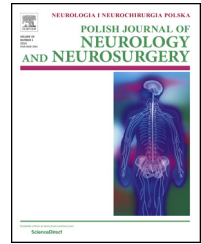


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## Original research article

# The correlation of clinical and chromosomal alterations of benign meningiomas and their recurrences



Waldemar Och<sup>a</sup>, Tomasz Szmuda<sup>b,\*</sup>, Kamil Kulbacki<sup>a</sup>, Krzysztof Witek<sup>a</sup>,  
Beata Sikorska<sup>c</sup>, Magdalena Zakrzewska<sup>c</sup>, Janusz Springer<sup>d</sup>,  
Joanna Reszeć<sup>e</sup>, Agnieszka Parda<sup>a</sup>, Paweł P Liberski<sup>c</sup>

<sup>a</sup>Neurosurgery Department, Regional Specialist Hospital, Olsztyn, Poland<sup>b</sup>Neurosurgery Department, Medical University of Gdansk, Gdansk, Poland<sup>c</sup>Department of Molecular Pathology and Neuropathology, Medical University of Łódź, Łódź, Poland<sup>d</sup>Department of Preventive Medicine and Education, Medical University of Gdańsk, Gdańsk, Poland<sup>e</sup>Department of Medical Pathomorphology, Medical University of Białystok, Białystok, Poland

## ARTICLE INFO

## Article history:

Received 14 March 2016

Accepted 13 July 2016

Available online 26 July 2016

## Keywords:

Meningioma

Loss of heterozygosity

LOH

Tumour recurrence

## ABSTRACT

Meningiomas (MGs) are the frequent benign intracranial tumors. Their complete removal does not always guarantee relapse-free survival. Recurrence-associated chromosomal anomalies in MGs have been proposed as prognostic factors in addition to the World Health Organisation (WHO) grading, tumor size and resection rate. The aim of this study was to evaluate the frequency of deletions on chromosomes in sporadic MGs and to correlate them with the clinical findings and tumor behaviour. Along with survival, the tumor recurrence was the main endpoint. Chromosomal loss of heterozygosity (LOH) was studied. 46 benign MGs were subjected to the analysis, complete tumor resection was intended and no early mortalities were observed. Incomplete removal was related to parasagittal location and psammomatous histopathology ( $p < 0.01$ ). Chromosomal alterations were present in 82.6% of cases; LOH at 22q (67.4%) and 1p (34.8%) were the most frequent and associated with male sex ( $p = 0.04$ ). Molecular findings were not specific for any of the histopathologic grade. Tumor recurrence (14 of 46) correlated with tumor size ( $\geq 35$  mm), LOH at 1p, 14q, coexistence of LOH at 1p/14q, 10q/14q, 'complex karyotype' status ( $\geq 2$  LOHs excluding 22q), patient age (younger  $< 35$ ), and Simpson grading of resection rate ( $\geq 3$  of worse prognosis). The last 3 variables were independent significant prognostic factors in multivariate analysis and of the same importance in recurrence prediction (Receiver Operating Characteristic curves comparison  $p > 0.05$ ). Among the cases of recurrence, tumor progression was observed in 3 of 14. In 2 cases, LOH on 1p and/or coexistence of LOH 1p/14q correlated with anaplastic transformation.

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\* Corresponding author at: Neurosurgery Department, Medical University of Gdansk, Debinki 7, 81-952 Gdansk, Poland.

Tel.: +48 583493330/669400733; fax: +48 583493320.

E-mail address: [tszmuda@gumed.edu.pl](mailto:tszmuda@gumed.edu.pl) (T. Szmuda).<http://dx.doi.org/10.1016/j.pjnns.2016.07.001>

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## 1. Introduction

According to the recent reports (2007–2011 USA registry), meningiomas (MGs) are the most frequently diagnosed brain tumor (36.1%), exceeding half of all intracranial benign tumors (53.7%) [1]. A MG is a frequent incidental finding in magnetic resonance imaging (MRI) of the head, but is revealed in only 2% of autopsies [2,3]. To date, the etiology of these tumors has not been sufficiently explored. Researchers attempted to use molecular biology to explain the recurrence phenomena of a completely removed MG. The criteria of the diagnosis of Grade 1–3 MG (respectively benign, atypical and malignant) were based on clinicopathological correlations made by the World Health Organisation (WHO) [4,5]. The 2007 revision of the WHO classification identified 14 heterogeneous histopathological subtypes [6]. According to WHO ~80% of all MGs are slowly growing Grade 1 tumors [7]. Whereas the Grade 2 or 3 are rarely diagnosed. Moreover, it has been suggested that 17–35% of Grade 2 and 54–70% of Grade 3 progress from benign subtypes [8,9]. The WHO grading of MGs remains controversial, nonetheless it has facilitated estimating patient management [10]. Parallel to the resection rate, the WHO classification is still regarded as the most potent prognosis-associated factor [10,11].

Almost 40% of the totally removed Grade 2 tumors relapse, comparing to only 5% of Grade 1 MGs [12]. However, the diagnosis of one of nine subtypes of benign MG (called Grade 1, non-cancerous) does not preclude its worse clinical behaviour in some patients [13]. Finally at least a quarter of patients harbouring a benign MG experience a tumor relapse within 20 years [14]. Therefore, some prognosis-related factors have been proposed to identify a clinically aggressive subset of benign MGs: familial occurrence, patient age, tumor location, Ki-67/MIB-1 labelling index, telomerase activity, proliferating cell nuclear antigen [15–17]. Recently the molecular biology has attempted to explain the recurrence phenomena of a completely removed MG [10,18–20]. The milestone was the identification of the NF2 gene on the long arm of chromosome 22 (22q12.2) that is responsible for the production of merlin (a cytoskeletal protein). Mutation or loss of NF2 is associated with the multiple tumor occurrence and is found in up to 70% of sporadic MGs [21,22]. Since 22q was established the most frequent aberration in MGs, authors focused on cytogenetic profiling of MG tumorigenesis or progression. A broad array of the loci was postulated, including 1p, 3q, 6q, 9p, 9q, 10p, 10q, 14q, 14p, 18p, 18q and 22q [10,20,23,24]. Moreover, single nucleotide polymorphism (SNP) demonstrated loss of heterozygosity (LOH) on several loci within one cytoband in MGs [21]. Surprisingly, the investigators sparsely focused on the correlation of molecular findings with clinical data. Benign tumors with known genomic status were rarely followed-up, thus we still are not certain of the relapse-associated chromosomal aberrations [18,20,25–27]. In clinical perspective, the disease-free survival is actually the most crucial outcome measure. The estimation of relapse-specific genomic landscape of MGs is strongly desired [18]. A prognosis based on molecular and histopathological findings is believed to prompt a decision-making process tailored to each individual patient [20].

## 2. Materials and methods

### 2.1. Study design

The aim of the study was to correlate the recurrence status and time to relapse with chromosomal alterations in sporadic Grade 1 MGs. The analysis included clinical data such as age, sex, tumor location and rate of recurrence. Multivariate analysis was used to bring reliable *molecular* recurrence-related prognostic factors.

### 2.2. Patients

Tumor tissues were obtained from patients presenting with non NF1/NF2-related intracranial MG. All consenting patients were managed operatively at the Neurosurgery Department (coded for peer review process) from 1999 to 2007. Intent-to-treat patient selection was applied; complete resection was intended in all cases and the Simpson grading was used for assessing the extent of tumor removal [28]. The study group consisted of 46 patients with benign (WHO Grade 1) sporadic MGs. The demographics are presented in Table 1. Pre- and postoperative imaging was evaluated, but was not standardised throughout the study (either computer tomography or MRI was performed based on the accessibility). The details of the surgical techniques performed are beyond the scope of this article purpose. Baseline and postoperative neurological status was not evaluated. Postoperative radiotherapy was not offered. All patients were followed-up either by outpatient clinic visits, mail, e-mail or phone.

**Table 1 – Patient characteristics, histopathological diagnoses and the extent of resection.**

	n	% or ratio
Demographics		
Sex (female:male)	33:13	Ratio:2.54
Age [mean ± SD median, min–max]	51.1 ± 12.9 50 (20–80)	
Location		
Supra-/infratentorial	38:8	ratio:4.75
Convexity	25	54.3%
Parasagittal or falx	15	32.6%
Cranial base	21	45.7%
Sphenoid	13	28.3%
Pyramid	5	10.9%
Size (mm)		
<35	4	8.7%
35–55	11	23.9%
>55	31	67.4%
Simpson grading		
1	26	56.5%
2	15	32.6%
3	4	8.7%
4	1	2.2%
Histopathology		
Transitional	24	52.17%
Fibroblastic	10	21.7%
Meningothelial	9	9.57%
Psammomatous	3	6.52%

### 2.3. Histopathology and LOH analysis

Tumor samples were collected intraoperatively for the standard histopathological examination and for the molecular analysis.

For the purpose of the molecular analysis, samples were stored at  $-20^{\circ}\text{C}$ . The genomic DNA was isolated from frozen tumor tissues and the corresponding peripheral blood leukocytes using a phenol-chloroform extraction protocol. Further quantification and analysis with respect to protein content and purity was performed. The markers were selected by using the NCBI database; paired normal and tumor DNA samples were analysed for LOH using 24 microsatellite markers obtained from HVD Holding AG (Ebersberg, Germany). The following polymorphic loci were tested: D<sub>1</sub>S<sub>508</sub>, D<sub>1</sub>S<sub>199</sub>, D<sub>1</sub>S<sub>197</sub>, D<sub>1</sub>S<sub>162</sub>, D<sub>9</sub>S<sub>156</sub>, D<sub>9</sub>S<sub>162</sub>, D<sub>9</sub>S<sub>319</sub>, D<sub>9</sub>S<sub>1748</sub>, D<sub>10</sub>S<sub>197</sub>, D<sub>10</sub>S<sub>209</sub>, D<sub>10</sub>S<sub>587</sub>, D<sub>10</sub>S<sub>1709</sub>, D<sub>14</sub>S<sub>292</sub>, D<sub>14</sub>S<sub>1010</sub>, D<sub>18</sub>S<sub>481</sub>, D<sub>22</sub>S<sub>257</sub>, D<sub>22</sub>S<sub>258</sub>, D<sub>22</sub>S<sub>268</sub>, D<sub>22</sub>S<sub>298</sub>, D<sub>22</sub>S<sub>303</sub>, D<sub>22</sub>S<sub>449</sub>, D<sub>22</sub>S<sub>609</sub>, D<sub>22</sub>S<sub>1150</sub>, and D<sub>22</sub>S<sub>1163</sub>. The polymerase chain reaction (PCR) was performed under standard conditions, the products were then electrophoresed on 6% denaturing polyacrylamide gel containing 7 mmol/L of urea and visualized using a LiCor automatic sequencer (LiCor Biotechnology, Lincoln, NE, USA). A  $>50\%$  reduction of intensity in the tumor lane compared to the corresponding blood lane was regarded as LOH. All samples with LOH were confirmed by repeated analysis.

Histopathological examination was performed; tumor samples were put in a 4% solution of buffered formaldehyde; dehydrated after 10–24 h of fixation, cleared and impregnated with paraffin in tissue processor and embedded in paraffin blocks. Slices of 4  $\mu\text{m}$  thickness were made and placed on microscope slides, stained by hematoxylin and eosin using an automatic stainer, then covered with coverglasses. After the initial histopathologic evaluation, immunostains with antibodies to progesterone receptor, epithelial membrane antigen (EMA) and MIB-1 were performed. Tumors were classified according to the WHO criteria (2002 and 2007 revision) [6,13].

### 2.4. Statistical analysis

The Chi-square with or without Yates correction for continuity, Fisher test, t-test or Mann-Whitney U test were used for testing statistical hypotheses. The independent MG

recurrence-associated factors were obtained by logistic regression in stepwise fashion. Odds ratios (ORs) were provided with 95% confidence interval (CI). Area under (AUC) receiver operating characteristic (ROC) curve was calculated for significant factors in multivariate analysis. GraphPad Prism v6.07 (GraphPad, La Jolla, CA, USA) and Statistica v12.0 (StatSoft, Tulsa, OK, USA) were applied for the calculations. A  $p$  value  $<0.05$  was considered statistically significant.

## 3. Results

### 3.1. Outcome

There were no early mortalities either related to the initial surgery. All 4 small and 72.7% (8 of 11) of medium MGs were excised completely (Simpson grade 1). Only 45.2% (14 of 31) of large tumors were resected totally with attached dura mater. However, the tumor size did not influence the resection rate (Chi-square 10.5,  $p = 0.10$ ). Convexity and cranial base MGs had a similar resection rate (Chi-square/Yates 2.4,  $p = 0.49$ ), likewise infra and supratentorial tumors (Chi-square/Yates 0.6,  $p = 0.89$ ). However, parasagittal (falx) tumor location correlated to its incomplete removal (Simpson grade  $\geq 3$ ). Of note, 3 of 15 (20.0%) parasagittal MGs were partially resected and 2 of 31 (6.5%) those occupying other location (Chi-square 17.9,  $p < 0.01$ ). All fibroblastic ( $n = 10$ ), almost every meningotheial (8 of 9; 88.9%) or transitional MG (22 of 24; 91.7%) was totally removed, while only 1 of 3 (33.3%) of psammomatous histopathology were (Chi-square 17.8,  $p = 0.04$ ). Simpson grading did not correlate with the side of tumor (Chi-square 3.4,  $p = 0.76$ ) or sex (Chi-square/Yates 0.63,  $p = 0.89$ ).

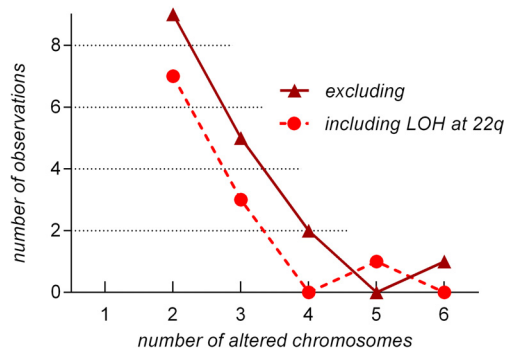
### 3.2. Genetic profile

Chromosomal alterations among the determined set of loci were present in 38 MGs (82.6%). The most frequent alteration was LOH at 22q, following 1p and 14q. Male sex significantly predisposed to LOH at 1p. No LOHs were specific for any of histopathology (Table 2).

No specific chromosomal localization of LOH was associated with tumor localization nor its primary size ( $p > 0.05$ ). Only tumors  $>35$  mm in diameter harboured mutation on 10q ( $n = 6$ )

**Table 2 – Distribution of loss of heterozygosity on determined cytobands. A correlation with sex and histopathology was presented. Statistically significant correlation in bold. LOH – loss of heterozygosity.**

LOH at	Sex					Histopathology				
	Entire group ( $n = 46$ )		Females ( $n = 33$ )	Males ( $n = 13$ )	$p$	Meningothelial	Fibroblastic	Mixtum	Psammomatous	$P$
	$n$	% of column	$(n; \% \text{ of column})$			$(n; \% \text{ of column})$				
1p	16	34.8%	8; 24.2%	8; <b>61.5%</b>	<b>0.04</b>	4; 44.4%	3; 30.0%	9; 37.5%	0; 0.0%	0.54
9p	3	6.5%	2; 6.0%	1; 7.7%	0.64	0; 0.0%	2; 20%	1; 4.2%	0; 0.0%	0.26
10q	6	13.0%	2; 6.1%	4; 30.8%	0.07	0; 0.0%	1; 10.0%	5; 20.8%	0; 0.0%	0.37
14q	13	28.0%	9; 27.7%	4; 30.8%	0.90	4; 44.4%	3; 30.0%	6; 25.0%	0; 0.0%	0.48
18p	1	2.2%	1; 3.0%	0; 0.0%	0.63	0; 0.0%	1; 10.0%	0; 0.0%	0; 0.0%	0.30
22q	31	67.4%	25; 75.8%	6; 46.1%	0.11	3; 33.3%	9; 90.0%	17; 70.8%	2; 66.7%	0.07



**Fig. 1 – Number of patients with total number of altered chromosomes.**  
**Light red line – 22q anomaly was included, dark red line – without 22q.**  
**LOH – loss of heterozygosity.**

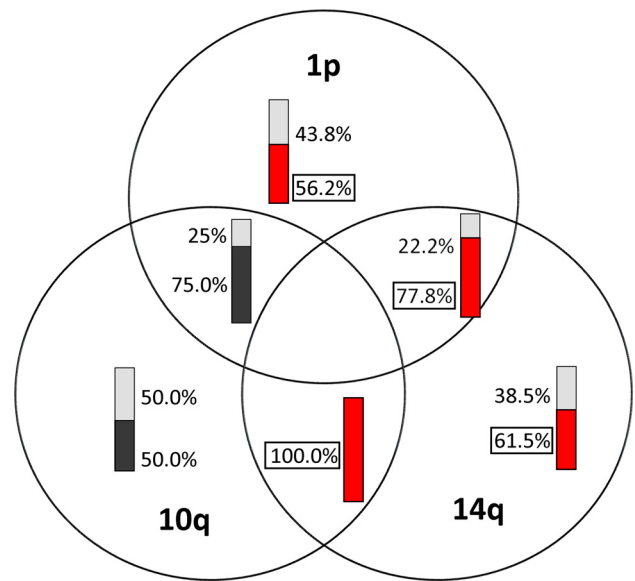
more frequently than smaller tumors (Chi-square 7.1,  $p = 0.03$ ). Of note, none of smaller MGs had LOH at 10q. Patient age did not correlate with any LOH ( $p = 0.16-1.00$ ).

Excluding LOH at 22q, 11 tumors (23.9%) had LOH on at least two cytobands (named herein 'complex karyotype'). The mean number of chromosomal alterations was 1.5 (median 1, maximum LOH at 6 chromosomes) (Fig. 1).

'Complex karyotype' was more frequent in males (6/13 46.1% vs. 5/33 15.2%;  $p = 0.07$ ) and significantly more frequent for MGs located parasagittally (7/15 46.7% vs. 4/31 12.9%;  $p = 0.03$ ). Neither age ( $p = 0.72$ ), histopathology ( $p = 0.68$ ) or Simpson grade ( $p = 0.26$ ) associated with LOH occurrence.

### 3.3. Tumor recurrence

Mean observation time to the first relapse after successful surgery was 5.7 years (16 months–13 years). 19.6% of patients (9/46) were followed for less than 30 months. In the time of observation tumor relapsed in 14 patients (30.4%), 9 of whom (9 of 14; 64.3%) experienced a second relapse. In the recurrence group, mean relapse-free survival was 31.5 months (median 25.5; 9 months–6 years). Slightly more males experienced MG relapse (7/13 53.9% vs. 7/33 21.2%;  $p = 0.07$ ). Convexity location had a tendency to recur was noted in the convexity location, though it was not statistically significant (11/25 44.0% vs. 3/21 14.3%;  $p = 0.06$ ). Supra/infratentorial location (Chi-square/Yates 0.1,  $p = 0.96$ ), cranial fossa (Chi-square 3.8,  $p = 0.42$ ), side of the body (Chi-square 0.0,  $p = 0.99$ ) as well as MG histopathology (Chi-square 0.7,  $p = 0.88$ ) did not demonstrate influence on the recurrence. MIB1 labeling index was evaluated in less than a half of cases thus not studied. 0 (0.0%), 1 (9.1%) and 13 (41.9%) of small, medium and large tumors relapsed respectively (Chi-square 6.1,  $p = 0.05$ ). As hypothesized, the rate of resection had an impact on the recurrence: 29.3% (12 of 41) of completely removed MGs (Simpson grade  $\leq 2$ ) versus 40.0% (2 of 5) of grade  $\geq 3$  relapsed (Chi-square 11.7,  $p < 0.01$ ). Younger patients experienced tumor recurrence more often, as mean age in the recurrence group was 54.1 (SD  $\pm 12.9$ ) and in the non-recurrence group was 44.1 (SD  $\pm 10.1$ ) (Z statistics = 2.6;  $p < 0.01$ ).



**Fig. 2 – Venn diagram demonstrating the influence of chromosomal anomalies on the meningioma recurrence. Tumors presenting with a selected chromosomal alterations or their coexistence are stratified for recurrent (black/red, black – insignificant, red – significant comparisons) and non-recurrent meningiomas (grey bar).**

The presence of LOH at any of the examined cytobands was not associated with the recurrence (Chi-square/Yates 2.7,  $p = 0.10$ ). The analysis of a single aberration revealed that only LOH at 1p, 14q as well as 'complex karyotype' (LOH at more than 2 loci) was related to the tumor recurrence. The greater number of chromosomes was damaged, the greater was the risk of recurrence; 8 of 14 (57.1%) recurrent tumors had complex karyotype (Fig. 2, Table 3).

Interestingly, 2 of the 7 tumors (28.6%) relapsed in the same location and 6 of 7 (85.6%) that relapsed in other location had complex karyotype (Fisher exact test 0.4,  $p = 0.38$ ).

Summarising the significant univariate analyses of relapse-associated factors, there were 5 genetic variables: 1p, 14q, complex karyotype (including coexistence of 1p/14q and 10q/14q), total number of damaged chromosomes (excluding 22q) as well as 3 other variables: size, Simpson grading, and patient age. In logistic regression (model Chi-square 24.6) only 3 factors independently and significantly influenced the recurrence behaviour: age (OR = 0.9; 95%CI: 0.8–1.0;  $p = 0.03$ ), complex karyotype (OR = 27.2; 95%CI: 3.3–223;  $p < 0.01$ ) and resection rate (Simpson grading) (OR = 4.8; 95%CI: 1.4–16.8;  $p = 0.01$ ). All of the above 3 factors had a comparable impact on the benign MG recurrence (ROC curves comparison:  $p < 0.01$ ) (Fig. 3).

Area under curves (AUC) of the following factors: complex karyotype AUC = 0.74; 95%CI: 0.59–0.86, Simpson grading AUC = 0.73; 95%CI: 0.58–0.85, patient age AUC = 0.74; 95%CI: 0.59–0.85.

Concerning the 14 patients who experienced tumor recurrence, time to relapse (in other words relapse-free survival) was analysed in terms of various factors (LOH, demographics, tumor location, rate of resection and histopathology).

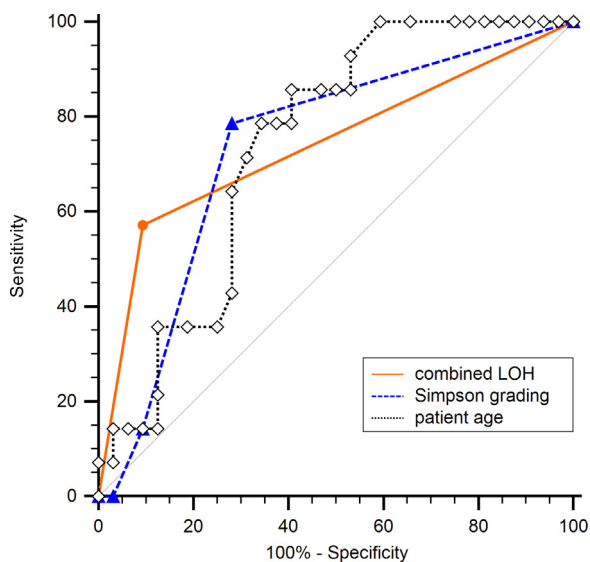
**Table 3 – The comparison of genetic profile of meningiomas in patients with and without relapse. Statistically significant correlations in bold.**

	Recurred (n = 14)	Non-recurred (n = 32)	p
LOH at	(n; % of column)		
1p	9; <b>64.3%</b>	7; 21.9%	<b>0.01</b>
9p	2; 14.3%	1; 3.1%	0.45
10q	3; 21.4%	3; 9.4%	0.52
14q	8; <b>57.1%</b>	5; 15.6%	<b>0.01</b>
18p	1; 7.1%	0; 0.0%	0.67
22q	9; <b>64.3%</b>	22; 68.8%	0.96
Complex karyotype	8; 57.1%	3; 9.4%	<0.01
1p/10q	3; 21.4%	1; 3.1%	0.14
1p/14q	7; <b>50.0%</b>	2; 6.3%	<0.01
1p/22q	5; 35.7%	5; 15.6%	0.26
10q/14q	3; <b>21.4%</b>	0; 0.0%	<b>0.04</b>
10q/22q	2; 14.3%	2; 6.3%	0.74
14q/22q	3; 21.4%	5; 15.6%	0.96
Number of damaged chromosomes (mean ± SD)			
Including 22q	1.6 ± 1.4	0.5 ± 0.8	0.02
Excluding 22q	2.2 ± 1.5	1.2 ± 0.9	<b>0.01</b>

Our calculations revealed that none of them had a statistically significant impact.

### 3.4. Tumor progression

Among all cases of MG recurrence, there were 3 (21.4%; 3 of 14) that progressed to higher grade according to WHO classification. In Patient 1 the tumor progressed to atypic and then to anaplastic MG. Patient 2 had a primary transitional MG, but on it relapsed as atypic. Patient 3 had a Grade 1 (meningothelial) recurrent tumor, whereas the second relapse was diagnosed as atypic MG. The molecular profile of the progressed tumors was



**Fig. 3 – Comparison of Receiver Operating Characteristic (ROC) curves between significant markers of benign meningioma recurrence.**

the same in 2 cases, whereas in Patient 1 the additional 1p mutation was noted upon the first relapse (Fig. 4).

## 4. Discussion

Leaving an apparently cured patient with Grade 1 MG unattended may be deleterious as the tumor can relapse. Irrespective of the WHO classification, two factors are undisputedly regarded as responsible for the recurrence – tumor size and the resection rate [29]. Considering only benign MGs, as in our series, a complete removal of the tumor mass could be achieved at least in half of patients. However, the neurosurgeons' self-assessment remains unreliable. In our series partially resected MGs were followed up by imaging without radiotherapy. Finally, 5% of the totally resected tumors recur [12], but almost 20% recur if observed for 20 years [13,14,30]. The resection rate correlated with recurrence rate in our series.

Younger patients are more prone to develop tumor recurrence, which can be connected with a longer survival time, but not elucidated. In our study, the multivariate analysis pointed to age as an independent and statistically significant factor responsible for the relapse status. Linsler's results does not support our findings even though he incorporated over hundred patients [31]. According to the Domingues series (302 cases), older patients experience longer relapse-free survival [20].

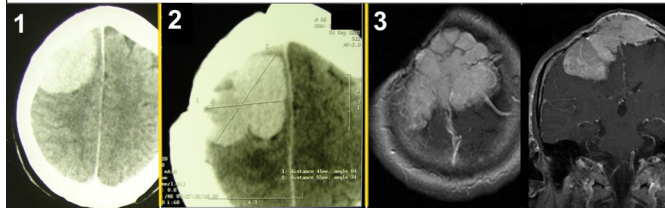
The third marker of recurrence in our study was the tumor size. One explanation is that more advanced technical skills are required to completely remove the larger tumor and therefore leaving a remnant is more probable. We proved that the size of the tumor did not influence the resection rate.

Tumor location correlated with the resection rate, but also relates to the recurrence rate itself. MGs originating at the cranial base recur more often than those of other locations [20,32]. In our sample, the recurrence of the convexity MGs was insignificantly more frequent than in cranial base tumors.

Molecular profiling is a recent and considered as powerful tool in explaining tumorigenesis of various brain tumors [10,33]. The aberration of chromosome 22 or mutation of NF2 (22q12.2) are the most commonly cited cytogenetic markers in MGs [34]. NF2 mutation is most frequent in fibroblastic, transitional and psammomatous histopathology [35]. In our series, 2/3 of tumors presented with LOH at 22q, which was the most frequent chromosomal aberration, though not associated with any particular histopathology. On the epigenetic level, the mutation or incorrect expression of the candidate genes (including, but not limited to, MN1, INI1, TIMP-1, TIMP3, p16, BCR, BAM22) was suspected in MG pathogenesis [31,36]. However, the above findings still show a limited prognostic value for patients harbouring Grade 1 MG. Genetic changes may bring better understanding of the relapse of completely removed benign tumors [23]. Recent studies consider the analysis of chromosomal LOH as the most crucial part of genetic evaluation in MGs [10,20,21].

For years the histopathological classification has been regarded as the most accurate parameter in the prognostication of these tumors. However, both the clinical and prognostic value of WHO grading is limited in cases of benign MGs

Patient	Type of meningioma	No of relapse	LOH on					Combined LOH on 1p/14q
			1	9	10	14	18	
✓ 1	Mixtum				X	X	X	
	Atypic	1	X		X	X	X	X
	Anaplastic	2	<i>not evaluated</i>					
2	Mixtum					X		
	Atypic	1				X		
3	Mixtum		X		X	X		X
	Meningothelial	1	X		X	X		X
	Anaplastic	2	<i>not evaluated</i>					



**Fig. 4 – The molecular characteristic of the 3 patients in who tumor progression was observed. The initial computer tomography (CT, axial plane image) of the Patient 1 (red tick) was presented (1) and axial plane CT at the time of tumor relapse (2) (atypic histopathology and additional 1p mutation). Second progression (3) magnetic resonance imaging (axial and coronal plane images) demonstrated sagittal sinus, bone and brain invasion. The coexistence of loss of heterozygosity at 1p/14q was marked.**

[5,26,37]. Due to the high frequency of occurrence, the absolute number of relapses after a seemingly successful removal among all MGs is the highest for benign histopathologies [26]. Moreover, some Grade 1 tumors behave more aggressively, similarly to atypic or anaplastic MGs. These findings demonstrate the need to identify additional recurrence-associated biomarkers, specific for benign tumors. Predicting the worst clinical scenario could streamline a tailored therapy.

Following 22q, the anomaly of 1p is the most frequent chromosomal aberration in sporadic MGs. However, opposite to 22q, LOH on 1p is considered as the most influential recurrence-associated chromosomal change [21,31]. Except for ELAVL4, the presence of any other suppressor gene among 1p was suggested, but as of yet was not sufficiently proven [38]. The expression of ELAVL4 was lower in males, explaining the higher recurrence rate in males [36]. LOH on 1p and male sex both correlated with tumor recurrence in univariate statistics in our study, which can indirectly confirm the deleterious role of ELAVL4 mutation.

Among the determined loci, LOH on 9p, 10q and 18p was not related to the aggressive behaviour of a benign MG. Other cytogenetic investigations described the role of 9q, 10q and 18p in MG progression and which are a characteristic finding in anaplastic histopathology [38]. The alteration of 10q is linked with the PTEN mutation (10q23.3), characteristic for Cowden syndrome [38], but rare in MGs. Larger tumors in our series displayed mutations on 10q more often. On the other hand, the coexisting LOH on 10q and 14q was a significant marker of recurrence in our series. The mutation or hypermethylation of NDRG2 and MEG3 both located on 14q were proposed in MG progression and recurrence [38]. Most of clinical studies confirmed 14q as a prognostic indicator for recurrence in Grade 1 tumors [20,38,39], as shown in our study.

Opposite to the results of the multivariate analysis, a variety of cytogenetic anomalies demonstrated the impact on the tumor recurrence in our series. LOH on 1p, 14q, 'complex karyotype,' combined LOH on 1p/14q, 10q14q as well as total number of aberrations (excluding the most common LOH – 22q) were significant in univariate comparisons. Domingues et al. performed an analysis similar to ours and out of many the numerous single prognostic factors selected only the 'complex karyotype', tumor location, tumor size and patient age as the best combination of independent variables for predicting relapse-free survival only [20]. These prognostic factors were incidental in our findings, except for the Simpson grade of resection. Domingues et al. created a tailored scale of prognostic importance which can be applied to any single patient post hoc. According to their findings, the role of a molecular biology in predicting a MG recurrence seems crucial, but likewise in our series it is limited to the number of altered loci. The statistical methodology applied in both studies (Domingues et al. and ours) revealed that univariate comparison seems valid, unless multivariate analysis is performed [20]. Multivariate analysis is a goal in the recurrence.

Unfortunately Pfisterer et al. [19] contradicted all of the above conclusions. He demonstrated that a regional variability of distribution in chromosomal changes exists in a single MG. For example, only 20% of benign MG presented with a homogeneous 14q aberration.

Our study did not aim to investigate the molecular pattern of tumor progression. Out of 14 relapsed tumors, 3 exhibited histopathological progression. The molecular findings revealed that acquired loss of genetic material on 1p and coexistence of LOH on 1p/14q lead to anaplastic transformation. These findings, based only on 3 progressed samples, support the deleterious role of mutation on 1p in progression of MG [20,39].

In sum, the aggressive phenotype of a benign MG is certainly correlated with its molecular status. Popular cytobands, including 1p, 14q and their coexistence, are recurrence-specific genomic alterations.

## 5. Conflict of Interest

None declared.

## 6. Acknowledgement and Financial Support

None declared.

## 7. Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at [doi:10.1016/j.pjnns.2016.07.001](https://doi.org/10.1016/j.pjnns.2016.07.001).

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