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CASE REPORT

Molecular progression in unusual recurrent non-pediatric intracranial clear cell meningioma

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ABSTRACT

We report a case of a recurrent clear cell meningioma (CCM) in the frontal lobe of the brain of a 67-year-old man. The patient developed three recurrences: at 3, 10, and 12 years after his initial surgery. Histopathology observations revealed a grade 2 CCM with positivity for vimentin and epithelial membrane antigen. Expression of E-cadherin was positive only in the primary tumour and in the first available recurrence. Fluorescence *in situ* hybridization analyses demonstrated 1p and 14q deletions within the last recurrence. Multiplex ligation-dependent probe amplification studies revealed a heterozygous partial *NF2* gene deletion, which progressed to total loss in the last recurrence. The last recurrence showed homozygous deletions in *CDKN2A* and *CDKN2B*. The *RASSF1* gene was hypermethylated during tumour evolution.

In this report, we show the genetic alterations of a primary CCM and its recurrences to elucidate their relationships with the changes involved in the progression of this rare neoplasm.

Key Words Clear cell meningioma, recurrence, intracranial disease, non-pediatric disease, genetics, *NF2*, tumour suppressor genes, molecular progression

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INTRODUCTION

Clear cell meningioma (CCM) classified as grade 2 is a rare, potentially aggressive entity that represents about 0.2% of all meningiomas, with most cases being described in young women[1–5]. Surgical resection and postoperative radiotherapy are the standard treatment for primary disease and local recurrences.

The genesis of meningioma is associated with loss of genetic material on chromosome 22. Monosomy of that chromosome is the most common genetic alteration in meningioma and is linked to mutations of the *NF2* gene, located in 22q12.2, which codes for the tumour suppressor protein merlin[6]. Losses of 1p and alterations in chromosome 14 are present in many atypical meningiomas and have been associated with tumour progression[7,8].

Here, we report a case of grade 2 CCM that arose in the right frontal lobe of a 67-year-old man and recurred three times. Histologic and genetic studies of the primary tumour and its recurrences showed changes in *NF2* and other tumour suppressor genes. We analyzed the evolution and implications of the various genetic aberrations present in this neoplasm with the aim of elucidating the genetic changes involved in the progression of CCM.

CASE DESCRIPTION

A 67-year-old man presented with a mass (12 cm^3) in the right frontal lobe, which was histologically diagnosed as a grade 2 CCM. The tumour, resected in a surgical procedure, was sporadic, and the patient did not show any feature of *NF2* disease. A control magnetic resonance imaging exam 3 years after the initial surgery revealed a recurrence of the tumour in the same location, which was treated with radiosurgery (15 Gy). No material from the recurrence could be saved. Another recurrence (37 cm^3), called the "first recurrence" in the present work, was resected 7 years after the second surgery. The frontal lesion again recurred (9 cm^3) 2 years later, and it (here called the "second recurrence") was resected. In all surgeries, the resection was maximal, and neither chemotherapy nor radiotherapy was applied. To date, further control exams have not shown new recurrences.

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Pathology Examination

Tumour sections were stained with hematoxylin and eosin. Antibodies against vimentin, epithelial membrane antigen, CD34, E-cadherin, and glial fibrillary acidic protein were assayed and valued as negative or positive. A Ki-67/MIB-1 antibody was used to estimate the proliferation index by determining the percentage of immunopositive nuclei. Results were defined as low proliferation (≤1% stained nuclei), moderate proliferation (1%–5%), or high proliferation (≥5%)[9]. All antibodies were purchased from Dako (Dako Diagnostics, Barcelona, Spain).

Fluorescence In Situ *Hybridization*

The fluorescence *in situ* hybridization (FISH) probes used were LSI-1p36/LSI-1q25 (red and green signals respectively) and IGH/CCND1-DF to detect loss of chromosome 14, with chromosome 11 acting as a control (Vysis: Abbott Molecular, Madrid, Spain). One signal or less per chromosome with respect to the control signal was considered to be a deletion, and a 2:2 ratio was considered normal[8].

Multiplex Ligation-Dependent Probe Amplification

Biopsy punches from selected areas of paraffin blocks from each tumour sample were used for DNA extraction with the QIAmp DNA FFPE tissue kit (Qiagen, Valencia, CA, U.S.A.). For multiplex ligation-dependent probe amplification (MLPA) assays, SALSA MLPA probemix P044-B1-NF2 and MS-MLPA (MLPA and methylation-specific MLPA) and probemix ME001-C2 tumour suppressor 1 kits were used (MRC-Holland, Amsterdam, Netherlands). Losses in 1p34 and 14q13 were detected with reference probes 14839-L16547 and 01063-L00061 included in the P044-B1-NF2 kit. All probe sequences, gene loci, and chromosome locations can be found at http://www.mlpa.com. Values between 0.7 and 1.3 were considered to be the normal genetic dose. Results between 0.7 and 0.3 were interpreted as heterozygous deletion. Results below 0.3 were interpreted as homozygous deletion[10].

Statistical Analysis

Data are expressed as means with standard error. Statistical differences were determined using the Student t-test for dual comparisons. Data were analyzed in the GraphPad Prism software application (version 6: GraphPad Software, San Diego, CA, U.S.A.). Significance was accepted at $p < 0.05$.

RESULTS

Pathology

The primary tumour, diagnosed as CCM, had moderate cellularity and was composed of sheets of polygonal cells with small round monomorphic nuclei and clear cytoplasm [Figure 1(A)]. Increase of cellularity, pleomorphism, nucleolar prominence, and focal micro-necrosis were observed in the recurrences [Figure 1(B,C)]. Additionally, the last recurrence showed chondroid areas [Figure 1(D)].

Immunohistochemistry

Vimentin and epithelial membrane antigen expression were strongly positive in all samples (Figure 2). In addition, expression of the glial fibrillary acidic protein was negative in both the original tumour and the two recurrences (data not shown). In the various samples, CD34 revealed regular (no hyperplastic) vascularity. Expression of E-cadherin showed important changes: It was expressed in the primary tumour and in the first recurrence, but was absent in the last recurrence. With respect to Ki-67 expression, the primary tumour presented a low proliferation index, the first recurrence was assessed as moderate, and the last recurrence had a high proliferation index (Figure 2).

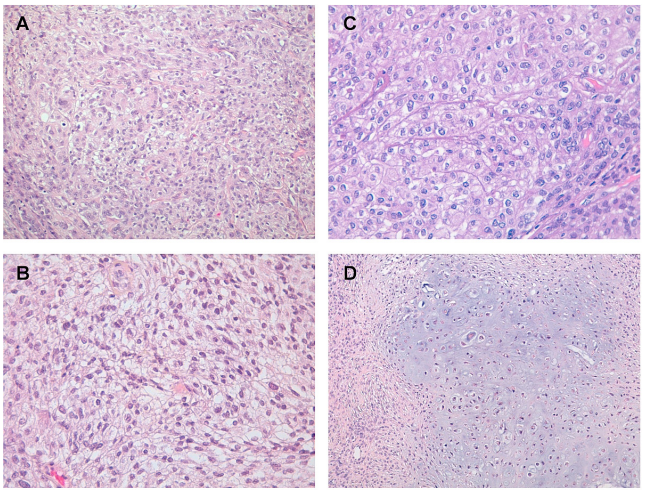


FIGURE 1 Histopathologic pattern of the reported clear cell meningioma: (A) primary tumour; (B) first recurrence; (C) second recurrence; (D) chondroid area in the second recurrence. Hematoxylin and eosin stain, 40× original magnification.

FISH

All samples underwent FISH studies for chromosomes 1p and 14q, but the quality of the primary tumour and first recurrence samples was suboptimal, being unsuitable for FISH. Analysis of the second recurrence revealed partial losses of chromosomes 1p and 14q [Figure 3(A)].

MLPA

Integrity of the *NF2* exons was studied by MLPA. The primary tumour and the first recurrence presented a heterozygous deletion of 15 exons from among the 17 total exons (exons 2–16); in the second recurrence, the entire codifying region of *NF2* was deleted. The *AP1B1* and *CABP7* genes that flank *NF2* were also deleted in the last recurrence [Figure 3(B)]. In the three samples studied, 1p34 was lost. On the other hand, chromosome 14q13 was in the normal range in the primary tumour and the first recurrence, but lost in the last recurrence [Figure 3(A,B)].

In addition to *NF2*, other tumour suppressor genes were studied [Figure 3(C)]. Heterozygous and homozygous losses of *CDKN2A/2B* were found within the first and the second recurrences respectively. Both recurrences presented *ESR1* and *TP73* heterozygous deletions. Moreover, the last recurrence also presented heterozygous deletions in *PTEN* and *TIMP3*. Study of the methylation status of tumour suppressor genes showed hypermethylation of *RASSF1* in the second recurrence, with *TP73* proving negative in every sample.

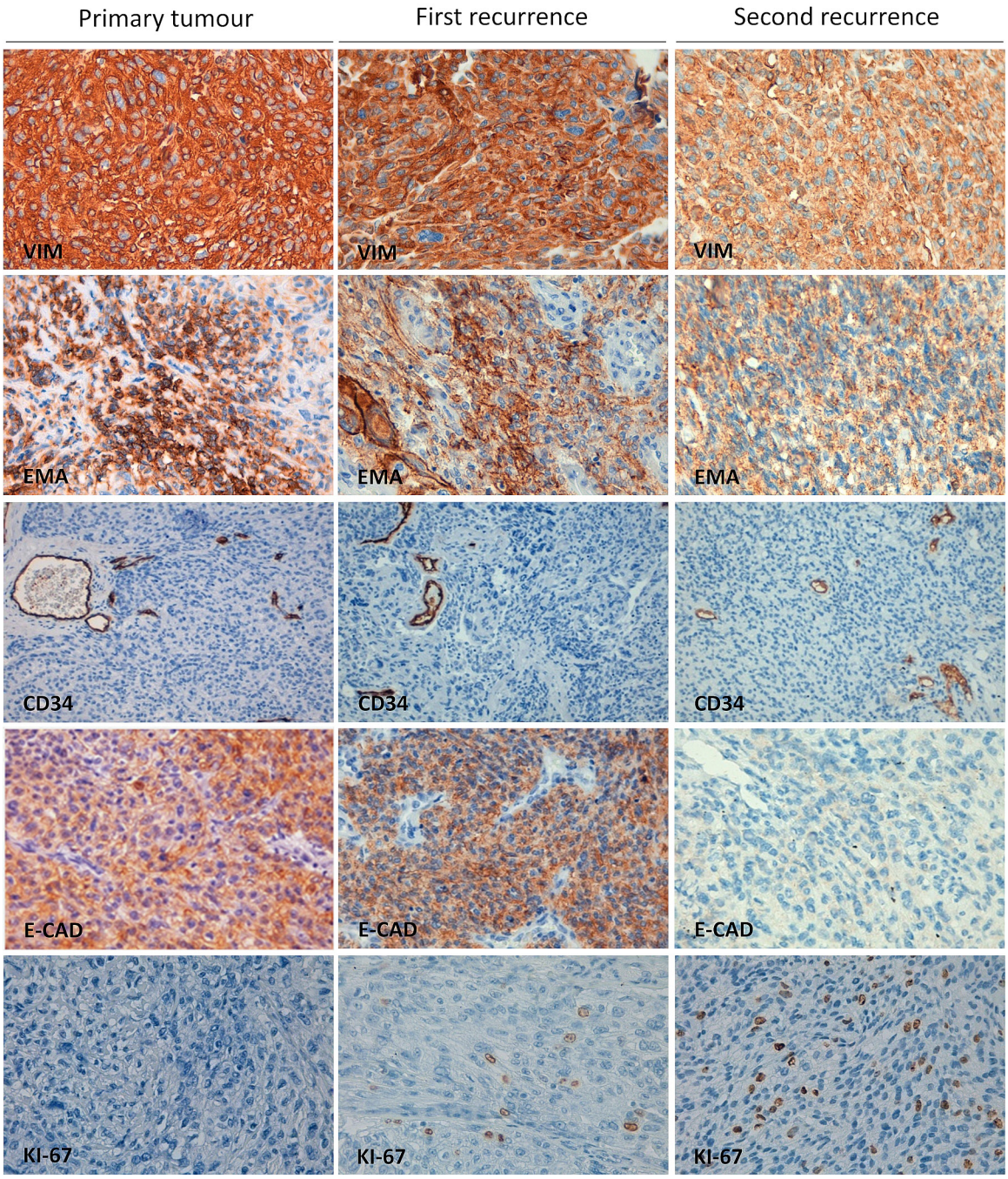


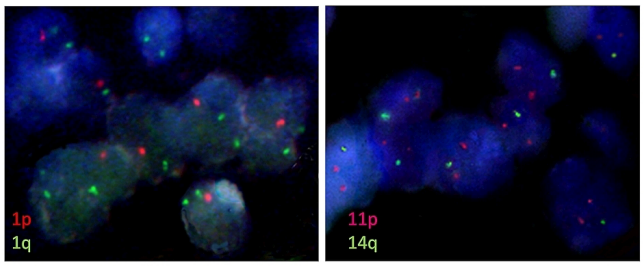
FIGURE 2 Immunohistochemical stains. Left column: Primary clear cell meningioma tumour. Middle column: First recurrence. Right column: Second (last) recurrence. VIM = vimentin; EMA = epithelial membrane antigen; E-CAD = E-cadherin. Each micrograph is representative from various areas of the same recurrence (40× original magnification).

DISCUSSION

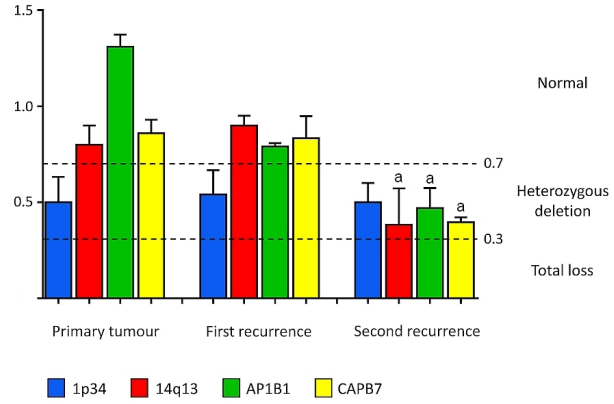
Although CCM has been considered to occur mainly in the pediatric age range, the literature describes a considerable number of cases of intracranial CCM in the middle-aged and elderly population (Table I). Tumour aggressiveness and recurrence rates are frequently greater than those seen with benign grade 1 meningiomas[17,26]. The second recurrence in our patient with CCM showed areas of chondroid differentiation. Some hypotheses explain the local differentiation as a reversible change in which some meningeal cells are replaced by chondroid cells[27], although the reason that such metaplasia occurs in specific tumours remains unclear.

Loss of E-cadherin expression with tumour progression suggests that CCM cells would have lost cellular junctions, which would be directly related to the biologic behaviour of tumour cells[28].

A



B



C Losses of tumour suppressor genes detected by MLPA

Gene	*Locus*	Primary tumour	First Recurrence	Second Recurrence
CASP8	2q33.1	N	N	N
FHIT	3p14.2	N	N	N
CTNNB1	3p21.3	N	N	N
RASSF1-1	3p21.3	N	N	N
MLH1	3p22.3	N	N	N
VHL	3p25.3	N	N	N
CASR	3q21	N	N	N
APC	5q22	N	N	N
ESR1	6q25.1	N	HTD[b]	HTD
PARK2	6q26	N	N	N
CDKN2A	9p21	N	HTD[b]	HD[c]
CDKN2B	9p21	N	HTD[b]	HD[d]
DAPK1	9q22	N	N	N
CREM	10p12.1	N	N	N
PTEN	10q23	N	N	HTD[c]
CD44	11p13	N	N	N
GSTP1	11q13	N	N	N
CHFR	12q24	N	N	N
BRCA2	13q12.3	N	N	N
MLH3	14q24.3	N	N	N
TSC2	16p13.3	N	N	N
CDH13	16q23	N	N	N
HIC1	17p13.3	N	N	N
TP73	1p36.32	N	HTD[b]	HTD
TIMP3	22q12.3	N	N	HTD[d]

HD: homozygous deletion; HTD: heterozygous deletion; N: normal gene dose.

FIGURE 3 (A) Fluorescence *in situ* hybridization in paraffin-embedded tissue sections from the second recurrence of the clear cell meningioma. Left panel: One red signal indicates the loss of 1p36 region; 1q25 region (two green signals) acts as control. Right panel: One green signal evidences the loss of one chromosome 14q; two red signals indicate the presence of chromosome 11 (control). (B) Multiplex ligation-dependent probe amplification (MLPA) study of chromosome 1p34, chromosome 14q13, *AP1B1,* and *CABP7* in primary tumour, first recurrence, and second recurrence. Values between 0.7 and 1.3 correspond to normal gene doses. Values between 0.3 and 0.7 correspond to heterozygous deletion. Values below 0.3 correspond to total loss. Results are expressed as mean ± standard error of the mean, from independent experiments performed on three fragments of each tumour. [a]$p < 0.05$ with respect to first-recurrence results. (C) Losses of tumour suppressor genes detected by MLPA. N = normal gene dose; HTD = heterozygous deletion; HD = homozygous deletion. Results from each gene—mean ± standard error of the mean—are obtained from independent experiments performed on three fragments of each tumour. [b]$p < 0.05$ with respect to primary tumour results. [c]$p < 0.05$ and [d]$p < 0.01$ with respect to first recurrence results.

Genetically, the most important abnormality in meningioma is the loss of heterozygosity of tumour suppressor gene *NF2*[29,30]. In the primary tumour and the first recurrence, 15 exons were deleted; in the last recurrence, every *NF2* exon was lost, showing that loss of *NF2* exons can progress during CCM progression. Furthermore, to our knowledge, no studies have considered the status of the *AP1B1* and *CABP7* genes in meningioma. Those genes, adjacent to *NF2,* also were found to be deleted, in heterozygosis, in the last recurrence of our patient's CCM. According to those results, we suggest that progressive deletions in *NF2* carry other associated deletions that involve different regions in chromosome 36.

The most frequent progression-associated genetic aberration in meningioma is the total or partial deletion of chromosome 1p[31]. Del(1p) was evidenced in the primary tumour by MLPA, but loss of chromosome 14q, homozygous deletion of *CDKN2A*/*2B* and heterozygous deletions of *PTEN* and *TIMP3* were evidenced only in the last recurrence. Those findings are associated with tumour progression. *PTEN* and *TIMP3* have been identified as candidates implicated in the progression of malignant meningiomas[26,32]. Heterozygous deletions of *ESR1* and *TP73* in the first recurrence suggest that they are earlier changes during the acquisition of aggressiveness.

Some earlier studies described *TP73* hypermethylation as the mechanism involved in the suppression of its expression[29], but other studies claim that *TP73* expression could be higher in anaplastic meningiomas, but lower because of hypermethylation in low-grade meningiomas[33]. In the present case, *TP73* was not methylated in any tumour. *ESR1* was deleted in heterozygosis in the recurrences, but its relationship with malignant progression remains unclear[34]. Hypermethylation in *RASSF1* occurs in 63% of atypical meningiomas[35]. In our case, we found hypermethylation in the last recurrence, thus indicating epigenetic differences along the evolutionary path of the tumour.

SUMMARY

Here, we describe the genetic changes occurring during the evolution of a CCM, through the primary tumour and two recurrences. A progressing heterozygous loss of *NF2*

TABLE I Brief literature review of cases of intracranial clear cell meningioma in patients more than 45 years of age

Reference	Patient characteristics		
	Age (years)	Sex	Tumour location
Shiraishi, 1991[11]	49	Female	Left frontal
Pimentel *et al.*, 1998[12]	61	Male	Convexity
Kuzeyli *et al.*, 2003[13]	52	Female	Right frontal and right occipital regions
	50	Male	Left temporal fossa
Jain *et al.*, 2007[14]	47	Male	Left Sphenoid wing
	65	Female	Left tentorium
Tena-Suck *et al.*, 2007[4]	62	Female	Right parasagittal
	45	Female	Right frontal lobe
	62	Female	Right frontal lobe
Cassereau *et al.*, 2008[15]	50	Male	Right lateral ventricle
Pizzoni *et al.*, 2008[16]	66	Female	Olfactory groove
Ohba *et al.*, 2008[17]	60	Male	Foramen magnum
	60	Male	Right posterior clinoid
Prayson *et al.*, 2008[18]	58	Male	Left sphenorbital
	72	Male	Left frontal
	76	Male	Right frontal
	56	Male	Left cavernous sinus
	80	Female	Left frontal lobe
	49	Male	Left cavernous sinus
	69	Female	Left cavernous sinus
	70	Male	Right frontal lobe
	72	Female	Right frontal lobe
	45	Male	Right temporal lobe
	79	Male	Right clinoidal
Tong-tong *et al.*, 2010[19]	65	Male	Right frontal
Chen *et al.*, 2011[20]	62	Male	Right parietal lobe
	56	Female	Left hypoglossal canal
	63	Male	Left tuberculum sellae
Chen *et al.*, 2011[21]	79	Male	Parasagittal
	77	Male	Parasagittal
	71	Female	Convexity
	56	Male	Frontal base
	69	Female	Tentorium
	56	Male	Parasagittal
	59	Male	Parasagittal
	47	Male	Parasagittal
	88	Male	Parasagittal
Hori *et al.*, 2012[22]	65	Male	Left frontal lobe
Wang *et al.*, 2014[23]	56	Male	Left tuberculum sellae
	53	Female	Left hypoglossal canal
	62	Male	Right parietal lobe
Li *et al.*, 2016[24]	50	Female	Right parietal lobe
	63	Female	Left temporal lobe
	77	Male	Left parietal lobe
	65	Female	Left frontal lobe
	64	Male	Parafalx
	48	Female	Foramen magnum
Yin *et al.*, 2016[25]	55	Male	Intrasellar
Present case	67	Male	Right frontal lobe

exons, together with deletions of 1p and 14q, was detected related with progression of the meningioma. *AP1B1* and *CABP7* deletions are described here for the first time in CCM. Additionally, deletions of *CDKN2A*/*2B* and suppression of *RASSF1* function were evidenced and should be considered factors involved in the advancement of the tumour and important with respect to the therapy for this neoplasm.

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Informed consent of the patient was obtained, and approval for the study reported here was given by the Institutional Ethics Committee of the University of Valencia and Clinic Hospital of Valencia. This work was supported by grants PROMETEOII/2015/007 from Generalitat Valenciana and PI14/01669 from the Instituto de Salud Carlos III and the Spanish Ministerio de Economía y Competitividad. We acknowledge the Fundación Investigación Hospital Clínico de Valencia and the Instituto de Investigación Sanitaria.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

AUTHOR AFFILIATIONS

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