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RESEARCH ARTICLE

Loss of SMARCE1 expression is a specific diagnostic marker of clear cell meningioma: a comprehensive immunophenotypical and molecular analysis

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Abstract

Clear cell meningioma (CCM) is a rare grade II histopathological subtype that usually occurs in young patients and displays high recurrence rate. Germline *SMARCE1* mutations have been described in hereditary forms of this disease and more recently in small syndromic and sporadic CCM series. The diagnostic value of SMARCE1 in distinguishing between CCM and other meningioma variants has not been yet established. The aim of our study was to investigate the status of SMARCE1 in a series of CCMs and its morphological mimickers. We compared the performance of an anti-SMARCE1 antibody and the molecular analysis of the *SMARCE1* gene in a retrospective multicenter series of CCMs. All CCMs lost SMARCE1 immunoexpression. Bi-allelic inactivating events were found by NGS-based sequencing in all of these cases, except for one, which was incompletely explored, but had a wild-type sequence. We then validated the anti-SMARCE1 antibody specificity by analyzing additional 305 pediatric and adult meningiomas of various subtypes and 15 non-meningioma clear cell tumors by SMARCE1 immunohistochemistry. A nuclear immunostaining was preserved in all other meningioma variants, as well as non-meningioma clear cell tumors. In conclusion, our series showed, for the first time, that SMARCE1 immunostaining is a highly sensitive biomarker for CCM, useful as a routine diagnostic biomarker.

INTRODUCTION

Meningiomas are the most frequent tumors of the central nervous system (CNS), representing 35.5% of all primary CNS tumors (12). They exhibit 13 different histopathological variants and are divided into three grades (grade I, II, and III) (23). Among them, CCM is a rare aggressive variant corresponding to grade II (23). Histopathologically, CCM may be difficult to distinguish from other morphological subtypes, especially grade I microcystic meningioma (MM) or other clear cell tumors (23).

The tumorigenesis of meningiomas, derived from arachnoid cells, depends on various germline and somatic gene mutations. The most common are germline *NF2* mutations, responsible for neurofibromatosis type 2 (NF2) (24, 38). Two genes (*SMARCB1* and *SMARCE1*), encoding two proteins of the SWI/SNF complex (BAF47 and SMARCE1), have also been recently implicated in hereditary meningiomatosis ((1, 2, 4, 5, 7–10, 12–14, 16), cf. review in (48)). Over the last three years, germline mutations in the *SMARCE1* gene have been described in 20 unrelated families ((4, 12–14), cf. review in (48)). These familial forms are linked to inherited germline mutations, mainly in exon 6 (38), with inactivation of the wild-type allele in the tumor cells, according to the Knudson hypothesis (20). *SMARCE1*-deficient meningiomas are of only the clear cell subtype ((4, 13–15), cf. review in (48)). In contrast, tumors of *NF2* and

SMARCB1-related meningiomas are morphologically heterogeneous with exceptional CCMs carrying *NF2* mutations ((1–3, 9, 10, 13), cf. review in (48)). The role of the loss of *SMARCE1* function in sporadic CCM has not yet been studied, but other SWI/SNF subunits (encoded by *SMARCA2, ARID1A, PBRM1*) have already been shown to be associated with other clear cell tumors, for example ovarian clear cell carcinoma (19, 33, 47).

In the literature, *SMARCE1*-deficient CCMs have been particularly described in children (approximately 36% of reported CCMs, from 8 months to 17 years of age) ((2, 4, 6, 8, 9, 11, 14, 17, 20, 23, 26–30, 32, 45), cf. review in (48)) and young adults (approximately 21% of reported CCMs, from 18 to 30 years of age) ((21, 27–29, 49), cf. review in (48)). A previous series of 72 pediatric meningiomas has also showed that clear cell subtype (7% of related cases) is much more common in children than adults proportionately (44). This is consistent with the low mean (30.9 years) and median (26 years) age of patients with CCM (from 8 months to 88 years) relative to the median age of meningioma in general (65 years) (23). In the literature, the female-to-male ratio of patients with CCM was 1.1 (124 females and 110 males) ((2, 4, 5, 7, 9–11, 13, 14, 17, 18, 21, 22, 26, 27, 30, 32, 34–39, 41, 46), cf. review in (48)). This characteristic is in contrast with classical meningioma epidemiology, showing a high frequency in females, particularly after menopause (23).

The existence of bi-allelic alterations of *SMARCE1* in some cases of clear cell meningiomatosis suggests that these alterations are oncogenic drivers in CCM, but this has not been tested in a large cohort of diverse histopathological variants of sporadic meningioma. Smith *et al*. tested 8 cases and identified 6 bi-allelic alterations including 3 new mutations in exons 3 and 10 (39). In this retrospective monocentric Asian cohort, a good correlation was found between immunohistochemical loss of SMARCE1 and *SMARCE1* molecular alterations.

The aim of our study was to perform a comparative immunohistochemical and molecular analysis of the status of *SMARCB1* and *SMARCE1*, as well as a molecular investigation of the *NF2* gene, in a retrospective Caucasian multicenter series of CCMs to determine whether the status of SMARCE1 is the same in adult or pediatric CCMs, and therefore, whether it represents a specific diagnostic biomarker of CCM.

MATERIALS AND METHODS

Clear cell and microcystic meningioma study cohort

Patients

A total of 27 cases (all surgical specimens) were retrieved from the consultation archive database (1982–2016) of the Sainte-Anne and Lariboisière Hospital pathology departments.

This retrospective and multicenter study included 27 tumors from 26 patients who underwent surgery at the Sainte-Anne ($n = 8$), Lariboisière ($n = 8$), Mondor ($n = 2$), Necker ($n = 3$), Val-de-Grâce Hospitals ($n = 1$) or the Rothschild foundation ($n = 3$). One patient, originated from Nancy, was part of the national pediatric meningioma study cohort. One patient (patient 5), presenting multiple meningiomas, underwent surgery for two meningiomas. Patient characteristics and clinical data were retrieved from hospital records. The location of tumors was based on operative reports.

Histopathological review

The central pathology review was performed by two neuropathologists (ATE and PV). The tumors were classified and graded according to the 2016 WHO classification and were subtyped into MMs and CCMs (23).

Immunohistochemical study

A representative section was selected for each case. Unstained 3-µm-thick slides of formalin-fixed, paraffin-embedded tissues were obtained and submitted for immunostaining. Somatostatin receptor 2a (SSTR2) was used as a diagnostic marker for meningioma. SSTR2 (Reference ab134152, clone UMB1, dilution 1:200, monoclonal, rabbit, Abcam) was evaluated as positive (moderate or dark diffuse staining) or negative (no staining or weak staining) (28). SMARCE1 (Reference HPA003916, dilution 1:800, polyclonal, rabbit, Sigma-Aldrich) and BAF47 (INI1) (Reference 612110, clone25/BAF47, dilution 1:200, mouse, BD Biosciences) were evaluated as positive when all nuclei were stained. Endothelial cells and lymphocytes were used as positive internal controls.

Targeted NGS genotyping of SMARCE1, SMARCB1 and NF2 genes

DNA extraction from 25 FFPE tumor samples was performed with the Maxwell® 16 FFPE Tissue LEV DNA Kit. DNA concentrations were determined using a Quant-iT dsDNA HS assay kit and a Qubit 2.0 Fluorometer (Life Technologies, Saint-Aubin, France).

Genotyping experiments were performed at the NGS facility of the Cochin hospital, Paris (*Assistance Publique - Hôpitaux de Paris*, France). A custom Ampliseq panel targeting *SMARCE1, NF2, SMARCB1,* and *SUFU* genes was developed (reference IAD51599_119, ThermoFisherScientific). Genomic DNA was amplified to generate the library using the Ion AmpliSeq Library Kit 2.0 (Life Technologies). NGS library preparation, followed by amplification and purification, emulsion PCR, enrichment, loading on Ion 318™ chips, sequencing with an Ion Personal Genome Machine® (PGM™) System (Life Technologies), and data collection were performed as already described (31).

Sequence alignment was performed using the Torrent Mapping Alignment Program (TMAP, https://github.com/iontorrent/TMAP, Ion Torrent for Life Technologies), which was specifically developed to analyze Ion Torrent data. Aligned reads from .bam files were visualized using the tool Integrative Genomics Viewer v2.3 (IGV, https://www.broadinstitute.org/igv/) from the Broad Institute (Cambridge, MA).

Single nucleotide variants (SNVs) and short insertion and/or deletion detection from the bam files was performed using the Torrent Suite Variant Caller (TSVC) plugin from the Torrent Suite Software v**5.0.4**. (https://ioncommunity.thermofisher.com/community/products/software/torrent_suite, Life Technologies). Major calling parameters were chosen as follows to avoid false negative results: minimum sequencing depth $\geq$ 5X for SNVs and multiple nucleotide or complex variants and $\geq$ 10X for short insertions and/or deletions, minimum allele frequency (MAF) $\geq$ 1% for all using the TSVC.

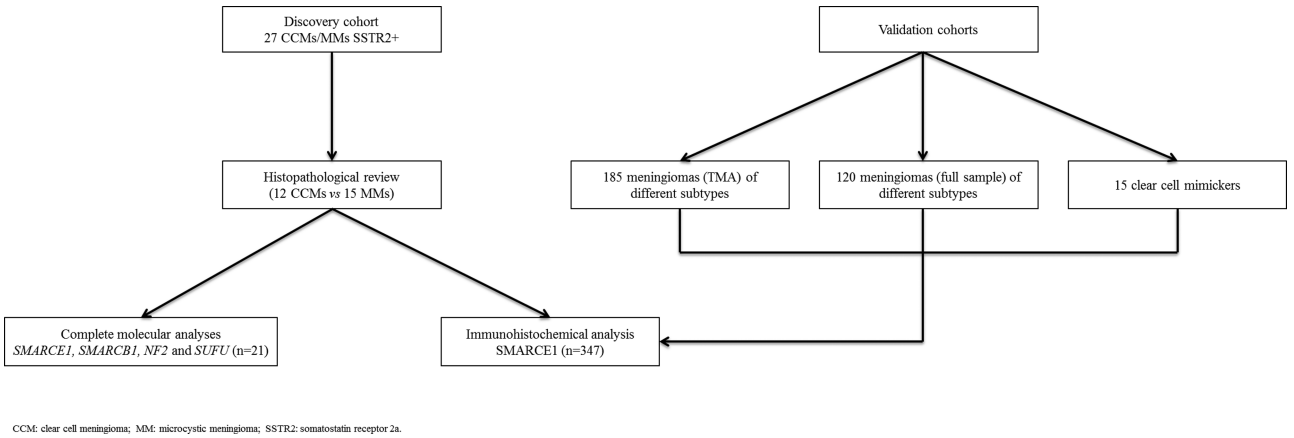

Figure 1. Workflow of the study.

Gene copy number analysis was performed using quantitative values (number of reads for each amplicon of each sample) of the *Coverage Analysis* plugin on the Ion Torrent Browser 5.0.4.0 (Life Technologies). Amplicon reads were first internally normalized for each sample: reads of target gene amplicons were individually divided by the total number of reads of the control gene amplicons. *SUFU* was used as the control gene for *SMARCE1*, *NF2,* and *SMARCB1* copy number analysis. Normalized reads obtained for each amplicon of a sample were then divided by the average number of normalized reads of control samples for the corresponding amplicon. Copy number ratios of <0.75 were defined as deleted.

In case which blood DNA was available a constitutional mutation in *SMARCE1* was looked for in peripheral blood lymphocytes.

Statistical analysis

Statistical analysis was performed using SPSS (version 20). Mean values and frequencies were used for the description of continuous and categorical variables, respectively. The degree of concordance between the results of SMARCE1 immunostaining and *SMARCE1* genotyping was assessed using the Cohen's kappa coefficient. The categorization values suggested by Altman were used for interpretation of the kappa value (2).

Meningioma validation cohort

We retrieved a cohort of 305 meningiomas of various subtypes, graded according to the 2016 WHO classification, consisting of 120 large surgical meningioma samples and a TMA of 185 tumors (kindly provided by the APHM tumor bank AC-2013-1786/CRB number BB-0033-00097) (23). Twenty patients of large surgical meningioma samples were children (ages 1–16). In total, 169 WHO grade I, 95 WHO grade II, and 41 WHO grade III meningiomas were studied. Among them, the following rare histopathological variants were included: angiomatous ($n = 2$), secretory ($n = 11$), psammomatous ($n = 9$), metaplastic ($n = 2$), microcystic ($n = 2$), chordoid ($n = 2$), lymphoplasmacyte-rich ($n = 1$) and rhabdoid ($n = 2$). The remaining meningiomas represented more common subtypes, such as meningothelial ($n = 73$), fibrous ($n = 28$), transitional ($n = 54$), atypical ($n = 80$) and anaplastic ($n = 39$) forms. No CCM was included in this cohort. A representative section (3-µm-thick slide) was selected for SMARCE1 immunostaining for each case.

Non-meningioma clear cell CNS tumor validation cohort

We retrieved an adult and pediatric cohort of 15 clear cell mimickers from the consultation archive database of the Sainte-Anne Hospital pathology department. The following histopathological entities were included: metastases of renal clear cell carcinoma ($n = 4$), hemangioblastomas ($n = 4$), clear cell ependymomas ($n = 4$), Ewing sarcoma ($n = 1$), choroid plexus carcinoma ($n = 1$) and schwannoma ($n = 1$). A representative section (3-µm-thick slide) was selected for SMARCE1 immunostaining for each case.

The study design is summarized in Figure 1.

RESULTS

Clinical data of the discovery cohort (CCM and MM)

The cohort included 22 adults (84.6%) and four children (15.4%). The mean age at surgery was 26.2 years (range, 7–72 years) for CCM and 55.2 years (range, 31–74 years) for MM. The female-to-male ratio was 0.8 for the CCM cohort and 6.5 (13 females and 2 males) for the MM cohort. The clinical data of patients with CCM are summarized in Table 1. Ten meningiomas (45.5%) were supratentorial, nine (40.9%) were located in skull-base region, two (9.1%) in the posterior fossa, and one (4.5%) in the spine. Multiple meningiomas were identified in four patients, three with MMs and one with CCMs. They were found for two of the patients at diagnosis and for two during follow-up. We obtained follow-up data for 24 patients. Total surgery was performed on 15 patients, who had no evidence of the disease at the end of the follow-up. The surgery was incomplete for two patients who then received adjuvant radiotherapy and were alive at the end of the follow-up. Two patients with CCM, both children, died of the disease. No familial history was mentioned in medical records except in one patient (case 3) which had a sister with a spinal ependymoma but no more clinical and histopathological data were available.

Table 1. Clinical data of the 11 patients with clear cell meningioma.

Case	Age of onset (years), sex	Tumor location(s)	Resection type	Adjuvant treatment	Recurrence/ progression	Recurrence interval (years)	Status at last follow-up	OS (years)	Meningiomatosis
1	13, F	Cervical C4-C5	GTR	0	0	NA	A	60.0	0
2	6, M	Petrous and clivus	GTR	0	1	1.9	D	5.6	0
3	18, F	Right temporal convexity	GTR	0	0	NA	A	1.7	0
4	7, F	Petrous	STR	0	1	2.8	D	6.9	0
5	23, F	Left jugum	STR	0	0	NA	A, stable residue	15.9	1
5	26, F	Lumbar spine	GTR	0	0	NA	A	15.9	1
6	72, M	Right trigeminal nerve	STR	0	1	3.2	A, stable residue	3.2	0
7	20, F	Right pontocerebellar angle	NK	NK	NK	NK	NK	NK	NK
8	41, M	Left pontocerebellar angle	STR	RT	1	2.1	A	10.4	0
9	61, M	Right cavernous sinus	GTR	0	0	NA	NK	NK	0
10	45, M	Right trigeminal nerve	GTR	0	1	3.5	A	3.5	0
11	13, M	Petrous and cavernous sinus	STR	0	1	1.3	A, stable residue	14.6	0

A: alive; D: died of progression of the disease; F: female; GTR: gross total resection; M: male; NA: not applicable; NK: not known; OS: overall survival; RT: radiotherapy; STR: subtotal resection.

Histopathological findings

The histopathological review classified 12 tumors as CCMs from 11 patients and 14 as MMs from 14 patients (Figure 2A,D). In one case, the extent of electrocoagulation artifacts did not allow discrimination between CCM and MM. One of the 14 MMs (case 21) was grade II because of high mitotic activity. All 12 CCMs were grade II, three with brain invasion (cases 3, 5 and 8), including one with high mitotic activity (case 3).

Immunohistochemical findings

Immunohistochemical analyses were performed on the entire cohort (27 tumors).

All meningiomas exhibited strong and diffuse SSTR2 staining with a preserved nuclear BAF47 staining. Preserved nuclear staining of SMARCE1 was present in all MMs (Figure 2B,C). In contrast, we observed a diffuse loss of nuclear SMARCE1 staining in all CCMs, which all included a strong vascular internal positive

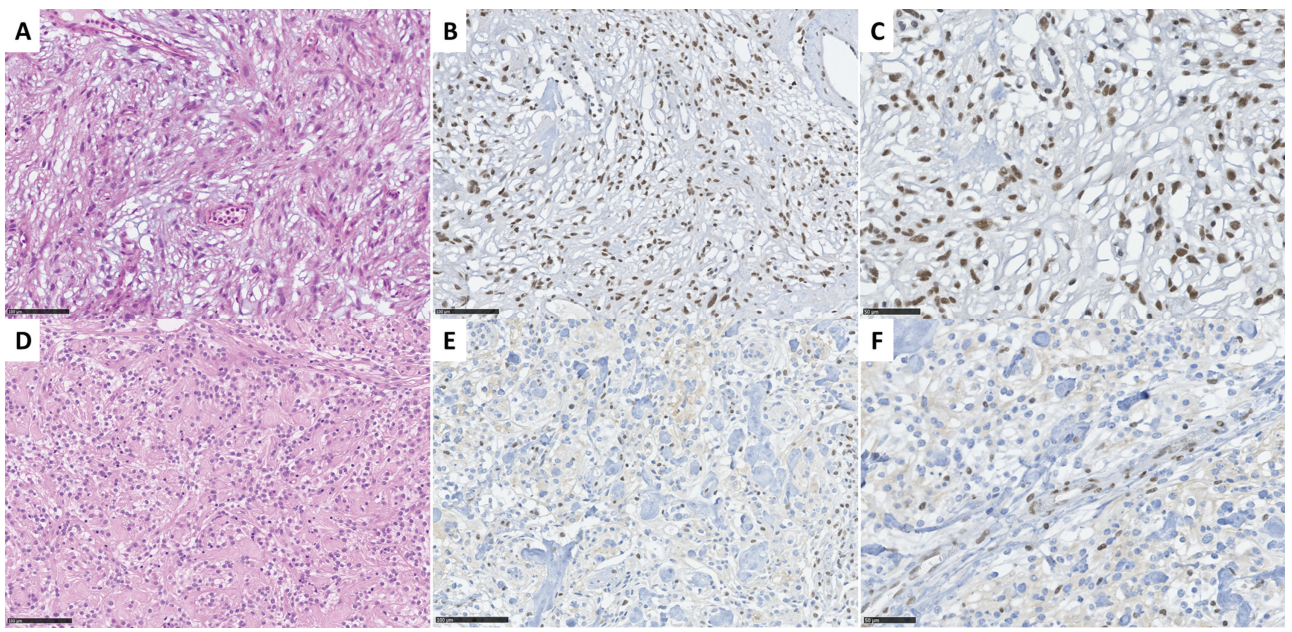

Figure 2. Histopathological and immunohistochemical findings. **A–C** a microcystic meningioma characterized by thin, elongated processes with ovoid nuclei (HES, 270×); preserved SMARCE1 nuclear staining in all tumor cells (280× and 400×). **D–F** a clear cell meningioma characterized by round to polygonal monotonous cells with clear cytoplasm, intermingled with thick interstitial collagen fibers (270×); loss of SMARCE1 nuclear staining in all tumor cells, note the preserved staining in endothelial cells (280× and 400×).

Table 2. Histopathological, immunohistochemical, and molecular findings of the CCM and MM study cohort.

Case	Histopathological subtype, grade	Mitotic index (/1.6 mm^2)	Loss of SMARCE1 immunoexpression	*SMARCE1(NM_003079)* sequence	Bi-allelic loss of function	*SMARCB1 (NM_003073)* sequence	*NF2 (NM_000268)* sequence	*SUFU (NM_016169)* sequence
1	CCM, II	1	1	Hit 1: Intron7 3′ acceptor site c.542–2A>G, p.? Hit 2: Exon 9 c.715C>T, p.(Arg239*)	1	WT	WT	WT
2	CCM, II	1	1	WT (CDS)/CNV: NI	NI	WT (CDS)/ CNV: NI	WT (CDS)/CNV: NI	WT (CDS)/CNV: NI
3	CCM, II	7	1	Hit 1: Exon7 c.472C>T, p.(Arg158*) Hit 2: Exon10 c.925_929delGAGCA, p.(Glu309Serfs*2)	1	WT	WT	WT
4	CCM, II	1	1	Hit 1: Intron 6 5′ donor site c.369 + 1G>A, p.? Hit 2: LOH by whole gene deletion	1	WT	WT	WT
5	CCM, II	0	1	Hit 1: Exon 5 c.197delC, p.(Pro66Glnfs*5) Hit 2: LOH by isodisomy	1	WT (CDS) CNV: deletion	WT (CDS) CNV: deletion	WT
5	CCM, II	0	1	Hit 1: Exon 5 c.197delC, p.(Pro66Glnfs*5) Hit 2: Exon 8 c.624_627delTGAG, p.(Ser208Argfs*26)	1	WT	WT	WT
6	CCM, II	0	1	Hit 1: Intron 7 3′ acceptor site = c.542–1G>A, p.? Hit 2: Exon 8 c.547_548delGA, p.(Asp183*)	1	WT	WT	WT
7	CCM, II	0	1	Hit 1: Exon 6 c.313C>T, p.(Arg105*) Hit 2: Intron 6 c.369 + 1G>T, p.?	1	WT	WT	WT
8	CCM, II	3	1	Hit 1: Intron 6 5′ donor site c.369 + 1G>T, p.? Hit 2: Exon 7 c.458T>G, p.(Leu153*) CNV: NI	1	WT (CDS)/ CNV: NI	WT (CDS)/CNV: NI	WT (CDS)/CNV: NI
9	CCM, II	3	1	Hit 1: Exon 8 c.633_659delGGTGCCA-GACGTTCGGTCAGTTGTCACinsT, p.(Val212Asnfs*3) Hit 2: LOH by whole gene deletion	1	WT	WT (CDS) CNV: deletion	WT
10	CCM, II	0	1	Hit 1: Intron 6 5′ donor site c.369 + 2_+5delTAGG, p.? Hit 2: Intron 6 3′ acceptor site c.370–4delTT, p.?	1	WT	WT	WT
12	MM, I	2	1	WT (CDS)/CNV: NI	0	WT	WT	WT
13	MM, I	0	0	WT	0	WT (CDS) CNV: deletion	WT (CDS) CNV: deletion	WT
14	MM, I	0	0	WT	0	WT	WT	WT
15	MM, I	0	0	WT	0	WT	WT	WT
16	MM, I	1	0	WT	0	WT	WT	WT
17	MM, I	1	0	WT	0	WT	WT	WT
18	MM, I	1	0	WT (CDS)/CNV: NI	0	WT (CDS) CNV: deletion	WT (CDS) CNV: deletion	WT

Table 2. *Continued.*

Case	Histopathological subtype, grade	Mitotic index (/1.6 mm^2)	Loss of SMARCE1 immunoexpression	*SMARCE1(NM_003079)* sequence	Bi-allelic loss of function	*SMARCB1 (NM_003073)* sequence	*NF2 (NM_000268)* sequence	*SUFU (NM_016169)* sequence
19	MM, I	0	0	WT	0	WT (CDS) CNV: deletion	Hit 1: Intron 11 c.1122 + 1G>C Hit 2: LOH by isodisomy (absence of deletion)	WT (CDS) CNV: deletion
20	MM, I	0	0	WT	0	WT (CDS) CNV: deletion	WT (CDS) CNV: deletion	WT (CDS) CNV: deletion
21	MM, II	8	0	WT	0	WT	WT	WT
22	MM, I	2	0	WT	0	WT	WT	WT
23	MM, I	1	0	WT	0	WT (CDS) CNV: deletion	WT (CDS) CNV: deletion	WT
24	MM, I	2	0	WT	0	WT	WT	WT

CCM: clear cell meningioma; CDS: coding sequence; CNV: cony number variation analysis; MM: microcystic meningioma; NI: noninterpretable; WT: wild-type (coding sequence and copy number).

control (Figure 2E,F). To note, tumor cells often presented a weak cytoplasmic staining. The tumor with an inconclusive histopathological subtype was SMARCE1 immunoreactive (sufficient material was not available for molecular analysis). The histopathological and immunohistochemical results are summarized in Table 2.

Targeted next generation sequencing (NGS) screening

Sufficient material for NGS-based sequencing was available for 25 meningiomas. Among them, we were able to perform a complete gene copy number analysis on 21 meningiomas (10 CCMs and and 11 MMs) due to the expected inconsistent quality of DNA extracted from the FFPE samples. The genotyping results are summarized in Figure 3 and Table 2.

We observed no mutational *SMARCE1* hits in any of the 11/11 MMs tested, whereas there were two losses of function *SMARCE1* hits in all SMARCE1-negative CCMs (10/10). By sequencing, *SMARCE1* alterations were predicted to induce loss of function of the protein, with three nonsense, six frameshift and six splice variants. We also observed LOH by gene deletion for only two CCMs and copy neutral LOH for one tumor. *SMARCE1* mutations did not always occur to the exclusion of *NF2/SMARCB1* alterations. We observed the co-occurrence of a *NF2* deletion alone, or combined with a *SMARCB1* deletion, in two *SMARCE1*-mutated CCMs. We also identified *NF2* whole gene deletions in conjunction with a *SMARCB1* deletion in 4/11 MMs. Of note, we were able to identify an exon-intron boundary *NF2* mutation c.1122 + 1G > C in one of these four MMs. Only one patient (case 5) could be analyzed for germline DNA: germline mutation of *SMARCE1* was evidenced.

We did not observe point mutation of *SUFU* gene but monoallelic deletions were identified in two *SMARCB1-NF2*-deleted MMs.

Statistical analyses

All SMARCE1 negative CCMs presented a bi-allelic *SMARCE1* alteration. The correlation coefficient (Cohen's kappa coefficient) for these methods was excellent (1.00).

Meningioma and clear cell non-meningioma validation cohorts

All 305 other various subtypes of meningiomas (100%) and 15 (100%) clear cell mimicking tumors were diffusely immunoreactive for SMARCE1.

DISCUSSION

Four meningioma variants, such as clear cell, chordoid (grade II), and papillary and rhabdoid (grade III) recur more frequently than others. The distinction between CCM from other variants is thus important in deciding on patient management and establishing a prognosis. Based on our extensive literature review of 234 CCMs (cf. Table S1 Supporting Information), we estimate that 45% of patients with CCM had tumor recurrence (local in 84%, local and other distant locations in 11%, and only distant location in 5% of reported cases) during the mean follow-up of 45 months ((3, 5, 6, 8, 10–12, 14, 15, 18, 19, 22, 23, 27, 29, 32, 34, 36–41, 43), cf.

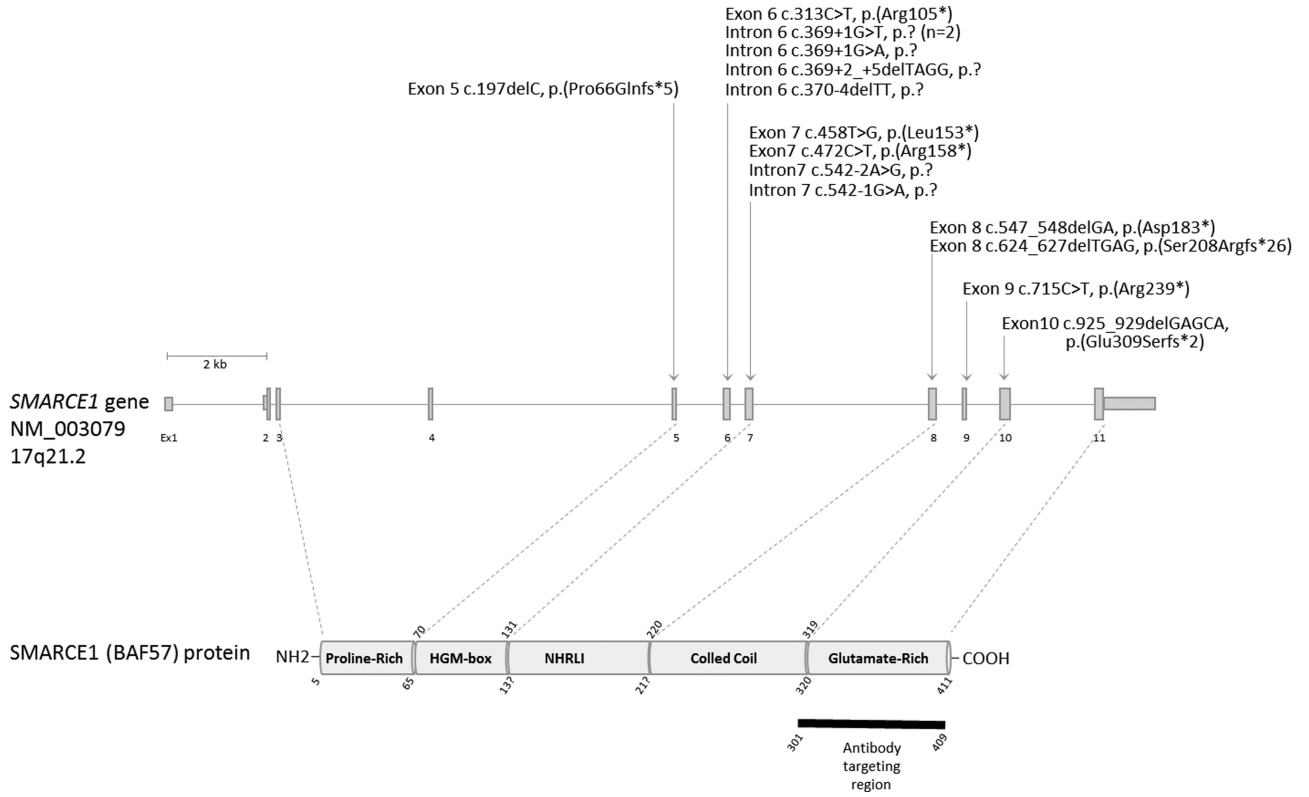

Figure 3. Schematic representation of the *SMARCE1* gene and the corresponding encoded protein. Exons are depicted as vertical bars and numbered from exon 1 to exon 11. The position of the *SMARCE1* variants identified in this study are indicated above the gene.

review in (48)). The locations of recurrence were distant intracranial in 40% of cases, distant neuraxis in 53% of cases and systemic (pulmonary) in 7% of cases ((18, 26), cf. review in (48)). This strongly contrasts with the recurrence rate of about 7–25% for benign meningiomas (23). Moreover, a retrospective literature analysis showed only 20 cases of multiple CCMs at diagnosis or during follow-up (20/234, 8.5%; 8.3% in our series). This proportion did not differ from the other histopathological subtypes (less than 10%) (23). These data suggest that the pejorative prognosis of CCM is not due to a higher frequency of meningiomatosis but rather to an intrinsic aggressivity. In light of the literature data, the proportion of hereditary forms of CCM *versus* sporadic cases still needs to be elucidated. Smith *et al*. have showed over the past years the presence of *SMARCE1* alterations in familial cases of CCM (39–41). Moreover, given the paucity of adjuvant treatment administered in our retrospectively discovery cohort, strong conclusions about prognosis cannot be made.

In our study, two thirds of the identified *SMARCE1* molecular variants are predicted to cause the truncation or absence of SMARCE1 protein, well explaining the negative SMARCE1 staining, targeting the COOH terminal amino acids 301—409. We were unable to investigate the functional consequences of the remaining third of *SMARCE1* variants, corresponding to splice variants. However, immunohistochemical analysis clearly showed that the corresponding proteins are likely truncated or absent. The loss of SMARCE1 protein expression appears to be a sensitive and specific test for the diagnosis of CCM, as SMARCE1 immunostaining was lost in 12/12 (100%) of the CCMs in this study. These results confirm the data of Smith *et al*. in a smaller series of syndromic and sporadic CCMs (39). Our study constitutes the first proof of the diagnostic value of the loss of SMARCE1 expression by validation on a cohort of 320 adult and pediatric meningiomas of various grades and subtypes. SMARCE1 immunostaining could be routinely used to aid diagnosis as it is easy to interpret with a positive internal nuclear control in endothelial and inflammatory cells. As the expression was homogeneous and easy to interpret, it was lawful to analyze this staining on TMA. In case of artifacts (as one case of our study) or if meningioma subtyping problems due to tumor heterogeneity (as two tumors of Smith's study), SMARCE1 immunoexpression permit to reclassification of the meningioma variant (39). Furthermore, none of the morphological mimickers of CCMs tested displayed a loss of SMARCE1 expression. Moreover, the loss of expression correlates well with the molecular status of *SMARCE1* (Cohen's kappa coefficient was 1.00). However, we were unable to identify the *SMARCE1* gene mutations in a single case of SMARCE1-negative CCM. This discrepancy may due to the low quality of the DNA due to long-term storage of FFPE samples (more than 30 years), making it impossible to assess the gene copy number. This may also be explained by the presence of a genetic alteration in non-coding sequences or a post-translational alteration, underlining the usefulness of protein expression testing in the diagnosis.

The epidemiological characteristics of our series are in agreement with the literature data, showing the lower CCM patients

mean age (26.2 years) than patients with "non clear cell" meningiomas (53.1 years) ($P < 0.001$). The female-to-male ratio (= 3.7) was also significantly higher than CCM patients ($P = 0.019$). The literature review showed that CCM is mainly located in the spine (26% of reported pediatric cases) ((8, 10, 11, 14, 15, 18, 19, 22, 23, 25, 27, 29, 34, 36–38, 40, 43), cf. review in (48)) or posterior fossa (49% of reported pediatric cases) ((5, 6, 32, 36, 37, 39, 43), cf. review in (48)). The anatomical distribution of reported CCM cases according to the age of patients is summarized in Figure S1 (Supporting Information). Our series is not consistent with the literature concerning the location of the CCMs, with a high proportion at the base of the skull (58%). This discrepancy is possibly due to the fact that some of our neurosurgical teams are more implicated in skull-base and cranial surgery than surgery of the spine.

In light of these data, we suggest genetic *SMARCE1* testing and counseling for patients with a familial history of CCM or CCM occurring in young patients (<26 years), particularly if the tumor is located in the posterior fossa or the spine. Although this tumor is rarely fatal (only 5% of the reported patients died of their disease and 8% in our series, all children) (6, cf. review in 48), the presence of this alteration may alter the management of these patients, with close radiological monitoring (magnetic resonance imaging –MRI- of the brain and the spine) to prevent the apparition of further tumors. In our series, a germline mutation of *SMARCE1* was identified in a young patient (case 5) with multiple meningiomas.

Somatic misactivation of the Hedgehog pathway is reported in meningioma (10, 42). Moreover, a germline *SUFU* gene variant has been reported in a large family presenting multiple meningiomas (1). Hence, *SUFU* gene was considered in our NGS custom design. No *SUFU* point mutation was identified in CCMs. We identified only copy number alterations in a subset of *SMARCB1* and *NF2*-deleted MMs. The consequence of the mono-allelic loss of *SUFU* gene in the tumorigenesis of these MMs remains to be studied. Likewise, we have showed that *SMARCE1* mutations identified in CCMs were not exclusive of *NF2/SMARCB1* alterations as co-occurrence of *NF2* solely or combined to a *SMARCB1* deletion was observed in two *SMARCE1*-mutated CCMs. How mono-allelic inactivation of *NF2* (and *SMARCB1*) contribute to the CCM tumorigenesis remains to be investigated.

In summary, our study showed that the loss of SMARCE1 expression is a useful tool, sensitive and specific for diagnosing CCM.

DECLARATION OF INTERESTS

All authors declare they have no conflicts of interest related to this work.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Clinical and molecular data of reported cases of clear cell meningiomas in the literature.

Figure S1. Anatomical distribution of reported clear cell meningiomas (CCMs) in the literature according to age: most pediatric tumors are located in the posterior fossa and the spine, whereas adult tumors are mainly cranial and in the posterior fossa.