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CLINICAL STUDY - PATIENT STUDY

# Allelic loss on chromosomes 1p32, 9p21, 13q14, 16q22, 17p, and 22q12 in meningiomas associated with meningioangiomatosis and pure meningioangiomatosis

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**Abstract** Meningioangiomatosis (MA) is a rare lesion appearing sporadically or as a part of neurofibromatosis 2. The occurrences of meningiomas arising from MA (MA-M) have raised doubts about the traditional concept of a hamartomatous origin for MA. Cytogenetic or molecular studies on MA, with or without meningiomas, are limited because of the rarity of MA. The current study was to evaluate the loss of heterozygosity (LOH) in seven cases of MA-M and two cases of pure MA. LOH on six chromosomes (1p32, 9p21, 13q14, 16q22, 17p, and 22q12) were investigated using 13 sets of microsatellite markers, including D1S193, D1S463, D22S193, D22S929, D22S282, TP53, D17S796, D16S421, D16S512, D13S118, D13S153, D9S162, and D9S104. PCR was performed using each marker and polymorphic analysis was accomplished by silver staining. Immunohistochemical stain for Ki-67 was carried out and labeling index was measured by using a semiquantitative manual counting method. The meningioma portions of MA-Ms showed LOH for loci on chromosomes 22q12, 9p21, and 1p32 in 57.1% (4/7), 28.6% (2/7), and 28.6% (2/7) of cases, respectively. The MA portions of MA-M had a LOH for loci on 22q12 in 28.6% (2/7) of cases, whereas each pure MA harbored one LOH on either chromosome 22q12 or 9p21. The proliferation indices of MA-Ms were significantly higher in the meningioma than in the MA components. Our data suggest that both the meningioma and the MA undergo the same overlapping clonal process, with the MA-M while undergoing additional genetic alterations that confer a greater proliferative potential.

**Keywords** Chromosome 1p32 · Chromosome 9p21 · Loss of heterozygosity · Meningioangiomatosis · Meningioma · *NF2*gene

## Introduction

Meningioangiomatosis (MA) is accompanied by neurofibromatosis 2 or occurs sporadically, and its biological nature has long been debated whether it is a hamartoma or a neoplasm [1, 2]. Meningiomas derived from MA (MA-M) in individuals without neurofibromatosis 2 have rarely been reported [3]. The most common association of meningiomas with MA suggested the possibility that MA may be neoplastic in nature. This suggestion was supported by demonstration of loss of 22q12 in an each case of MA and MA-M [4, 5]. However, recent largest genetic series demonstrated no *NF2* gene mutation in 24 sporadic MAs and two neurofibromatosis-associated MAs. These observations supported the hypothesis of the hamartomatous nature of MA [6, 7]. Perry et al. [6] demonstrated identical *NF2* gene deletions and protein loss in both components of MA-M patients. Their data suggest that the MA component of MA-M cases may represent cortical perivascular spread of an overlying meningioma, rather than an underlying hamartoma. However, their data on proliferation index on MA-Ms were against this hypothesis because Ki-67 labeling index was consistently higher in the meningioma

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rather than MA components of MA-M cases. Also, it cannot be explained by the lack of EMA-positive meningothelial cells in perivascular areas in some MA cases [8]. These different observations among the previous studies may be partly caused by the limited numbers of samples as well as genetic loci such as *NF2*, *4.7B* gene (chromosome 18p), and chromosome 1p. We, therefore, investigated genetic alterations of MA-M and pure MA, through loss of heterozygosity (LOH) studies with expanding markers to gain insight into the histogenesis of MA-M.

In general, early genetic alterations occur in polymorphic markers on 22q in 30–70% of the meningiomas [9, 10], and chromosome 1p has been reported as the second most commonly deleted region; it is the critical step for meningioma progression, and is highly correlated with recurrence [11]. Cytogenetic alterations of chromosomes 6q, 10q, 14q, and 17q are also known to be related to progression-associated genes [12]. Markers used in this study included chromosomes 9p21, 13q14, 16q22, 17p, 22q12 and 1p32.

## Materials and methods

Selected cases were collected from four tertiary centers: four cases were from Seoul National University Hospital (cases 1, 2, 3, and 4), three cases from Samsung Medical Center (cases 5, 8, and 9), one case from Daejeon St. Mary's Hospital of the Catholic University of Korea (case 6), and one case from Gil Medical Center of the Gachon University of Medicine and Science (case 7) in Korea. Cases 1, 2, 3, 4, and 9 had been previously reported [13, 14]. All the tumors were fixed in 10% buffered formalin and stained with hematoxylin–eosin. The immunohistochemical stainability of Ki-67 (prediluted; DAKO, Glostrup, Denmark) was investigated by using a semiquantitative manual counting method i-Solution 7.5 (version 7.5, IMT i-Solution, Coquitlam, Canada). The proliferation indices were determined as follows; at least 1,000 nuclei were counted and the Ki-67 proliferation indices were defined as the percentage of positively stained nuclei. Differences of proliferation indices between MA and meningioma were estimated using the paired samples *t* test.

The formalin-fixed, paraffin-embedded tissue was used for molecular analysis. All the tumor specimens were microdissected with the aid of a light microscope. Genomic DNA from the dissected tissue was extracted using a standard method [15]. Briefly, DNA was extracted from the meningioma and from peripheral blood lymphocytes of the patient by standard methods as described. The allelic alterations were assessed by 13 microsatellite markers of chromosomes 22q12, 1p32, 13q14, 9p21, 16q22, and 17p13 (D1S193, D1S463, D22S193, D22S929, D22S282, TP53, D17S796, D16S421, D16S512, D13S118, D13S153, D9S162, and D9S104). The primers for these markers are commercially available (GIBCO, Carlsbad, CA, USA). PCR was carried out in thermal cycler (Perkin Elmer Cetus 9700, USA). PCR amplifications were performed in 10 µl containing 2 µl DNA template, 0.25 µl each primer, 1.25 mM NTP with 1/2dCTP, 1.5 mM $MgCl_2$, 0.6 U Taq polymerase 0.07 µl, and 10× PCR buffer. Every cycle consist of denaturation at 94°C for 30 s, annealing at 55–60°C for 30 s and extension at 72°C for 40 s, followed by the last extension at 72°C for 10 min. Amplified PCR products 3 µl were analyzed by 12% polyacrylamide gel electrophoresis. By using silver staining, the gain or loss was assessed as described previously [16]. The band intensity of two alleles of each case was determined by scanning densitometric analysis (Pharmacia, San Francisco, CA, USA). LOH in the tumor samples were determined by loss of one allele compared with those from adjacent non-neoplastic cerebral tissue. Scanning densitometry was performed to determine the allelic status of the markers analyzed, and LOH in an informative case was defined as a greater than 50% reduction in band intensity relative to the non-tumorous tissue control. PCR was performed using each marker and polymorphic analysis was accomplished by silver staining.

## Results

### Clinical results

Seven cases of MA-M included five males and two females. Pure MAs involved one male and one female. Gross total resection was done in seven cases [7/9 (77.8%)] and subtotal resection was achieved in two cases (22.2%). The ages of the patients were ranged from 3 to 23 years (mean, 10.3 years). All except one patient, who presented with acute intracranial hemorrhage (case 4), were accompanied by long-standing intractable seizures and well-defined calcified masses demonstrated on brain computed tomography. During the follow-up period, a recurrence occurred in one case which had been subtotally resected. One patient died due to an unrelated cause. The clinical data are summarized in Table 1.

### Pathological and immunohistochemical results

MA-M had the following histologic subtypes: cases 2 and 5 were transitional types; cases 3, 6 and 7 were meningothelial types; and cases 1 and 4 were fibrous types. Meningioma portion of case 2 showed focal increased cellularity with mitotic activity (up to 3 per 20 high power

**Table 1** Clinical summary of nine cases of MA with or without meningiomas

| Case no. | Age (years), gender | Site | Procedure | Histologic type of meningioma | Ki-67 proliferation index (%) | | Association with NF2 | Outcome during follow-up |
|---|---|---|---|---|---|---|---|---|
| | | | | | MA | Meningioma | | |
| 1 | 3, M | Fr-P | GTR | Fibrous | 0.32 | 1.1 | – | NR, alive, LFU (7 year) |
| 2 | 6, M | T | STR | Transitional | 0.2 | 10.1 | – | R, alive (4 year) |
| 3 | 9, M | Fr | GTR | Meningothelial | 0.1 | 1.3 | – | NR, alive (13 month) |
| 4 | 4, M | Fr | GTR | Fibrous | 0.1 | 2.2 | – | NR, alive (1 year) |
| 5 | 6, F | PO Fr | GTR | Transitional | 0.36 | 8.53 | – | NR, alive (1 year) |
| 6 | 9, F | T | STR | Meningothelial | 0.38 | 7.62 | – | NR, dead due to unrelated cause |
| 7 | 10, M | T | GTR | Meningothelial | 0.1 | 2.2 | – | NR, alive (14 month) |
| 8 | 23, F | P | GTR | No meningioma | 0.66 | No meningioma | – | NR, alive (5 month) |
| 9 | 23, M | T | GTR | No meningioma | 0.91 | No meningioma | – | NR, alive (5 year) |

*MA* Meningioangiomatosis, *GTR* gross total resection, *STR* subtotal resection, *M* male, *F* female, *Fr-P* fronto-parietal lobe, *PO* parieto-occipital lobe, *T* temporal lobe, *Fr* frontal lobe, *NF2* neurofibromatosis type 2, *NR* no recurrence, *R* recurrence, *LFU* lost to follow up

fields). All the meningiomas belonged to WHO grade I tumors. The tumors had surrounding MAs that were composed of elongated spindle cells around haphazardly arranged vessels (Fig. 1). Calcification was present in all cases except cases 8 and 9. Case 9 was accompanied by co-existing Taylor-type focal cortical dysplasia type IIA, as well as MA.

Ki-67 labeling indices of MA ranged from 0.1 to 0.91% (mean 0.35%), whereas those of meningiomas ranged from 1.1 to 10.1% (mean 4.7%). In MA-M, proliferation indices of meningioma portions were significantly higher than those of MA portions ($P = 0.01$). The results are summarized in Table 1 and shown in Fig. 2.

## Molecular results

Four cases of MA-M showed LOH for loci on chromosome 22q12 [4/7 (57.1%)] in meningioma components; allelic loss was observed in three cases with D22S193, one with D22S282, and one with D22S929. Two cases of MA-M had LOH on chromosome 9p21 in meningioma components [2/7 (28.6%)]: one was LOH at locus D9S162 and the other one LOH at locus D9S104. Two cases of MA-M had LOH for loci on chromosome 1p32 in meningioma components [2/7 (28.6%)]: one had LOH at locus D1S193 and the other one had LOH at locus D1S463. The MA component of two MA-M cases had a LOH at loci D22S282

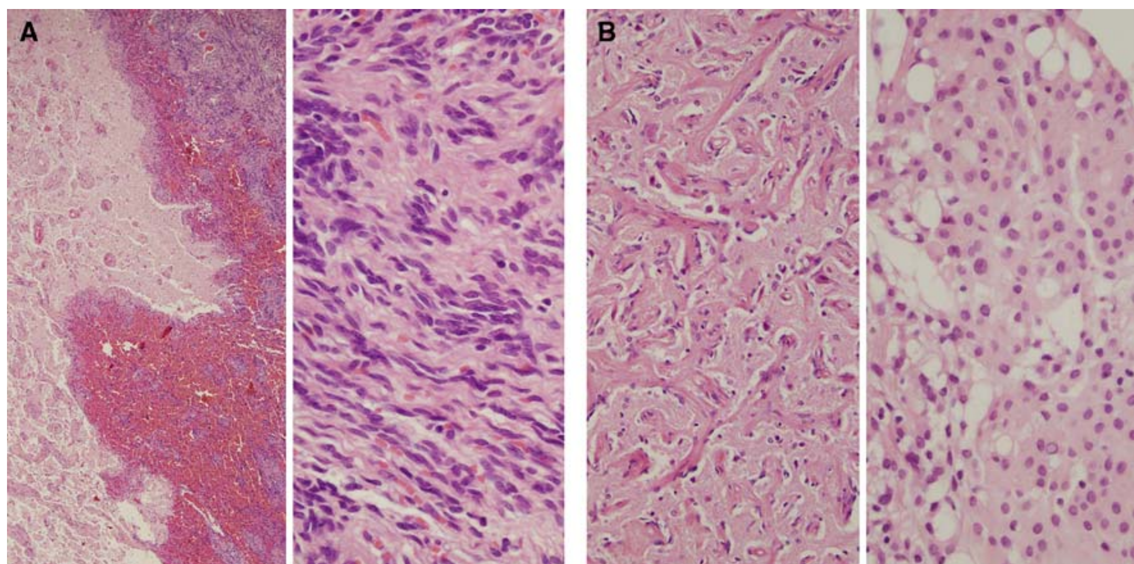


**Fig. 1** Cases 4 (**a**) and 5 (**b**) show transition from MA (*left*) to meningioma (*right*). The majority of the calcific mass consists of the MA component with proliferation of small blood vessels and perivascular spindle cells (Hematoxylin–eosin stain)

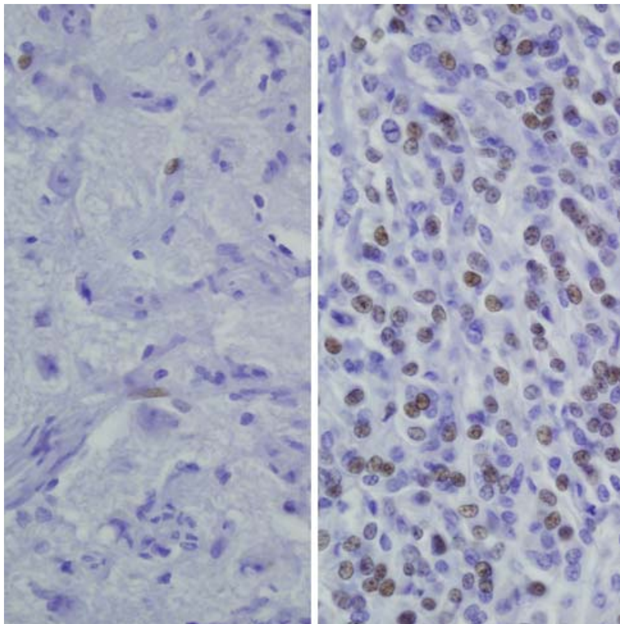

**Fig. 2** Case 5 shows higher proliferation index in the meningioma component (*right*) than in MA portion (*left*) of MA-M (Ki-67 immunostain)

and D22S193 [2/7 (28.6%)], and two cases of pure MA had a LOH at loci D22S929 and D9S162 [2/2 (100%)]. Two MA-M cases (cases 3 and 7) had the same allelic loss at locus D22S193 or D22S282 in both meningioma and MA components [2/7 (28.6%)], of which one (case 3) had additional allelic loss on locus D9S104 other than locus at 22q12 in meningioma component. None of the normal portions showed allelic losses. These results are summarized in Table 2 and Fig. 3.

## Discussion

In the present study, allelic loss was observed in the meningioma component of six (85.7%) cases of MA-M, and in the MA component of two (28.6%) out of seven MA-Ms at least one of 13 microsatellite markers was analyzed. Two pure MAs included in this study demonstrated the allelic loss at one microsatellite marker. The number of specific loci lost ranged from one to three. The microsatellite markers used in this study are specific loci on chromosomes 22q12 (D22S193, D22S282, D22S929), 1p32 (D1S463, D1S193), 13q14 (D13S118, D13S153), 9p21 (D9S162, D9S104), 16q22 (D16S421, D16S512), and 17p13 (TP53, D17S796). The current study demonstrated allelic loss at 22q12, 1p32, and 9p21 in the meningioma and/or MA components. The frequency of allelic loss at 22q12, 9p21, and 1p32 in meningioma components of MA-Ms was 57%, 28.6%, and 28.6%, respectively. The

**Table 2** Molecular results of LOH in nine cases

| Case no. | MA portion | Meningioma portion |
|---|---|---|
| 1 | No LOH | D1S193, D22S193 |
| 2 | No LOH | No LOH |
| 3 | D22S282 | D22S282, D22S929, D9S104 |
| 4 | No LOH | D1S463 |
| 5 | No LOH | D22S193 |
| 6 | No LOH | D9S162 |
| 7 | D22S193 | D22S193 |
| 8 | D22S929 | – |
| 9 | D9S162 | – |

– No tissue obtained, *MA* meningioangiomatosis

frequency of allelic loss at 22q12 in this study is similar to that (56%; 5/9 MA-Ms) observed in the largest series of Perry et al.[6]. This frequency also parallels that of molecular genetic findings in meningiomas unrelated to MA. Approximately half of meningiomas have allelic losses that involve chromosome 22q12 [9, 10].

Allelic loss at 1p32 that was observed in the meningioma component of MA-M cases in the present study has not been demonstrated in previous genetic or molecular studies. Our study showed no loss of D16S512, which is true of the previous studies [17]. Losses of 16q22 have not yet been detected in the meningiomas and are predominantly reported in breast carcinomas or prostatic adenocarcinoma.

In the present study, we have identified losses of D9S162 in the meningioma component of a MA-M case and in a case of pure MA. Losses on 9p are often linked to alterations of CDKN2A that in turn occur predominately in grade II or III of sporadic meningiomas [18]. However, there was no clinical and pathological difference from others without losses of D9S162 in the present study.

The present study revealed the same allelic loss at loci D22S193 and D22S282 in both MA and meningioma components of two MA-M cases, which is similar to those of Perry et al.'s study [6]. Perry et al. demonstrated deletion of *NF2* gene and protein losses between meningioma and MA components in 56% of nine MA-M cases, but no deletion in pure MAs by FISH and immunohistochemical study. They suggested that MA pattern may be perivascular spread of underlying meningioma rather than neoplastic transformation of MA in MA-M cases. However, this suggestion is not supported by our data on LOH at loci D22S929 and D9S162 in two pure MA cases or by a previous report of MA with LOH on 22q12 [4]. The genetic alterations in pure MAs support the possibility that MA originates from the monoclonal meningothelial cells of neoplastic nature, although limited number of samples have been studied. Our two cases of MA-M had identical allelic loss in both components, of which one MA-M had

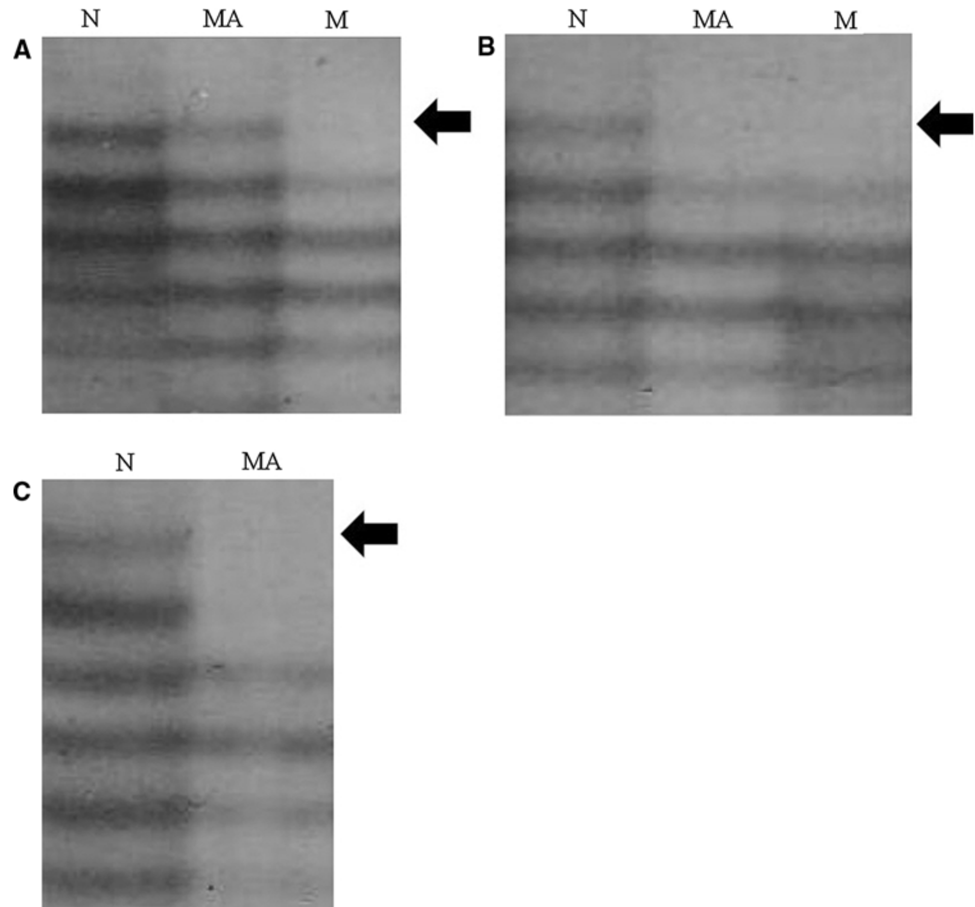


**Fig. 3** **a** Microsatellite analysis of MA-M (case 3). The meningioma portion (*M*) shows LOH on D9S104 (*arrow*). **b** Microsatellite analysis of MA-M (case 7). Both the meningioma (*M*) and *MA* show LOH on D22S193. **c** Microsatellite analysis of pure *MA* (case 8) shows allelic loss on D22S929 (*arrow*). *N* is the PCR product from the non-neoplastic cerebral tissue or peripheral blood

**Table 3** Summaries of the previous studies about meningiomas with or without MA

| Authors | Materials | Association with NF2 | Method of study | Studied markers | Summary of results |
|---|---|---|---|---|---|
| Takeshima et al. [4] | MA (1 case) | – | LOH (2 microsatellite markers) | 22q11-12 | LOH on chromosome 22q in pure MA, supporting neoplastic theory |
| Sinkre et al. [5] | MA-M (1 case) | – | FISH | 22q12 region, 1p32 region | Loss of chromosome 22q12 in both components, supporting neoplastic theory |
| Perry et al. [6] | MA-M (10 cases)<br>MA (14 cases) | + (2 cases out of 14 MAs) | FISH and ICH | 22q12.2<br>18p11.3 | 1. Gene deletions (*NF2/4.7B*) and protein losses (merlin/protein 4.1B) in both components, supporting neoplastic theory<br>2. No gene deletions in pure MAs, supporting hamartomatous nature of pure MA |
| Stemmer-Rachamimov et al. [7] | MA (12 cases) | – | SSCP | *NF2* gene | Absence of somatic mutation of the *NF2* gene, supporting hamartomatous nature |
| Kim et al. (this study) | MA-M (7 cases)<br>MA (2 cases) | – | LOH (13 microsatellite markers) | 22q11-12<br>1p32<br>13q14<br>9p21<br>16q22<br>17p13 | 1. LOH on 1p32, 9p21, 22q11-12 in meningioma component of MA-M, supporting neoplastic theory<br>2. LOH for loci on D22S929 and D9S162 in pure MA, supporting neoplastic theory |

*NF2* Neurofibromatosis type 2, *LOH* loss of heterozygosity, *FISH* fluorescence in situ hybridization, *ICH* immunohistochemistry, *SSCP* single strand conformation polymorphism

additional allelic loss on chromosome 9p21 rather than chromosome 22q12. These results are distinctly different from those of Perry et al.'s study. In our opinion, these findings support the evidence that meningioma develops from additional genetic alterations on the preexisting MAs having genetic alterations in itself. The previous studies are summarized in Table 3.

In the literature pertaining to Ki-67 proliferation indices of MAs, high indices were detected in the meningioma portions of MA-M, while low or absent indices were detected in the MA portions of MA-M or pure MA [5–7, 19]. Absent Ki-67 immunoreactivity or relatively low proliferation indices in pure MAs or MA-M support the clinical impression of a slow-growing lesion. In the present study, as measured by morphometric analysis, a similar pattern of Ki-67 results was obtained in MA-M cases, and the proliferation indices of MA-M cases were significantly higher in the meningioma portion than in the MA components. The Ki-67 labeling index of our cases are ranged in low grade meningiomas [20] except a case (case 2) that showed higher labeling index (10.1%). That patient had a recurrent tumor but both original and recurrent tumors were histologically benign.

Microsatellite alterations on *NF2*, 1p32 and 9p21 might contribute to the progression from MA to meningiomas, similar to conventional de novo meningiomas. Our observation on allelic losses on chromosome 22q12 or chromosome 9p21 in pure MAs, albeit a small series, suggests that the MA and MA-M may in part share the same genetic event and undergo the same overlapping clonal process, with the meningioma arising from MA probably undergoing additional and genetic alterations. These genetic alterations of MA may confer a more proliferative potential. It was also supported by the higher Ki-67-indices in the meningioma portions of MA-M.

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