunits, including SMARCE1. SMARCE1 has many functions, including the induction of apoptosis by stimulating expression of the cylindromatosis tumor suppressor gene, *CYLD*[20]. Therefore, loss of its activity might lead to the uncoupling of apoptotic control, consistent with the previous identification of *SMARCE1* somatic mutations in breast cancer[21–25].

Loss of SMARCE1 expression in spinal meningioma tissue is consistent with a tumor suppressor mode of action. Both SMARCB1 and SMARCE1 are Gli-interacting proteins, and loss of SMARCB1 leads to activation of the Hedgehog-Gli pathway, which might in turn lead to uncontrolled cell growth[26]. In addition, the known Hedgehog-Gli pathway inhibitor gene *SUFU* has recently been identified as the causative gene in a single family with multiple meningiomas, supporting a role for this pathway in meningioma pathology[27].

In contrast to meningiomas in individuals with *SMARCB1* mutations, where somatic *NF2* mutation occurs, only one individual with a germline *SMARCE1* mutation showed somatic LOH at the *NF2* locus. This suggests a different mechanism of action that does not require mutation of both *NF2* and *SMARCE1*. Although familial multiple-cranial-meningioma disease is well recognized[3,28], familial spinal-meningioma disease has not been previously described. Our studies define it as a discrete, genetically heterogeneous disorder, as *SMARCE1* mutations were only identified in four of nine affected families. Because all of the tumors from our mutation-positive spinal meningioma cases showed clear-cell histology, it is likely that *SMARCE1* mutations are specific to this meningioma subtype. Indeed, somatic mutations in the SWI/SNF subunit genes *ARID1A* and *PBRM1* are major drivers in both ovarian and renal clear-cell tumor subtypes, respectively[29–31].

Heterozygous germline mutations in multiple members of the SWI/SNF complex, including *ARID1A*, *ARID1B*, *SMARCA2*, *SMARCA4* and *SMARCB1*, have been described recently in individuals with Coffin-Siris syndrome, a neurodevelopmental disorder characterized by growth and intellectual disability, microcephaly, coarse facial features and nail hypoplasia[32,33] A single germline *de novo* missense *SMARCE1* mutation (encoding p.Tyr73Cys) was also reported in an individual with Coffin-Siris syndrome[32]. Apart from a single case report of an individual with Coffin-Siris syndrome and schwannomatosis[34], CNS tumors are rare in Coffin-Siris syndrome, suggesting that loss-of-function mutations in *SMARCE1* predispose to a specific disease phenotype, as none of the individuals we studied with spinal meningiomas had additional neurodevelopmental problems.

Our studies define a new role for *SMARCE1* in the pathogenesis of multiple spinal meningiomas and reinforce the importance of the SWI/SNF complex in tumors with clear-cell histology. These studies will allow accurate risk estimation in affected families, facilitating appropriate screening and investigation into the role of modulators of chromatin remodeling in treatment.

URLs. Exome Variant Server, National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ESP) (accessed 5 October 2012), http://evs.gs.washington.edu/EVS/.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Human *SMARCE1* cDNA, NM_003079.4; human SMARCE1 protein, NP_003070.3. The anonymized exome sequencing data for our mutation-positive cases are available upon request.

Note: Supplementary information is available in the online version of the paper.

ACKNOWLEDGMENTS

We thank the families for their contributions to this study. We also thank N. Bowers and A. Wallace for *NF2* mutation screening and T. Gledhill for immunohistochemistry images. M.J.S. is supported by a Young Investigator Award (2011-01-006) from the Children's Tumor Foundation. We also acknowledge support from the Association for International Cancer Research (12-0275) and the Manchester Biomedical Research Centre. D.G.E. is a National Institute for Health Research (NIHR) Senior Clinical Investigator. Finally, we thank the NHLBI GO Exome Sequencing Project and its ongoing studies, which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the Women's Health Initiative (WHI) Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the Heart GO Sequencing Project (HL-103010).

AUTHOR CONTRIBUTIONS

M.J.S., W.G.N. and D.G.E. designed the study and wrote the manuscript. J.O. and S.S.B. performed exome sequencing and bioinformatics analysis. M.J.S. and K.D.H. performed Sanger sequencing. D.G.E., G.P., D.F., S.S., D.R., D.E. and J.C. provided detailed clinical information and samples for analysis. D.d.P. performed histopathological review and immunohistochemistry studies. All authors reviewed the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/doifinder/10.1038/ng.2552.
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ONLINE METHODS

Subjects. All multiple-meningioma cases in this study had previously tested negative for constitutional mutations in both *NF2* and *SMARCB1* in genomic DNA from peripheral lymphocytes with whole-gene sequencing and multiplex ligation–dependent probe amplification (MLPA) probe sets P258-B1 and P044 (MRC-Holland). The study was approved by the North West 7 Research Ethics Committee–GM Central–10/H1008/74. Informed consent was obtained from all participants.

Exome sequencing. Whole-exome targeted enrichment and sequencing were performed on lymphocyte DNA extracted from three individuals from three families with multiple spinal meningiomas. Enrichment was performed using the SureSelect Human All Exon kit v.1 (Agilent) for the Applied Biosystems SOLiD system. Emulsion PCR (ePCR) was carried out on the enriched sample library, and products were sequenced on a SOLiD 4 sequencer (Life Technologies). Sequence data were mapped to the hg19 reference human genome using SOLiD BioScope software (Life Technologies). SNPs were called using the diBayes tool in the BioScope software suite with the medium stringency setting, which requires at least 1–25× coverage and that the variant be seen in either the forward or reverse strand. An initial filter was used to remove SNPs with an overall sequence depth of <5×. This strategy retains sufficient numbers of variants without unreasonably increasing the false negative rate. Indels were included with a sequence depth of >5× and a range of end positions between 15–40 bp. A more stringent filter was applied to SNPs to ensure that all novel allele counts were ≥18× with a corresponding novel mean quality value of ≥18×. Subsequent interpretive analysis prioritizing novel putative loss-of-function variants (nonsense and frameshift mutations) was used to decide which genes should be analyzed further. After eliminating all candidate variants, the stringency was reduced to 17× coverage to allow for a lower novel allele read count.

Sanger sequencing. Mutations identified by exome sequencing were confirmed by direct Sanger sequencing. Primers were designed to amplify individual exons, including approximately 50 bases of flanking intronic sequence, and samples were sequenced using the BigDye Terminator v3.1 system (Life Technologies).

RNA analysis. RNA was extracted from peripheral lymphocytes using PAXgene Blood RNA tubes and the PAXgene Blood RNA Extraction kit (Qiagen). RNA was converted to cDNA using the High-Capacity RNA-to-cDNA kit (Life Technologies). A fragment of the *SMARCE1* transcript was amplified in the sample from family 2 and in a control cDNA sample using primers designed for exons 3 and 7. PCR products were electrophoresed on a 2% agarose gel, and three bands were resolved. Each band was excised from the gel and purified using a QiaEx II Gel Purification column (Qiagen). Purified products were analyzed by Sanger sequencing.

Immunohistochemistry. Spinal meningiomas from affected individuals from families 2 and 4 were immunostained for SMARCE1 protein. Briefly, paraffin sections were cut at 4 µm, dried for 4 h at 37 °C and incubated for 1 h at 60 °C. Sections were then stained with a 1:100 dilution of antibody to SMARCE1 (HPA00396, Sigma-Aldrich) using an indirect peroxidase method on the Roche Ultra IHC Autostainer. Cells were counterstained with hematoxylin II and bluing reagent. Sections were then dehydrated through three changes of absolute 100% methylated spirits and cleared in three changes of xylene, before being mounting in Pertex mountant.