Clinical Characteristics and MRI-based Prediction of the *KLF4*^{K409Q}-mutation in Meningioma

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Abstract

Purpose: Meningioma is the most common primary brain tumor in adults. In recent years, several non-NF2 mutations, i.e. *AKT1, SMO, TRAF7,* and *KLF4* mutations, specific for meningioma have been identified. This study aims to analyze the clinical impact and imaging characteristics of the KLF4^{K409Q} mutation in meningioma.

Methods: Clinical, neuropathological, and imaging data of 170 patients who underwent meningioma resection between 2013 and 2018 were retrospectively collected and tumors were analyzed for the presence of the *KLF4^{K409Q}* mutation. We collected imaging characteristics, performed semiautomatic volumetric analysis of tumor size and peritumoral edema (PTBE), and calculated the edema index (EI, i.e. ratio of PTBE to tumor volume). Receiver operating characteristic (ROC) curve analysis was performed to identify cut-off EI values to predict the mutational status of KLF4.

Results: Eighteen (10.6%) of the meningiomas carried the KLF4^{K409Q}mutation; these were significantly associated with a secretory subtype ($p<0.001$) and sphenoid wing location ($p=0.029$). Small tumor size ($p=0.007$), an increased PTBE ($p=0.012$), and an increased EI ($p=0.001$) proved to be significantly associated with the *KLF4^{K409Q}* mutation. In receiver operating characteristic (ROC) curve analysis, EI predicted the *KLF4^{K409Q}* mutation with an AUC of 0.728 ($p=0.0016$).

Conclusion: The KLF4^{K409Q} mutation is associated with a distinct small tumor subtype, prone to substantial PTBE. EI is a reliable parameter to predict the *KLF4^{K409Q}* mutation in meningioma, thus providing a tool for improvement of pre- and perioperative medical management.

Introduction

With an incidence of 8-14/100,000, meningioma are the most common primary brain tumor in adults.[1] About 20% of meningioma are classified as WHO grade II° or III°, while 80% correspond to WHO I°.[2] Meningioma arise from the arachnoid cap-cells and may harbor a distinct subset of mutations, the most common being the bi-allelic inactivation of *NF2.* [2, 3] Due to its frequency, the clinical and biological impact of *NF2*-inactivation has been a focus of meningioma research. In recent years, "non-*NF2*" mutations (*AKT1, SMO, TRAF7* and *KLF4*) have been shown to correlate with certain locations, frequency of recurrence and some are currently investigated as candidates for targeted therapy (ClinicalTrials.gov: NCT02523014). [4-7]

A subgroup of meningioma causes peritumoral brain edema (PTBE) which itself can lead to an amplification of tumor related symptoms or cause new neurologic deficits and seizures.[8]

Meningiomas of the angiomatous and secretory subtype may be associated with increased PTBE. While the direct cause for this remains elusive, cellular processes such as hypoxia induced upregulation of HIF1a and VEGF have been correlated with increased PTBE in meningioma as well as in metastases.[9,

10] We have recently shown that the *KLF4*^{K409Q} mutation renders meningioma cells susceptible to hypoxia and triggers an upregulation of HIF1a dependent pathways, suggesting that these tumors may be a subgroup prone to cause PTBE.[11]

While our previous research on *KLF4*^{K409Q} mutated meningiomas focused on the impact of the *KLF4*^{K409Q} mutation on cellular processes on the molecular level, this present work investigates clinical features of KLF4^{K409Q} mutated meningiomas.

Materials And Methods

Patient selection

Clinical, neuropathological, and preoperative imaging data on 170 patients who previously underwent meningioma resection between 2013 and 2018 at our center were collected. Only patients with available preoperative MRI-scans (consisting of T1-/T2-weighted, T1-weighted contrast-enhanced (T1 CE), and fluid-attenuated inversion recovery (FLAIR) sequences) and frozen tumor samples were included.

Clinical Data and Imaging analysis

Preoperative MRI scans of 170 patients were analyzed. Tumor location, tumor surface (smooth/irregular), the arachnoid plane (clear/disturbed), and the T2 intensity levels of the tumor were determined. The amount of PTBE was classified into four groups according to the Steinhoff classification[12] and computer-assisted volumetric analysis was used to measure the tumor- and edema volume. For each axial MRI slice, the tumor/edema margins were traced using iPlan software (Brainlab AG, Feldkirchen, Germany). Volumes were calculated as the product of the area traced and the corresponding slice thickness. The sum of each slice volume gave the total tumor or PTBE volume in cubic millimeters. All measurements and classifications were performed independently by 2 neurosurgeons.

Human specimens

Study of human tumor specimens was performed after written consent was received from individual patients. The study was approved by the local ethics board of University Hospital of Cologne (Application No. 03-170).

Statistics

Statistical analysis was performed using SPSS, release 22 and GraphPad Prism 7. ANOVA, t-test, Chi-square, and Fisher's exact test were used for gaussian distributed data, Mann-Whitney-U and Kruskal-Wallis for non-normally distributed variables. Tests were performed two-tailed. Log-rank (Mantel-Cox) test was used for comparison of survival curves. Significance level was defined as $p<0.05$, Confidence Interval: 95%. Error bars in figures represent SD.

Targeted Sanger Sequencing

Targeted Sanger sequencing of the region encompassing codon 409 of the human *KLF4* gene was performed after PCR amplification with the primers GCTCATGCCACCCGGTTC (forward) and CTGTGTGGGTTCGCAGGTG (reverse) on an ABI-Prism-310C capillary sequencer (Applied Biosystems, Foster City, CA, USA), using Big-Dye-Terminator technology (Applied Biosystems).

Results

Patient and tumor characteristics

Data on 170 patients who previously underwent meningioma resection at our center were collected and the corresponding frozen tumor samples sequenced for the $KLF4^{K409Q}$mutation. The median age of all patients was 59.1 years (min. 21, max. 84 years). The majority of patients were female (69.4%, n = 118). 87.6% (n = 149) of the analyzed tumors were classified as meningioma (WHO I°), 11.8% (n = 20) as atypical meningioma (WHO II°), and 0.6% (n=1) as anaplastic meningioma (WHO III°). In 10.6% (n = 18) of the tumors, the $KLF4^{K409Q}$ mutation was found, all of which were WHO I°.

Clinical presentation and follow-up in consideration of the $KLF4^{K409Q}$ mutational status

When comparing patients with $KLF4^{wt}$ and $KLF4^{K409Q}$ tumors, there was no difference in age at presentation (p=0.984) while the *KLF4* mutation was more frequent in female patients, even though significance was not reached (p=0.06). (**Table 1**) The histological subtype of $KLF4^{K409Q}$ tumors differed significantly (p<0.001) from that of $KLF4^{wt}$ tumors. A large portion (50% (n=9)) of the $KLF4^{K409Q}$ tumors were classified as secretory, 33.3% (n=6) as meningothelial, 11.1% (n=2) angiomatous and 5.6% (n=1) as fibroblastic. (**Table 1**)

Symptoms at onset did not differ between the two groups (**Table S1**) including seizures at which were not significantly more frequent in patients bearing KLF^{K409Q} tumors (p=0.603, **Table S1**).

Throughout the first 30 days after surgery, patients with $KLF4^{K409Q}$ mutated tumors had a significantly increased rate of surgical reintervention (including placement of an external ventricular drain (EVD) for hydrocephalus) (p=0.035) and a significantly higher rate of postoperative CSF leaks (p<0.001). Regarding overall complications, there was no significant difference between the two groups (p=0.121) (**Table S2**).

While there was no significant difference in PFS (p=0.308, p=0.322 when excluding WHO II°-III° tumors), there were no recurrent $KLF4^{K409Q}$ tumors within the follow-up time in our cohort. (median follow-up 3.4 years, min. 0.09, max. 11.5 years) **Fig. 1.** An overview of all clinical parameters evaluated is given in supplementary **Tables S1** and **Table S2**.

$KLF4^{K409Q}$-mutated meningioma cluster at the sphenoid wing, are characterized by small tumor size and large peritumoral edema and mutational status can be predicted by the edema index (EI)

66.6% (n=12) of *KLF4*^{K409Q} mutated tumors were skull base meningiomas, and 9 (50%) were sphenoid wing based (**Fig. 2a**), confirming previously reported results.[4, 7]

Image analysis of common MRI parameters such as tumor surface, clear arachnoid plane or T2 intensity did not correlate with mutational status (**Table 2**). However, further analysis of preoperative MRIs revealed that significantly more *KLF4*^{K409Q} tumors harbored larger peritumoral brain edema (PTBE) when classified into 4 categories according to Steinhoff et al.) ($p<0.05$) **Fig. 2b.** [12] This was consistent with the previously reported tendency of secretory meningiomas to cause severe PTBE.[8] A semiautomatic volumetric analysis of both, PTBE and tumor volume (**Fig. 2c,d**) confirmed *KLF4*^{K409Q} tumors to be smaller ($p<0.05$) and yet cause more PTBE ($p<0.05$).

Finally, we calculated the Edema Index (EI, defined as the ratio of PTBE to tumor volume) and found this to be a reliable predictive marker for the *KLF4*^{K409Q}-mutation in Receiver Operating Characteristic (ROC) curve analysis (AUC = .728, p=0.0016; Sensitivity: 61.1%, Specificity 84.7% for criterion >1.4) (**Fig. 2e, f**).

Discussion

This is the first study which correlates clinical data with MRI, neuropathological, and molecular features in a comprehensive series of 170 meningioma patients. In these patients, 10.6% of the tumors harbored the *KLF4*^{K409Q} mutation and all these mutated tumors were graded as meningioma (WHO I°). The frequency of KLF mutations and the associated WHO grade were in line with previously reported mutational rates of about 10%. [4, 7] Similarly, our data confirm previously reported female predominance of *KLF4*^{K409Q} mutation and a prevailing secretory subtype. However, in contrast to previous studies which hypothesized that the *KLF4*^{K409Q} mutation is exclusive to the secretory subtype our cohort suggest that this mutation is not confined to that subtype. [4, 13]

With respect to the clinical aspect of *KLF4*^{K409Q} mutated meningiomas, we observed the only significant correlations of the mutational status to be with an increased incidence of postoperative surgical reintervention (including placement of EVDs for hydrocephalus or brain swelling) and CSF leaks in comparison to *KLF4*^{wt} tumors. The increased PTBE and the inherent increased risk for postoperative brain swelling in secretory meningioma [8] might partially explain the increased incidence of postoperative surgical reintervention. As the underlying causes for postoperative CSF leaks are diverse (e.g. tumor location, bone involvement, surgeon's experience, and technique of dural closure), the observed difference in CSF leaks may be based on the small sample size. [14] .

Other than the increased postoperative risks, *KLF4*^{K409Q} mutated meningiomas appear to represent a slowly growing benign subgroup, since all of the mutated tumors correspond to meningioma (WHO I°) and none recurred within the follow up interval. However, as previously reported, we confirmed that these tumors occur with significantly larger PTBE than *KLF4*^{wt} tumors. The latter may explain the smaller tumor size in initial diagnosis, as an increased edema may cause earlier symptoms.

Previous studies reporting on edema or tumor size in connection with genomic mutations relied on inaccurate volumetric methods. Youngblood at al. calculated tumor volume by measuring the maximum orthogonal diameters in three anatomical planes and employed the ellipsoid formula (V = (4/3)πabc) to calculate tumor size.[7] Especially in the context of scull base tumors which tend to grow in more complex shapes, the ellipsoid formular is an inadequate approximation of the actual volume. Therefore, we performed semiautomatic volumetric analysis of both the tumor and PTBE volume allowing for direct comparison of PTBE and tumor volume. In addition, we were able to calculate the edema index as the ratio of PTBE to tumor volume and found this to be a reliable predictive marker allowing for the prediction of *KLF4* mutational status on the basis of preoperative MRI (AUC of 0.728,a sensitivity of 61.1%, and specificity of 84.7% for an EI > 1.4 in ROC-Analysis). Youngblood et al. reported on a machine learning based approach for genomic subgroup prediction. Their model performed well in distinguishing *NF2* from Non-*NF2* tumors, based on preoperative MRI. It failed however, to predict other subgroups.[7] Our simple method therefore adds a valuable tool to predict the *KLF4* mutation in meningioma. Especially the combination of tools like this with machine learning approaches[7] and newly developed quantitative MRI techniques such as MRI-Fingerprinting could enable precise prediction specific mutations in the future.[15]

While no therapeutic consequences can be drawn form a known mutational status to date, the prediction of genomic mutations might facilitate targeted treatment in the future, especially in complex scull base lesions prone to relevant perioperative complications as well as elderly patients with high perioperative risks.

Thus, our work provides a first towards non-invasive molecular diagnostics with a novel and simple predictive tool for the $KLF4^{K409Q}$ mutational status.

Limitations

The studies retrospective nature and limited set of patients is an inherent limitation to the conclusions made in this study and should be validated through further studies of a different cohort.

Declarations

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Conflicts of interest/Competing interests The authors declare no conflicts of interest

Availability of data and material Not applicable

Code availability Not applicable

Authors' contributions Draft of the study and writing of the Manuscript: NvS. Compilation, analysis and interpretation of clinical data: NvS and PS with contributions from MT, LG, DR, KL, LP, SG, CM and RG. Targeted Sanger Sequencing: NvS, NW, MT, EK and CM. Contribution of paraffin embedded tissue blocks: MD. All authors critically reviewed the manuscript

Ethics approval The study was approved by the local ethics board of University Hospital of Cologne (Application No. 03-170).

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Tables

Table 1: Patient/Tumor characteristics in regard to KLF4 mutational status

Patient/Tumor characteristics		$KLF4^{K409Q}$ n= 18	$KLF4^{wt}$ n=152	p-value
Age	mean (years)	58.6	59.23	0.984
Gender	female	16 (88.9%)	102 (67.1%)	0.63
	male	2 (11.1%)	50 (32.9%)	
WHO Grade	I°	18 (100%)	131 (86.18%)	0.226
	II°	0	20 (13.16%)	
	III°	0	1 (0.7%)	
Histological subtype	secretory	9 (50%)	1 (0.7%)	<0.001
	meningothelial	6 (33.3%)	101 (55.5%)	
	transitional	0	4 (2.63%)	
	angiomatous	2 (11.1%)	13 (8.55%)	
	fibroblastic	1 (5.6%)	12 (7.9%)	
	atypical	0	20 (13.16%)	
	anaplastic	0	1 (0.7%)	

Table 2: Image parameters

Image parameters		KLF4^{K409Q} n= 18	KLF4^{wt} n=152	p-value
Tumor surface	smooth	17 (94.4%)	116	0.127
	irregular	1 (5.6%)	36	
Arachnoid plane	clear	12 (66.7%)	121	0.230
	disturbed	6 (33.3%)	31	
T2 intensity	hypointense	0	6	1.000
	isointense	8	67	
	hyperintense	10	79	
Tumor volume	mean in ml (SD)	13.6 (14.7)	28.4 (28.9)	0.007
Edema volume	mean in ml (SD)	45.2 (48.3)	22.9 (39.6)	0.012
EI (Tumor/Edema)	mean in ml (SD)	5.2 (7.4)	1.2 (3.1)	0.001

Figures

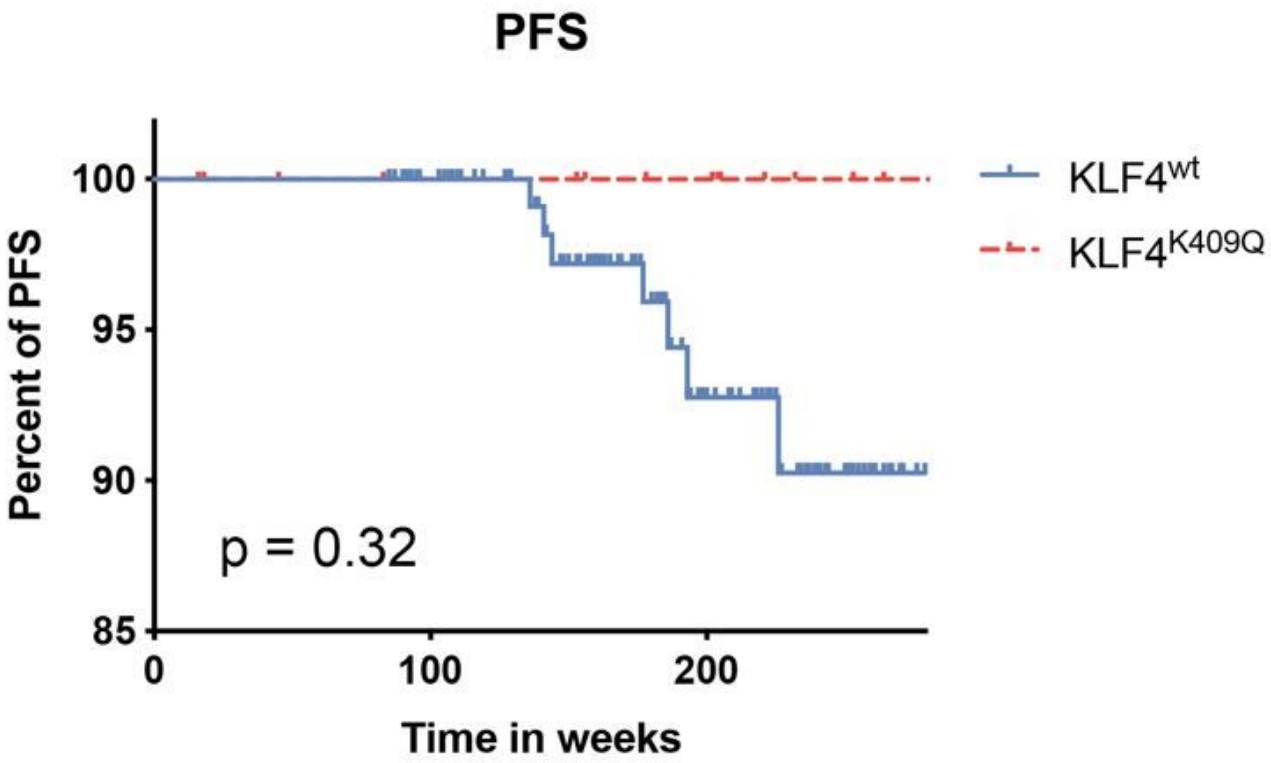

Figure 1

Kaplan-Meier survival curve of KLF4wt and KLF4K409Q meningiomas Kaplan-Meier survival analysis for progression free survival (PFS) comparing WHO I° KLF4wt and KLF4K409Q tumors (p = 0.32). While the difference is not significant, there were no recurrent KLF4K409Q mutated tumors within the timeframe of follow-up.

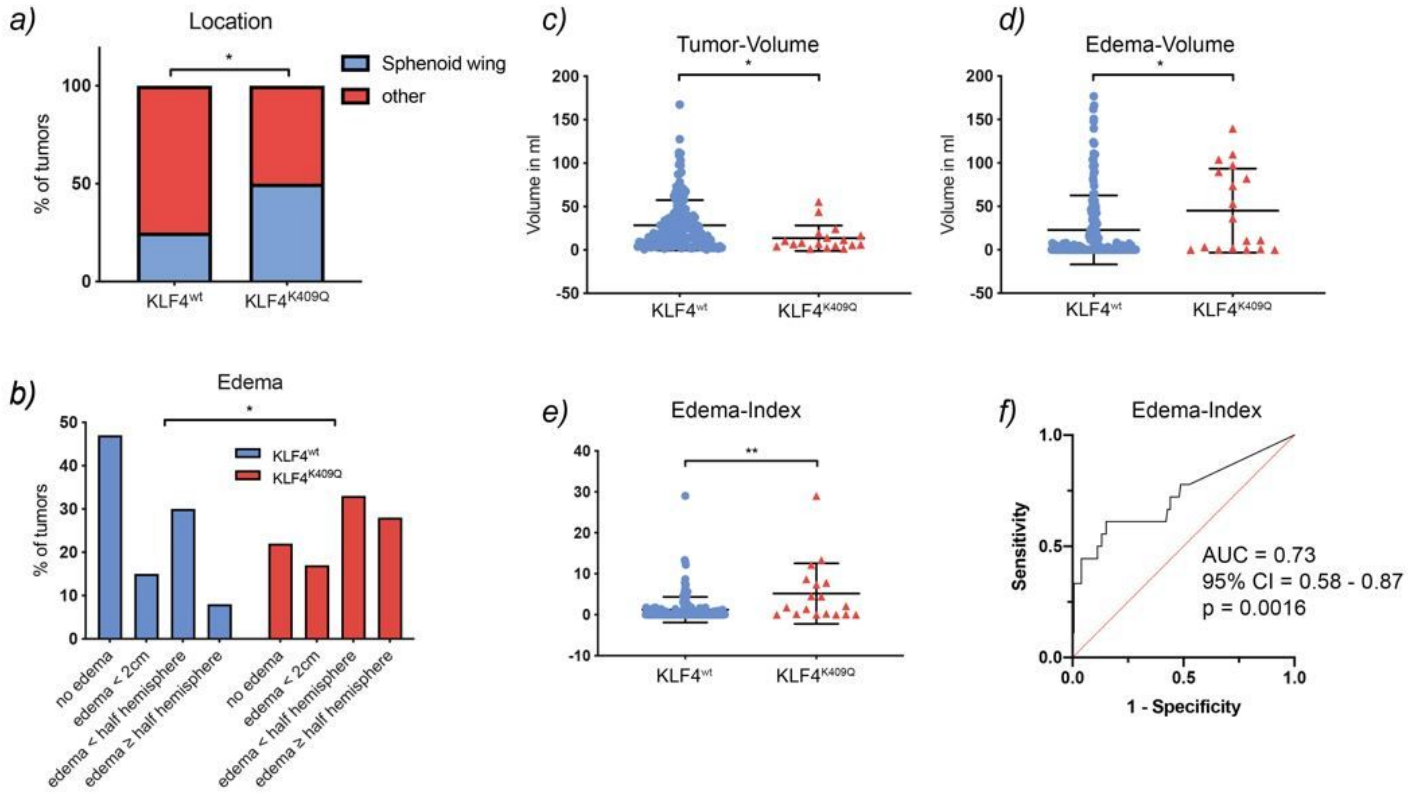

Figure 2

Analysis of preoperative MRI imaging and prediction of KLF4 mutational status a) Difference in tumor location, sphenoid wing vs. other (p=0.025). (b) Comparison of PTBE divided into 4 categories (p=0.033). (c, d, e) Volumetric analysis of preoperative MRI. KLF4K409Q tumors were significantly smaller (p=0.007), had larger PTBE (p=0.012) and a higher Edema-Index (p=0.001) (f) Receiver Operating Characteristic (ROC) curve analysis of the Edema Index (EI) as a predictive factor for KLF4K409Q mutation. (AUC = 0.728, SE = 0.075, 95% CI = 0.581 - 0.874, p=0.0016)

Supplementary Files

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- SupplementaryTables.docx