Acta Neuropathologica Communications

RESEARCH

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Refining prognostic stratification of atypical meningiomas: significance of chromosome 1p deletion and brain invasion

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Abstract

Atypical meningiomas display heterogeneous clinical outcomes, necessitating prognostic markers to identify cases that would benefit of adjuvant treatment. This study investigated the prognostic value of chromosome 1p deletion, assessed by fluorescent in situ hybridization (FISH), in a cohort of 98 primary atypical meningiomas. The accuracy of FISH was validated by comparison with next-generation sequencing (NGS) results. Chromosome 1p deletion was significantly associated with parafalcine/tentorial location, high mitotic index, recurrence, and shorter recurrence-free survival (RFS). Multivariate analysis confirmed the presence of 1p deletion as an independent prognostic factor for shorter RFS. The study also evaluated the immunohistochemical expression of MCM2 and ACADL, which were more frequent in 1p-deleted tumors, but could not reliably predict 1p status. Brain-invasive otherwise benign (BIOB) meningiomas had significantly lower rates of 1p deletion, MCM2 expression, and recurrence, than mitotically active atypical meningiomas. However, recurring BIOB meningiomas showed higher frequencies of MCM2 expression, spontaneous necrosis, and 1p deletion, suggesting that these features may identify BIOB cases with a higher recurrence risk. In conclusion, FISH-detected 1p deletion is a reliable prognostic marker for atypical meningiomas, and its assessment, along with histopathological and immunohistochemical features, can refine the prognostic stratification of these tumors.

Keywords Meningioma, 1p, Atypical, Recurrence, FISH

Introduction

Meningiomas are the most common primary tumors of the Central Nervous System (CNS), accounting for approximately 41% of intracranial neoplasms in adults [26]. According to the fifth edition of the World Health Organization (WHO 2021) classification of CNS tumors, they are categorized into 15 histological subtypes and three grades of malignancy [32]. The WHO grading of meningiomas represents a major prognostic determinant for these tumors; it is currently based on histopathological features as well as on the presence of *CDKN2A/B* homozygous deletion and *pTERT* mutation [17]. However, additional factors are required to refine the

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prognostic stratification of WHO grade 2 meningiomas, which exhibit a widely heterogeneous clinical outcome, to identify high-risk cases that would benefit of adjuvant therapy.

Several factors may contribute to the variable clinical outcomes of WHO grade 2 meningiomas. Firstly, the atypical subtype of WHO grade 2 meningioma encompasses tumors with a broad range of histopathological features, including a mitotic index between 4 and 19 mitoses per 1.6 mm^2 or brain invasion, or other minor atypical criteria [32]. These differences might be associated with varying levels of biological aggressiveness. Indeed, among atypical meningiomas, those featuring brain invasion in the absence of other "atypical" criteria, so-called brain-invasive otherwise benign (BIOB) meningiomas, demonstrate a more indolent behavior, similar to that of grade 1 tumors [6, 22, 28, 36]. Moreover, tumors with overlapping histopathological features may harbor different genetic and epigenetic characteristics, which can result in different clinical outcomes [8, 10, 20, 25, 27, 34]. For instance, several studies have demonstrated the prognostic significance of chromosome 1p deletion in meningiomas [10, 19, 20, 34, 38], and two studies have suggested that 1p deletion could be used to stratify the recurrence risk of atypical meningiomas [20, 38]. A key drawback of these studies is that 1p deletion was analyzed using highly sophisticated technologies, such as DNA methylation or genomic arrays, which may limit its widespread application in routine practice. To the best of our knowledge, only one study conducted in 2001 investigated 1p deletion in atypical meningiomas by Fluorescent In Situ Hybridization (FISH) using two probes against 1p36 and 1p32. However, this study did not find any prognostic value associated with this genetic alteration [7]. Another study classified meningiomas into four molecular groups (MG1, MG2, MG3 and MG4), each with a distinct prognosis and enriched in the immunohistochemical expression of a specific protein [25]. Of note, 1p deletion was exclusive to MG3 and MG4, which were enriched in ACADL and MCM2 expression and displayed poorer prognosis [25].

Based on these premises, this study aimed to evaluate the prognostic value of chromosome 1p deletion determined using FISH in meningiomas classified as atypical based only on histopathological criteria. Given that FISH is commonly used for detecting 1p deletion in oligodendrogliomas, this technique could be quickly integrated into routine practice for meningioma analysis. The experimental approach was set to validate the accuracy of FISH by comparison with next-generation sequencing (NGS) results. Moreover, we aimed at analyzing the correlation of 1p deletion and the immunohistochemical expression of MCM2 and ACADL, utilized as surrogates for MG3 and MG4, to verify whether the latter can be employed to identify meningiomas with a higher probability of harboring 1p deletion.

Finally, we aimed at exploring the prevalence and prognostic value of 1p deletion or MCM2 and ACADL expression in BIOB meningiomas compared to other atypical meningiomas.

Materials and methods

Cases

We carried out a comprehensive review of all primary (*de novo*) atypical meningiomas diagnosed between 2000 and 2021 at the Unit of Pathology of the University and Hospital Integrated in Verona, Italy.

The inclusion criteria for this study were: (i) confirmation of histological diagnosis of atypical meningioma; (ii) available follow-up information, with a minimum follow-up of 36 months for non-recurring cases; (iii) absence of multiple meningiomas and neurofibromatosis type 2; (iv) complete surgical removal (Simpson grades 1, 2, or 3 [35]); and (v) availability of paraffin blocks.

Twenty-two cases were analyzed for 1p deletion using NGS in a previous study [4].

Additionally, we included 12 atypical meningiomas diagnosed between 2023 and 2024, for which 1p deletion was assessed in the diagnostic workup using FISH.

Ethical issues

This study was approved by Comitato Etico per la Sperimentazione Clinica delle province di Verona e Rovigo (protocol n. 40400, 2019/07/19).

Histological revision

We reviewed the histological slides of all cases to assess the number of mitoses per 1.6 mm^2, brain invasion, and "minor atypical criteria" (hypercellularity, macronucleoli, small cells with a high nuclear-to-cytoplasmic ratio, spontaneous necrosis, and sheeting), as previously described [3, 11]. The cases were subdivided into: (1) mitotically active (mitotic index $\geq$ 4/1.6 mm^2); (2) BIOB (if showing brain invasion in the absence of a mitotic index $\geq$ 4/1.6 mm^2 and minor atypical criteria); (3) cases with minor atypical criteria in the absence of a mitotic index $\geq$ 4/1.6 mm^2.

According to WHO classification, brain invasion was defined as "the presence of irregular, tongue-like protrusions of tumor cells into underlying GFAP-positive parenchyma, without intervening lepromeninges" [32].

Clinical data

Information on tumor localization, extent of surgical resection, and recurrence-free survival (RFS) was retrieved from clinical records and operatory registries.

Tumor sites were classified into four groups: (1) convexity, (2) parafalcine/tentorial/, (3) skull base, and (4) ventricle.

Immunohistochemistry

All samples were immunostained using antibodies against MCM2 (clone 1E7, dilution 1:200, Cell Signaling Technology) and ACADL (polyclonal, dilution 1:200, Sigma) and an automated immunostainer. The immunostained slides were first scanned at low-power magnification to identify areas with positive cells. Thereafter, we counted positively stained cell nuclei among 1000 tumor cells.

Cases with >5% stained cells were considered to be positive for MCM2 or ACADL.

Based on MCM2 and ACADL positivity, meningiomas were subdivided into three immunohistochemical groups (IHC-G): (i) IHC-G1, comprising cases negative for both ACADL and MCM2 (corresponding to MG1 and MG2); (ii) IHC-G2, including cases positive for ACADL, but negative for MCM2 (corresponding to MG3); and (iii) IHC-G3, consisting of cases positive for MCM2 regardless of ACADL immunostaining (corresponding to MG4) [2].

Fluorescent in situ hybridization (FISH) analysis

For each case, a 3 µm section was processed with LSI 1p36/1q25 Dual-Color Probe Set assay (Vysis/Abbott, Molecular Europe, Wiesbaden, Germany), following the manufacturer's protocol. Slides were examined using an Olympus BX61 fluorescence microscope equipped with a 100x oil immersion objective and a triple band pass filter for simultaneous detection of Spectrum Orange, Spectrum Green, and DAPI signals. A total of 500 non-overlapping nuclei were counted.

Cases were classified as follows: (i) 1p deleted when two reference probe signals (1q) and one target probe signal (1p) were detected in at least 50% of cells; (ii) 1p non-deleted when two reference probe signals (1q) and two target probe signals (1p) were present in >50% of cells.

Next-generation sequencing

NGS was performed for 65 meningiomas overall.

Twenty-two cases were analyzed in a previous study using the targeted NGS panel Oncomine Tumor Mutational Load (TML) assay (ThermoFisher), which targets 1.65 Mb of genomic space, including all exons of 409 cancer-related genes, for mutational, copy number, and tumor mutational burden assessment [4].

The remaining forty-three meningiomas were explored using the SureSelectXT HS CD Glasgow Cancer Core assay (www.agilent.com), hereafter referred to as CORE [5]. This spans 1.85 Mb of the genome and interrogates 174 genes for somatic mutations, copy number alterations and structural rearrangements (details in Supplementary File 1).

Sequencing libraries were prepared by targeted capture using the SureSelect kit (Agilent Technologies), according to the manufacturer's instructions. Genomic DNA was enzymatically fragmented using the SureSelect Enzymatic Fragmentation Kit (Agilent Technologies). The quality and quantity of pre-capture libraries were assessed using the Qubit BR dsDNA assay (Thermo Fisher). Hybridization-capture and purification of the libraries were performed on 16-library pools (1.6 µg of total pooled DNA), using 100 ng of each pre-capture library to prepare the pools. The captured library pools were enriched by PCR, purified, and quantified using the Qubit dsDNA HS assay. The quality and fragment size of the library pools was verified using an Agilent 4200 Tape Station and High Sensitivity D1000 ScreenTape (Agilent Technologies). Sequencing was performed on a NextSeq 500 (Illumina) loaded with two captured library pools using a high-output flow cell and 2×75 bp paired-end sequencing. Demultiplexing was performed using BaseSpace Sequence Hub (https://basespace.illumina.com). Paired-end reads were aligned to the human reference genome (version hg38/GRCh38) using BWA and saved in BAM file format [15]. BAM files were sorted, subjected to PCR duplicate removal, and indexed using biobambam2 v2.0.146 [37]. Coverage statistics were produced using samtools [16]. Single nucleotide variants were called using Shearwater [12]. Small (<200 bp) insertions and deletions were called using Pindel [40]. Small nucleotide variants were further annotated using a custom pipeline based on vcflib (https://github.com/ekg/vcflib; last access 11/30/2020), SnpSift [9], Variant Effect Predictor (VEP) software [23], and NCBI RefSeq transcripts database (www.ncbi.nlm.nih.gov/refseq/). Annotated variants were filtered by retaining only missense, nonsense, frameshift, or splice-site variants with the exclusion of the *TERT* gene for which also variants affecting the promoter were retained. All candidate mutations were manually reviewed using Integrative Genomics Viewer (IGV) version 2.18 [30] to exclude sequencing artifacts. Gene copy number alterations were detected using the GeneCN software (https://github.com/wwcrc/geneCN). Whole-chromosome or chromosome-arm alterations were assessed by measuring the ratio of normalized GC-adjusted coverage of tumor sample alignments to the mean normalized GC-adjusted coverage of 20 non-neoplastic samples for all targeted regions of a chromosome arm. The targeted regions included both targeted genes and a set of "backbone" regions probing each chromosome at 1 megabase intervals. Each large alteration was further confirmed by checking the copy number status of the targeted genes included in the large alteration, as reported by the GeneCN software.

Copy-neutral LOH events and gain after LOH events were detected by examining b-allele frequency (BAF) for all SNPs (population frequency) detected in targeted regions by the Varscan2 software (PMID: 22300766). Sequencing artefacts were removed by subtracting SNPs calls recurring in all samples and visual verification of alignments. A minimum of 5 contiguous SNPs on independent target regions showing a coherent median absolute deviation (Wilcoxon signed rank test $p < 0.05$) from the expected 50% variant allele frequency was used as threshold for copy-neutral LOH calling. BAF analysis was also used to verify GeneCN calls.

Following the five-tier classification system recommended by the joint consensus of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) [29], variants were classified: Benign (class 1); Likely Benign (class 2); Variant of Un-certain Significance (VUS – class 3); Likely Pathogenic (class 4); Pathogenic (class 5). When available, variant classification was retrieved from the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) and accepted when the record complied with the following criteria: review by an expert panel according to the ACMG/AMP guidelines and/or report by multiple submitters with evaluation criteria according to the ACMG/AMP guidelines, and no conflicts. When a consistent classification was unavailable or when the variant was not present in the ClinVar database, variants were evaluated in-house, according to the ACMG/AMP guidelines using also the following databases and software to gather and integrate all relevant information: My Cancer Genome (https://www.mycancergenome.org), Intogen [13] (https://www.intogen.org/), QIAGEN Clinical Insight (QCI) software (https://variants.Qagenbioinformatics.eu/qci/) and Franklyn (https://franklin.genoox.com).

Statistical analyses

We used the chi-squared test to analyze the correlations between the presence of 1p deletion and clinico-pathological (age and sex of the patients; Simpson grade; localization; tumor recurrence; histological group; IHC-G) or genetic features (i.e., gene mutations or copy number variation, CNV) and to assess the differences between and within the histological groups.

We calculated the sensitivity (1pdel-MCM2+/ACADL+/ total 1pdel) and the specificity (1pnondel-MCM2-/ACADL-/ total 1pnondel) of MCM2 and ACADL immunostainings for predicting 1p-deletetion.

Finally, RFS was assessed using the Kaplan-Meier method, with the date of primary surgery as the entry point and the detection of a recurrent tumor as the endpoint. The Mantel-Cox log-rank test was applied to assess the strength of the association between clinical parameters, histological group, IHC-G, 1p deletion and RFS.

A probability (P) value of less than 0.05 was considered as significant. Statistical analyses were performed using the MedCalc 12.1.4.0 statistical software (MedCalc Software, Mariakerke, Belgium).

Results

Cases

Ninety-eight primary meningiomas classified as atypical subtype according only to the histological criteria of the WHO 2021 classification were included in this study.

Tumors were resected from 54 male and 44 female adult patients (median age: 63 years; range: 23–88 years;). None of the patients had received adjuvant treatment after surgery.

Forty-six meningiomas were localized at the brain convexity, 35 were parafalcine/tentorial, 16 were located at the skull base and one was ventricular (Fig. 1).

The extent of resection was Simpson grade 1 in 64 cases, grade 2 in 22 and grade 3 in 12.

Follow-up information was available for 87 patients. Fifty-six meningiomas recurred during follow-up. RFS ranged between 3 and 151 months (median: 25 months) for recurring cases, and between 36 and 194 months (median 53 months) for cases that did not recur.

Histological features

Sixty-two meningiomas were mitotically active (mitotic index between 4 and 18 mitoses/1.6 mm^2). Twenty-five cases were BIOB, 9 had only minor atypical criteria, and 2 had the co-occurrence of minor criteria and brain invasion.

Immunohistochemical groups

Fifty-one (52%) meningiomas were positive for MCM2 (Fig. 2A), with the percentage of stained cells ranging between 8 and 90%.

Forty-one (42.8%) cases were positive for ACADL (Fig. 2B).

Based on the immunohistochemical stainings for the two proteins, 28 meningiomas were classified as IHC G1 (MCM2-/ACADL-), 18 as IHC G2 (MCM2-/ACADL+), and 52 as IHC G3 (MCM2+/ACADL+/-) (Fig. 1).

Chromosome 1p deletion is accurately detected by FISH

Using FISH, we detected 1p deletion in 53 neningiomas (Figs. 1 and 3). Eight cases, all resected between 2001 and 2005, could not be assessed owing to technical artifacts.

NGS results confirmed FISH findings in all but one case (Fig. 1), which was reported as non-deleted by FISH, but was found to harbor 1p copy neutral loss of heterozygosity by NGS.

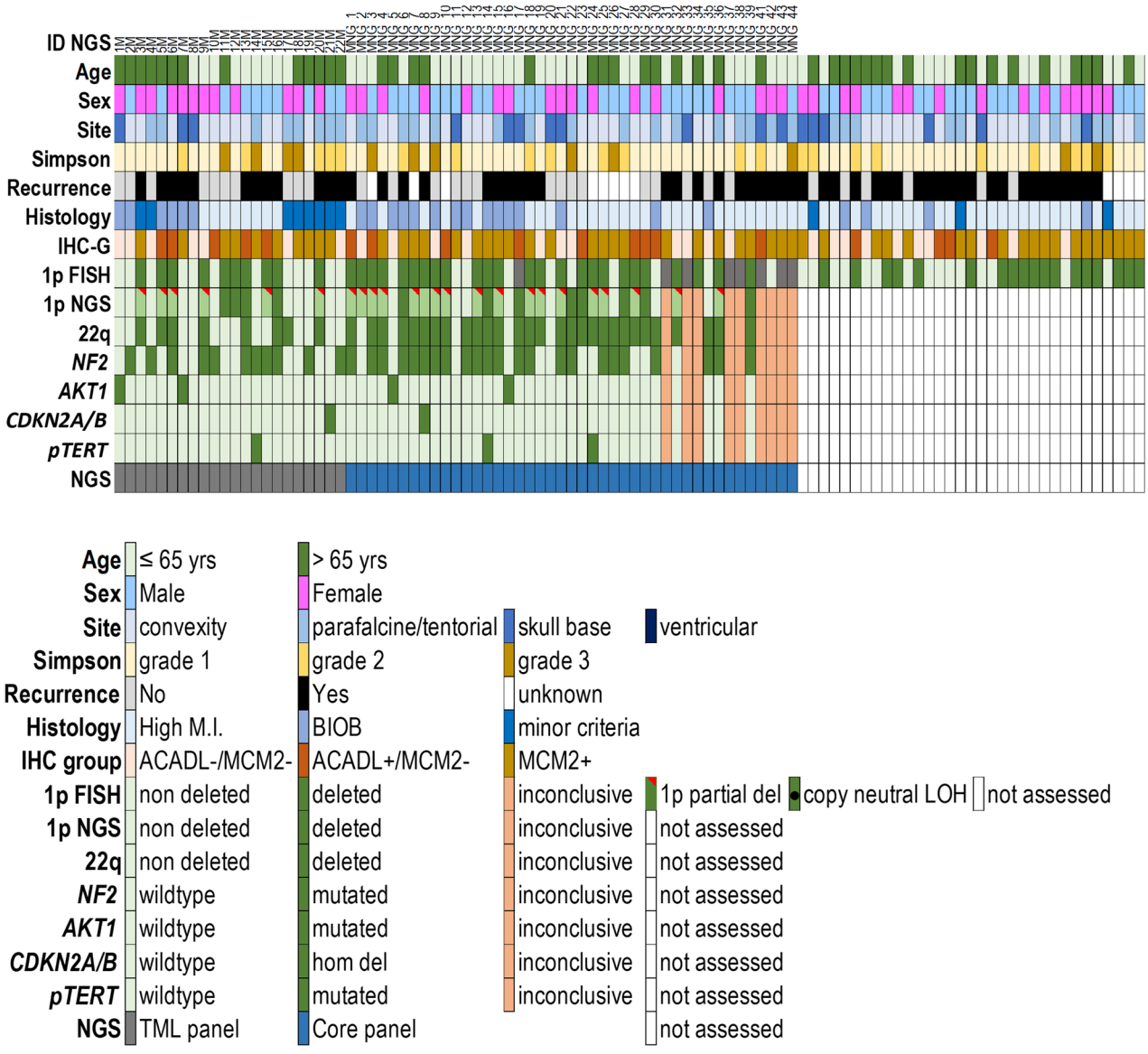

Fig. 1 Clinical, pathological, immunohistochemical and genetic findings of 98 atypical meningiomas included in this study

Nine meningiomas, all resected between 2003 and 2005, could not be assessed by NGS because of excessive DNA fragmentation.

Of the 35 cases with 1p deletion on NGS, 22 harbored a segmental deletion of chromosome 1p, invariably involving the 1p36 region. For the following analyses, both cases with complete and segmental deletions were classified as "1p-deleted".

Chromosome 1p deletion is associated with parafalcine/tentorial site, high proliferation and recurrence

Based on FISH and NGS findings, 55 atypical meningiomas harbored chromosome 1p deletion.

This was significantly more frequent in parafalcine/tentorial than in skull base meningiomas ($P = 0.0178$) (Table 1), mitotically active tumors ($P = 0.0007$) and IHC-G2 and IHC-G3 meningiomas ($P = 0.0251$). The sensitivity and specificity of ACADL and MCM2 immunostainings for predicting 1p deletion were 82% and 44%, respectively. In addition, chromosome 1p deletion was significantly associated with recurrence ($P = 0.0097$) (Table 1). The association between 1p deletion and recurrence remained significant, limiting the analysis to meningiomas analyzed using NGS and excluding cases with *pTERT* mutation or *CDKN2A/B* homozygous deletion ($P = 0.0168$).

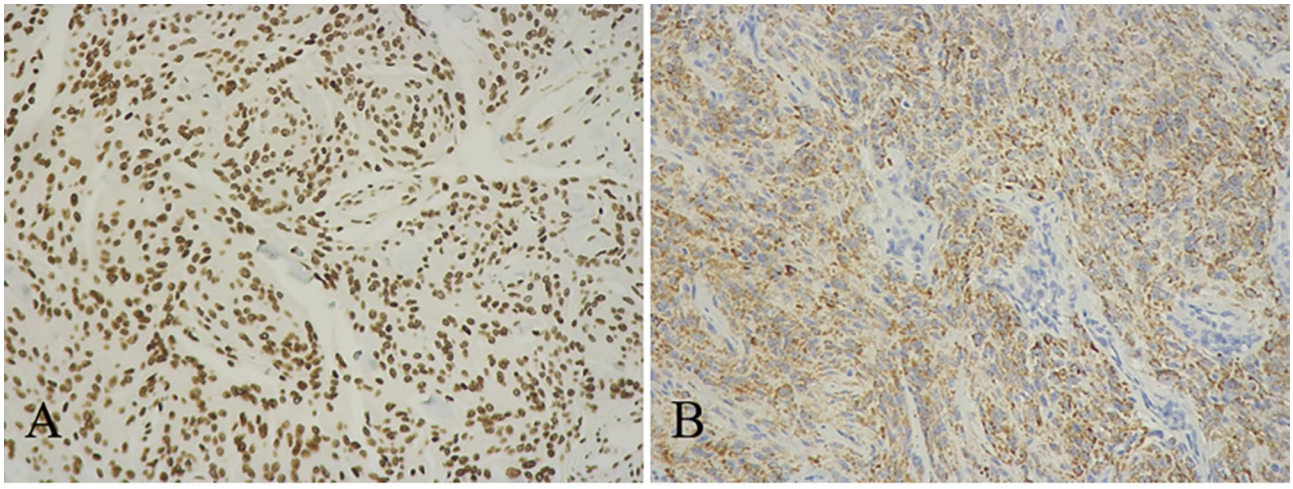

Fig. 2 Immunohistochemical expression of MCM2 (**A**) and ACADL (**B**) in atypical meningioma. (**A**) Diffuse and strong nuclear expression of MCM2 in an atypical meningioma. (**B**) Cytoplasmic expression of ACADL in an atypical meningioma

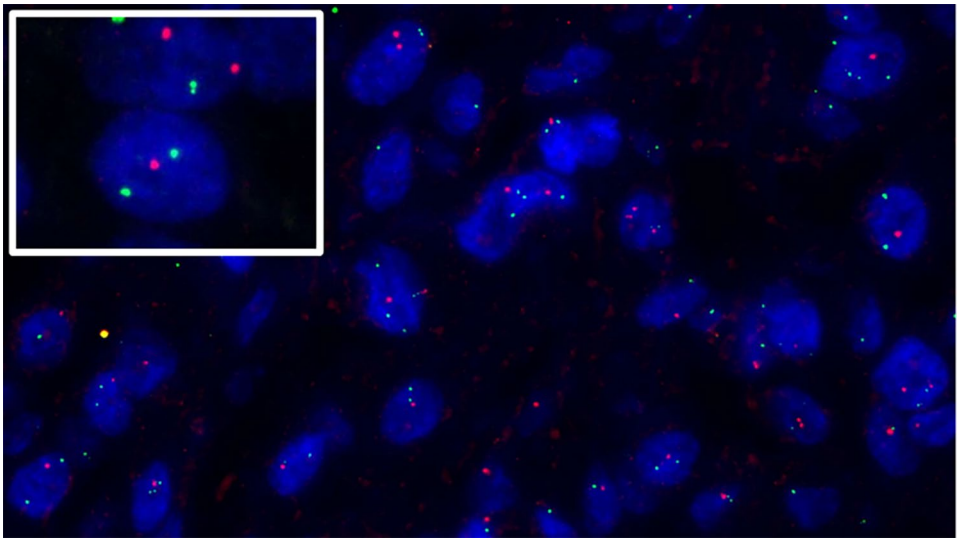

Fig. 3 Atypical meningioma with 1p deletion identified by FISH. FISH using 1p36/1q25 dual color probes. Tumor cells exhibited 2 green signals corresponding to 1q and one red signal, corresponding to 1p

Association between chromosome 1p deletion and other genetic alterations

The genetic alterations of cases analyzed using the TML Oncomine panel have been detailed in a previous paper [4]. The copy number variations and mutations detected in the remaining cases are listed in Supplementary Tables.

The chromosome 22q deletion was the most frequent genetic alteration, detected in 35/54 meningiomas. Thirty cases had a complete deletion of 22q and five had a partial deletion of 22q.

Thirty-two meningiomas had concurrent 1p and 22q deletion, whereas four tumors with 1p deletion lacked 22q deletion.

NF2 was the most frequently mutated gene in this cohort (36/56 meningiomas), with 24 mutant cases harboring 1p deletion (Table 1).

AKT1 mutation (p. E17K) was identified in four cases, all localized at the skull base and none harboring 1p deletion ($P = 0.0073$) (Table 1).

CDKN2A/B homozygous deletion was present in two cases, both recurring and one of which had concurrent 1p deletionFig. . 1).

pTERT mutation was identified in three cases (c. 146 C > T in two cases c. 124 C > T mutation in one case), two of which harbored 1p deletion. Recurrence occurred in both cases with available follow-up information.

One meningioma had *KLF4* mutation (MNG_11). The tumor was localized at the olfactory groove, lacked 1p

Table 1 Statistical correlations between 1p deletion and clinico-pathological, immunohistochemical, and molecular features of atypical meningiomas

Parameter	1p deletion		*P*
	Absent	Present	
Age			
≤ 65 years	17	31	0.395
> 65 years	19	24	
Sex			
Male	16	33	0.147
Female	20	22	
Site			
Convexity	13	31	**0.0178**
Parafalcine/tentorial	13	20	
Skull base	10	3	
Ventricular	0	1	
Recurrence			
Absent	18	11	**0.0097**
Present	16	34	
Histological group			
Mitotically active	14	41	**0.0007**
Brain invasive otherwise benign	13	12	
Minor atypical criteria	9	2	
Immunohistochemical group			
Group 1 (MCM2-/ACADL-)	16	10	**0.0251**
Group 2 (MCM2-/ACADL+)	5	12	
Group 3 (MCM2+)	15	33	
NF2 mutation			
Absent	10	12	0.225
Present	10	24	
AKT1 mutation			
Absent	16	34	**0.0073**
Present	4	0	

deletion and did not develop recurrence during a follow-up period of 182 months.

Chromosome 1p deletion is associated with shorter RFS regardless of concurrent 22q deletion

RFS length was significantly shorter in patients with meningiomas harboring chromosome 1p deletion than in patients with meningiomas lacking 1p deletion ($P = 0.0048$) (Table 2; Fig. 4). The association between 1p deletion and shorter RFS remained significant limiting the analysis to meningiomas analyzed using NGS and excluding the cases with *pTERT* mutation or *CDKN2A/B* homozygous deletion ($P = 0.0268$; hazard ratio: 2.6; 95% confidence interval: 1.1–6.2).

No significant difference in RFS length was found between patients with 1p-deleted meningiomas according to the presence of concurrent 22q deletion ($P = 0.431$) (Fig. 4).

The mitotically active histological group ($P = 0.0054$) (Fig. 5), parafalcine/tentorial or ventricular location

Table 2 Univariate and multivariate analyses for recurrence-free survival in 86 patients with atypical meningioma

	Univariate analysis		Multivariate analysis	
Parameter	HR (95% CI)	*P*	HR (95% CI)	*P*
Age				
≤ 65 years	1	0.975		
> 65 years	1 (0.5–1.7)			
Sex				
Male	1	0.650		
Female	0.8 (0.5–1.5)			
Site				
Convexity	1	**0.0021**	1	
Parafalcine/tentorial	1.7 (0.9–3.1)		2.1 (1.1–4.1)	**0.0177**
Skull base	0.8 (0.4–1.6)		1.7 (0.6–5.1)	0.297
Ventricular	13 (0-12186)		10 (1.2–97.1)	**0.0316**
Simpson grade				
1	1	0.452		
2	1.4 (0.7–2.8)			
3	1 (0.3–2.8)			
Histological group				
Mitotically active	2.7 (1.5–4.8)	**0.0054**	1	
Brain invasive otherwise benign	1		0.2 (0.1–0.6)	**0.0036**
Minor atypical criteria	1.8 (0.7–4.7)		0.7 (0.2–1.9)	0.539
Immunohistochemical group				
Group 1 (ACADL-/MCM2-)	1	**0.0007**	0.4 (0.2-1)	0.0689
Group 2 (ACADL+/MCM2-)	2.5 (1.2–5.2)		0.7 (0.3–1.6)	0.529
Group 3 (MCM2+)	3.6 (2-6.5)		1	
NF2 mutation				
Absent	1	0.794		
Present	0.8 (0.3–2.1)			
AKT1 mutation				
Absent	1	0.882		
Present	1.1 (0.2–5.3)			
1p deletion				
Absent	1	**0.0048**	0.4 (0.2–0.9)	**0.0290**
Present	2.3 (1.2–4.1)		1	
1p deletion/22q deletion				
1pdel/22q non-del	1	0.431		
1p del/22q del	1.7 (0.3–8.8)			

HZ: hazard ratio. C.I.: confidence interval. Del: deleted

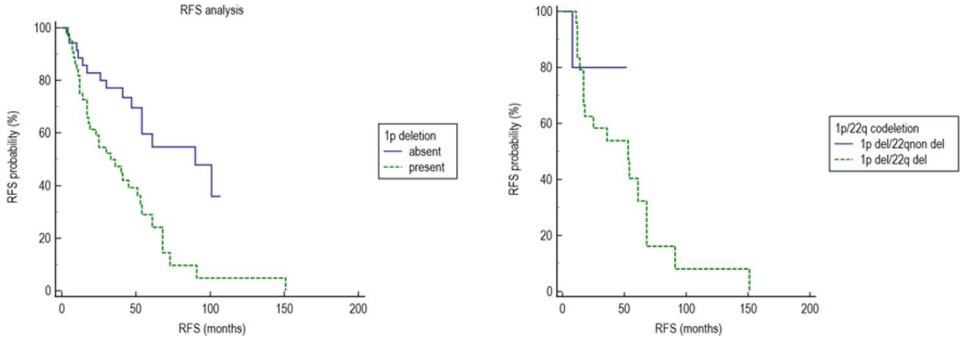

Fig. 4 RFS was significantly shorter in patients with 1p-deleted meningiomas, regardless of concurrent 22q deletion. Patients with atypical meningioma harboring 1p deletion had significantly shorter RFS than patients with meningiomas lacking 1p deletion. Among patients with 1p-deleted meningioma, no difference in survival length was found according to concurrent 22q deletion

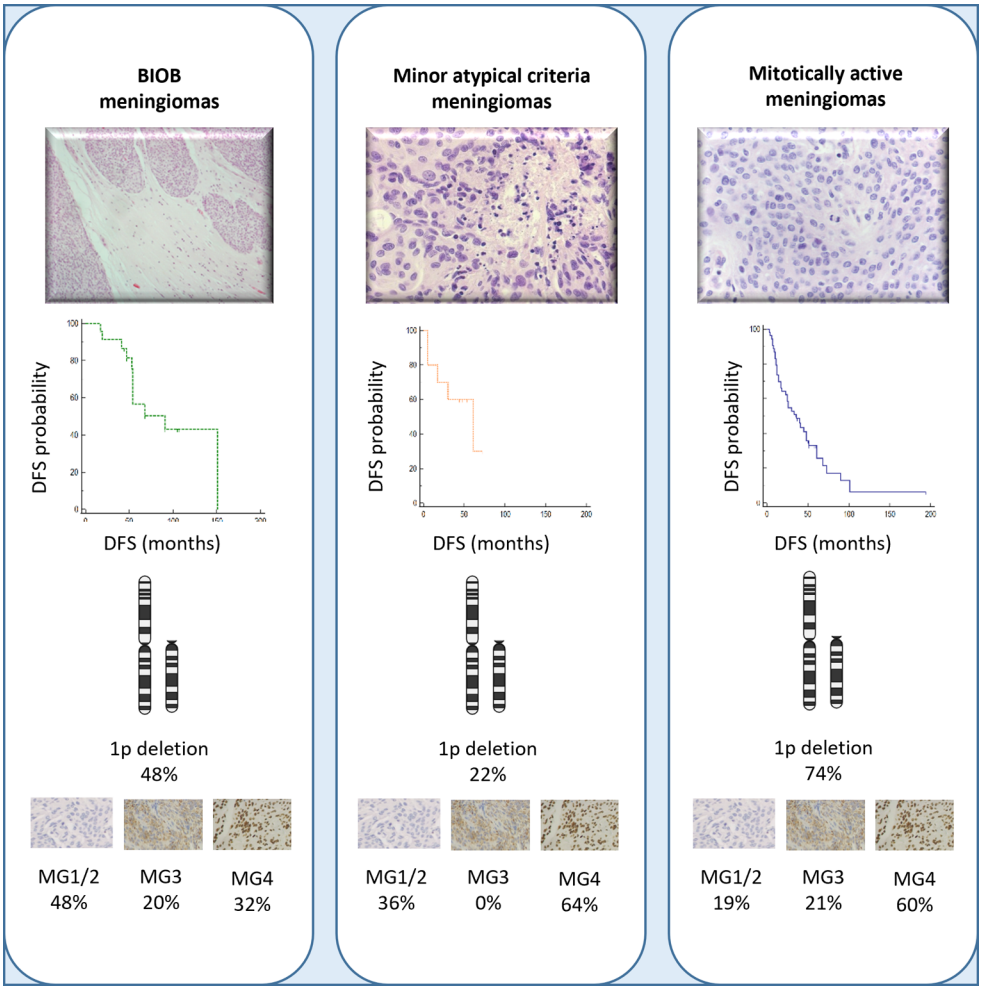

Fig. 5 Recurrence-free survival (RFS), 1p deletion and distribution across molecular groups (MGs) of atypical meningioma according to histopathological features. BIOB meningiomas had significantly lower frequency of 1p deletion and MCM2 immunostaining (used as a surrogate for MG4). Patients with these tumors had significantly longer RFS

Table 3 Clinical, immunohistochemical and molecular features of atypical meningiomas according to their histopathological features

Parameter	Histological group			*P*
	Mitotically active	Brain invasive otherwise benign	Only minor atypical criteria	
Age				
≤ 65 years	38	14	2	**0.0298**
> 65 years	24	11	9	
Sex				
Male	37	13	4	0.335
Female	25	12	7	
Site				
Convexity	37	7	2	**0.0034**
Parafalcine/tentorial	18	9	8	
Skull base	6	9	1	
Ventricular	1	0	0	
Recurrence				
Absent	14	12	5	0.0617
Present	39	11	5	
Immunohistochemical group				
Group 1 (ACADL-/MCM2-)	12	12	4	**0.0328**
Group 2 (ACADL+/MCM2-)	13	5	0	
Group 3 (MCM2+)	37	8	7	
NF2 mutation				
Absent	9	8	5	0.394
Present	16	13	3	
AKT1 mutation				
Absent	25	17	8	**0.0336**
Present	0	4	0	
1p deletion				
Absent	14	13	9	**0.0007**
Present	41	12	2	

($P = 0.0021$), and IHC G2/G3 ($P = 0.0007$) were also significantly associated with shorter RFS (Table 2).

Multivariate analysis demonstrated that the lack of chromosome 1p deletion ($P = 0.0290$) and BIOB ($P = 0.0036$) were independent prognostic factors associated with longer RFS, whereas parafalcine/tentorial ($P = 0.0177$) and ventricular ($P = 0.0316$) sites were independently associated with significantly shorter RFS (Table 2).

BIOB meningiomas have lower rates of 1p deletion, MCM2 immuno-expression and recurrence

Twenty-five meningiomas were classified as atypical owing to brain invasion in the absence of a mitotic index of at least 4 mitoses/1.6 mm^2 or at least three minor atypical criteria (Fig. 1). Compared to mitotically active meningiomas, BIOB meningiomas displayed a significantly lower prevalence of 1p deletion ($P = 0.0007$) and MCM2 positivity ($P = 0.0328$) (Fig. 5) and had a higher frequency of skull base localization ($P = 0.0034$) (Table 3). All four *AKT1*-mutated meningiomas in this study were BIOB tumors ($P = 0.0336$). Of the 23 cases with available follow-up data, 11 BIOB meningiomas recurred, compared to 39/53 (74%) mitotically active meningiomas ($P = 0.0617$).

Recurrence of BIOB meningiomas was significantly associated with IHC-G2 and IHC-G3 (9/11 recurring vs. 2/12 non-recurring, $P = 0.0072$) and with spontaneous necrosis (6/11 recurring vs. 0/12 non-recurring, $P = 0.0036$). 1p deletion showed a trend to enrichment in recurring BIOB meningiomas (7/10 recurring vs. 4/13 non-recurring), but the difference was not statistically significant ($P = 0.0678$).

RFS analysis suggested that 1p deletion (Hazard Ratio: 2.9; 95% Confidence Interval: 0.7–11.1 $P = 0.111$), IHC-G2 (Hazard Ratio: 3.8; 95% Confidence Interval: 0.8–18.1; $P = 0.0512$) and IHC-G3 (Hazard Ratio: 5.3; 95% Confidence Interval:1.3–21; $P = 0.0512$) were associated with shorter RFS, although statistical significance was not reached. Spontaneous necrosis was strongly associated with shorter RFS in patients with BIOB meningiomas (Hazard ratio: 33.7; 95% Confidence Interval: 5.8-195.9; $P = 0.0001$).

Discussion

The fifth edition of WHO classification of CNS tumors introduced genetic features as additional criteria for meningioma grading [32]. Notably, *pTERT* mutations and *CDKN2A/B* homozygous deletion, due to their association with aggressive clinical outcomes, now designate CNS WHO grade 3 of meningioma regardless of histological features [14, 24, 33]. However, these genetic alterations are uncommon (< 10%) in histologically grade 1 and 2 meningiomas [22]. Consequently, even with the application of the WHO 2021 criteria, meningioma grading continues to rely heavily on histological features. This approach leaves the challenge of predicting recurrence risk largely unresolved, particularly for grade 2 (atypical) meningiomas. To address this limitation, alternative systems incorporating genetic, epigenetic, or transcriptomic data, have been proposed to improve the prognostic stratification of meningiomas [22]. The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy-Not Official WHO (cIMPACT-NOW) recently published an update with recommendations for meningioma grading, informed by findings from the latest molecular studies [31]. Given the strong evidence supporting the prognostic relevance of chromosome 1p deletion in meningiomas, cIMPACT-NOW recommends assigning CNS WHO grade 2 to BIOB meningiomas, as well as to meningiomas with histological features borderline between grade 1 and 2, that display combined deletion of chromosome 1p and 22q [31]. Notably, DNA methylation profiling demonstrated that a proportion of meningiomas histologically classified as atypical subtype fall into methylation classes associated with a more favorable clinical outcome [34]. These tumors typically lack 1p loss [34], suggesting that analysis of this genetic alteration may be beneficial for the prognostic subgrouping of atypical meningiomas. Based on this premise, thisinvestigation aimed to assess the potential of chromosome 1p deletion, as evaluated by FISH, in predicting recurrence risk for atypical meningiomas. The study examined a cohort of 98 primary meningiomas, categorized as atypical based on WHO 2021 histological criteria, with all cases undergoing complete surgical excision and no subsequent adjuvant therapies. Our findings revealed that 1p deletion, detected through FISH analysis, exhibited a significant correlation with tumor recurrence and emerged as an independent indicator of RFS.

These findings align with those of other studies that analyzed 1p deletion in grade 2 meningiomas using DNA methylation profiling or microarray techniques [20, 38]. Additionally, by comparing findings obtained by FISH and NGS in 65 cases, this study is the first to demonstrate that FISH is an accurate method for detecting 1p copy number variation in meningiomas in routine practice. Indeed, all segmental deletions of 1p, identified in 22 of 35 1p-deleted meningiomas by NGS, encompassed the 1p36 region and could therefore be identified using FISH with 1p36/1q25 probes. Since a loss over than 5% of chromosomal arm 1p is considered prognostically significant in meningiomas [20], we classified meningiomas as "1p-deleted" regardless of whether 1p deletion was segmental or complete.The chromosome 1p36 region is estimated to encompass approximately 30 Mb (24%) of the 125 Mb genomic space associated with chromosome 1p. Consequently, FISH using a probe for 1p36 is unable to detect smaller segmental deletions in this region or in other areas of chromosome 1p, which may constitute a limitation compared to DNA methylation profiling. However, it should be noted that the majority of segmental deletions of chromosome 1p involve the telomeric region, specifically the 1p36 region, and losses < 24% are exceedingly rare [18]. Accordingly, none of the cases investigated by NGS in this study exhibited segmental deletions outside 1p36. However, both FISH and DNA methylation profiling [18] are unable to identify chromosome 1p copy neutral LOH, which was observed in one of the meningiomas analyzed with NGS.

Since 22q deletion is the most frequent genetic alteration in meningioma, c-IMPACT-NOW suggests that the co-occurrence of 1p and 22q deletion designates grade 2 meningiomas [31]. In this cohort, 32 meningiomas hadconcurrent 1p and 22q deletion and 4 meningiomas had 1p deletion in the absence of 22q deletion. Although the number of cases with 1p deleted/22q balanced chromosome status is too small to draw any significant conclusions, these patients exhibited RFS length similar to that of patients with 1p/22q deleted meningiomas.

In support of the negative prognostic value of 1p deletion in atypical meningiomas, this genetic alteration was significantly more frequent in cases characterized by a mitotic index ≥ 4/1.6 mm^2 and parafalcine/tentorial location, which were identified as negative prognostic determinants in other studies [11, 39]. Moreover, the loss of chromosome 1p was significantly associated with the immunohistochemical expression of ACADL and MCM2, used as surrogates for the unfavorable molecular groups referred to as "hypermetabolic" (MG3) and "hypermitotic" (MG4), previously described by Nassiri et al. [25]. However, 1p deletion was not exclusive to ACADL or MCM2 positive tumors. Although MCM2 and ACADL immunostaining had high sensitivity for identifying 1p deleted meningiomas, their specificity was low, indicating that these markers cannot be used to predict the status of 1p deletion.

Notably, none of the skull base *AKT1*-mutant meningiomas harbored 1p deletion, consistent with findings from another study that reported 9 *AKT1*-mutated meningiomas (including 7 secondary and 2 primary tumors; 8 grade 1 and 1 grade 3), none of which had 1p deletion [1].

This suggests that these genetic alterations are mutually exclusive. Although *AKT1*-mutated meningiomas mostly have indolent behavior [34], two of four cases in this cohort unexpectedly recurred. Notably, both cases were positive for MCM2, suggesting that immunohistochemical assessment of this protein may be valuable in predicting the recurrence risk of *AKT1*-mutated meningiomas.

This study demonstrated that within atypical meningiomas, BIOB feature significantly lower rates of 1p deletion and MCM2 immuno-expression, along with longer RFS, compared to mitotically active tumors. These findings support the hypothesis that BIOB tumors are more similar to grade 1 meningiomas [6, 21, 28, 36]. Notably, recurring BIOB meningiomas exhibited a significantly higher frequency of spontaneous necrosis. Additionally, they harbored a higher rate of 1p deletion and increased expression of MCM2 and ACADL compared to non-recurring cases, although the difference was not statistically significant.

Conclusions

This study demonstrates that 1p deletion can be accurately assessed using FISH in routine practice, and that this genetic alteration, as detected by FISH, is associated with an increased recurrence risk in atypical meningiomas.

A comparison of BIOB meningiomas and mitotically active meningiomas revealed significantly lower frequencies of 1p deletion, MCM2 expression and recurrence rates in BIOB tumors, consistent with their more indolent behavior. The evidence of higher frequencies of MCM2 expression, spontaneous necrosis and 1p deletion observed in recurring BIOB meningiomas suggest that these features may help identify cases at a greater risk of recurrence, for which a grade 2 designation could be more appropriate.

Abbreviations

NGS	Next Generation Sequencing
FISH	Fluorescent In Situ Hybridization
RFS	Recurrence-Free Survival
BIOB	Brain-Invasive Otherwise Benign
CNS	Central Nervous System
WHO	World Health Organization
IHC-G	Immunohistochemical Group
TML	Tumor Mutational Load
CNV	Copy Number Variation
C-Impact-NOW	Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy-Not Official WHO

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40478-025-01973-6.

Supplementary Material 1

Supplementary Material 2

Author contributions

G.Z., G.H.G. and V.B. performed the study concept and design; G.H.G, A.M., S.P., and S.A. developed the methodology; G.Z., G.H.G, A.M., S.P., D.M., S.A, M.C., and V.B. contributed to the acquisition, analysis and interpretation of the data; G.Z. and V.B. performed the statistical analyses; G.Z. and V.B. wrote the original manuscript draft; G.H.G., A.M., S.P., D.M., S.A. revised and approved the final version of the manuscript.

Funding

This study is supported by Italian Ministry of University and Research, through the "Project of significant national interest" (PRIN 2022; code: 22022H73242; CUP: B53D23008120006).

Data availability

Data are available from the corresponding author on reasonable request.

Declarations

Ethical approval

Ethical approval was granted by the Comitato Etico per la Sperimentazione Clinica delle province di Verona e Rovigo (protocol n. 40400, 2019/07/19).

Consent for publication

The patients signed informed consent for using surgical material and anonymous health information for scientific purposes and publication. All authors have approved the publication of the study.

Clinical trial number

not applicable.

Competing interests

The authors declare no competing interests.

Received: 3 January 2025 / Accepted: 22 February 2025
Published online: 06 March 2025

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