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Case Report

The Role of Genetic Analysis in Distinguishing Multifocal and Multicentric Glioblastomas: An Illustrative Case

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Keywords
Genetic analysis · Glioblastoma · Multifocal · Multicentric

Abstract
Introduction: Glioblastomas can manifest as multiple, simultaneous, noncontiguous lesions. We genetically analyzed multiple glioblastomas and discuss their etiological origins in this report. ***Case Presentation:*** We present the case of a 47-year-old woman who presented with memory impairment and left partial paralysis. Radiographic imaging revealed three apparently noncontiguous lesions in the right temporal and parietal lobes extending into the corpus callosum, leading to diagnosis of multicentric glioblastomas. All three lesions were excised. Genetic analysis of the lesions revealed a *TERT* promoter C228T mutation, a roughly equivalent amplification of *EGFR*, and homozygous deletion of *CDKN2A/B* exclusively in the two contrast-enhanced lesions. Additionally, the contrast-enhanced lesions exhibited the same two-base pair mutations of *PTEN*, whereas the non-enhanced lesion showed a partially distinct 13-base pair mutation. The other genetic characteristics were consistent. Rather than each having arisen de novo, we believe that they had developed by infiltration and are therefore best classified as multifocal glioblastomas. ***Conclusion:*** Our findings underscore anew the possibility of infiltration by glioblastomas, even within regions devoid of signal alterations on T2-weighted images or fluid-attenuated inversion recovery images. Genetic analysis can play a crucial role in differentiating whether multiple glioblastomas are multifocal or multicentric.

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Introduction

Glioblastomas are the most common type of primary malignant brain tumor in adults. Furthermore, multiple lesions can occur simultaneously; these are classified as multifocal or multicentric. The former term is used when multiple enhancing lesions are identified within a continuous area of high signal intensity on T2-weighted imaging (T2WI) or fluid-attenuated inversion recovery (FLAIR) magnetic resonance images. In contrast, the latter term typically refers to multiple lesions without imaging continuity [1–3]. The prognosis of multiple glioblastomas is much poorer than the prognosis of glioblastomas overall [3]. Investigating multifocal or multicentric glioblastomas is considered useful in elucidating the origin of glioma development and progression and has been the subject of previous research [4, 5]. The debate has centered on whether simultaneous multiple lesions have different origins or if a single lesion has invaded multiple sites. In the case of invasion, various pathways of progression, such as white matter fibers, ventricles, and the cerebral cortex, have been hypothesized. However, the poor prognosis associated with multiple glioblastomas has resulted in publication of very few reports of evaluation of all lesions pathologically or genetically. In the present case, we resected three noncontiguous glioblastomas simultaneously and conducted genetic testing on each of them. We here present our findings along with a literature review.

Case Presentation

A 47-year-old woman presented to a nearby neurosurgical clinic because of memory loss, left hand weakness, and gait disturbance. Multiple tumor-like lesions were identified on cranial magnetic resonance imaging. The lesions were as follows: (1) a non-enhancing lesion in the anterior part of the right temporal lobe; (2) an enhancing lesion in the right temporal lobe; and (3) the largest enhancing lesion in the right parietal lobe, extending through the corpus callosum to the left parietal lobe (Fig. 1). These three lesions appeared to be noncontiguous and independent on T2WI and FLAIR images (Fig. 1). The two enhancing lesions exhibited ring enhancement. The largest lesion, located in the right parietal lobe, had been partially resected at a different hospital. Histopathological examination had revealed proliferation of atypical cells with enlarged hyperchromatic nuclei, accompanied by microvascular proliferation and partial necrosis (Fig. 2). Immunohistochemical staining demonstrated retained *ATRX* expression, with a high Ki67 index of 40%. *IDH1-R132* and *IDH2-R172* were wild type, leading to diagnosis of glioblastoma, *IDH*-wild type, classified as World Health Organization Grade 4. The patient was transferred to our hospital for further treatment and received radiation therapy and temozolomide (Stupp regimen) [6].

The tumor initially decreased slightly in size and the patient was discharged. However, 1 month later the tumor began to enlarge again, accompanied by worsening impairment of consciousness and left hemiparesis. Lesion (3) had extended further to the contralateral side. Lesions (1) and (2) were completely resected, and lesion (3) underwent partial resection within the right hemisphere, including a part of the splenium of corpus callosum. The ventricle was not opened. Carmustine wafers were placed and photodynamic therapy administered. Lesions (2) and (3) resected during the second surgery exhibited typical findings of glioblastoma with degenerative changes attributable to chemoradiation therapy, including fibrosis, ghost-like cellular changes, and calcification. Similar atypical proliferating cells were identified in some regions of lesion (1) (Fig. 2).

All lesions were *IDH1-R132* and *IDH2-R172* wild type, with unmethylated *MGMT*. Lesion (1) did not show *TERT* promoter mutation or *EGFR* amplification, whereas both lesions (2)

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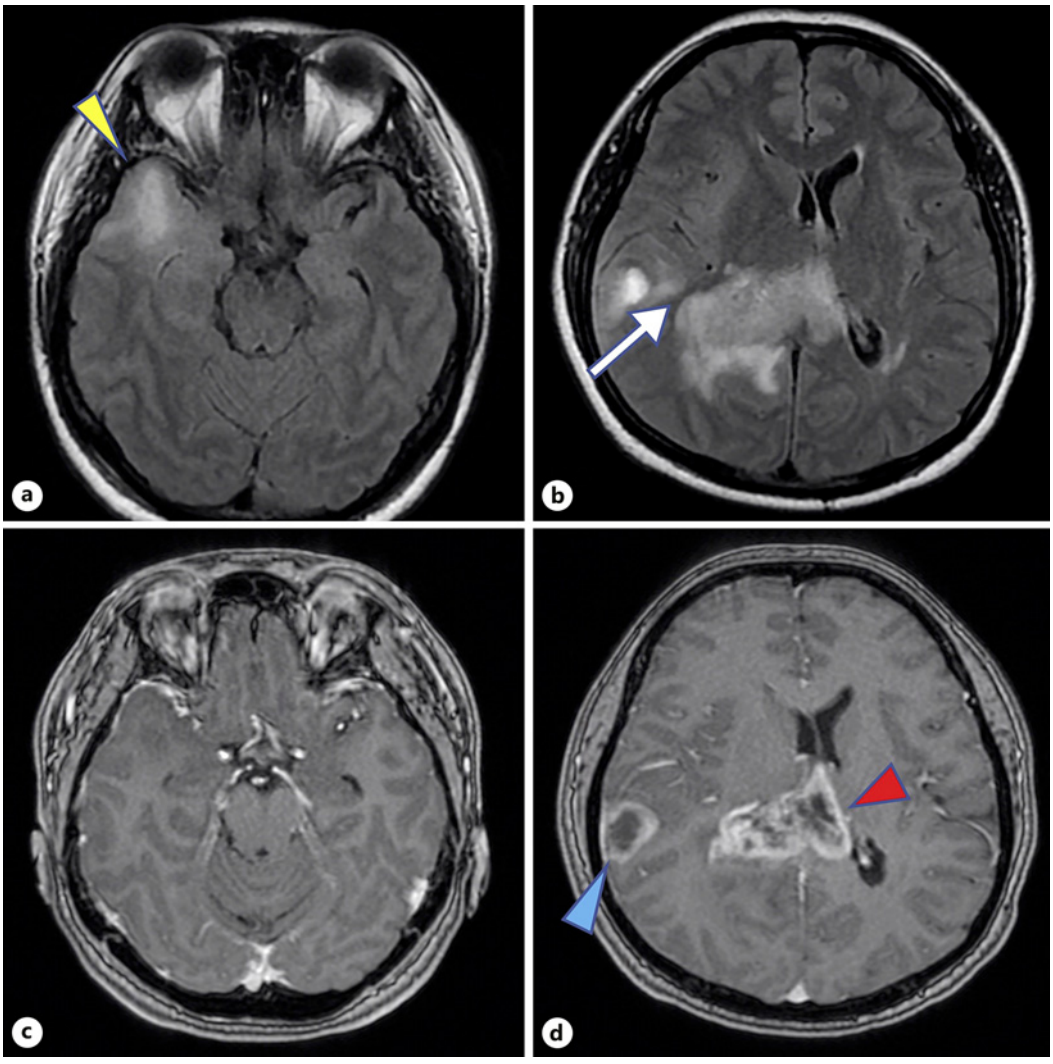

Fig. 1. a–d Preoperative FLAIR and contrast-enhanced T1-weighted images (upper panel: FLAIR, lower panel: Gd+, right panel: enhanced lesions, left panel: non-enhanced lesion) showing the three lesions, which are highlighted by three arrowheads; yellow arrowhead: lesion (1), blue arrowhead: lesion (2), red arrowhead: lesion (3). The white arrow indicates a lack of continuity between lesions (2) and (3) in a FLAIR image. FLAIR, fluid attenuated inversion recovery; Gd, gadolinium.

and (3) exhibited *TERT* promoter mutation (C228T), lesion (2) showing *EGFR* amplification of 50 copies and lesion (3) showing amplification of 55 copies (Table 1). Moreover, both lesions (2) and (3) showed homozygous deletion of CDKN2A/B. In addition, mutations of PTEN C720T and T405C were observed in lesions (2) and (3); however, mutation T405C was not present in lesion (1). A mutation involving a 13-base sequence that included *C720T* was also detected in lesion (1) (Table 1). Postoperatively, short-term memory impairment persisted, but our patient's conscious state improved and her left hemiparesis resolved. She was discharged home on postoperative day 19. Following discharge, maintenance therapy with NovoTTF-100A system (tumor treatment fields), temozolomide, and bevacizumab was continued in an outpatient setting. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000536051).

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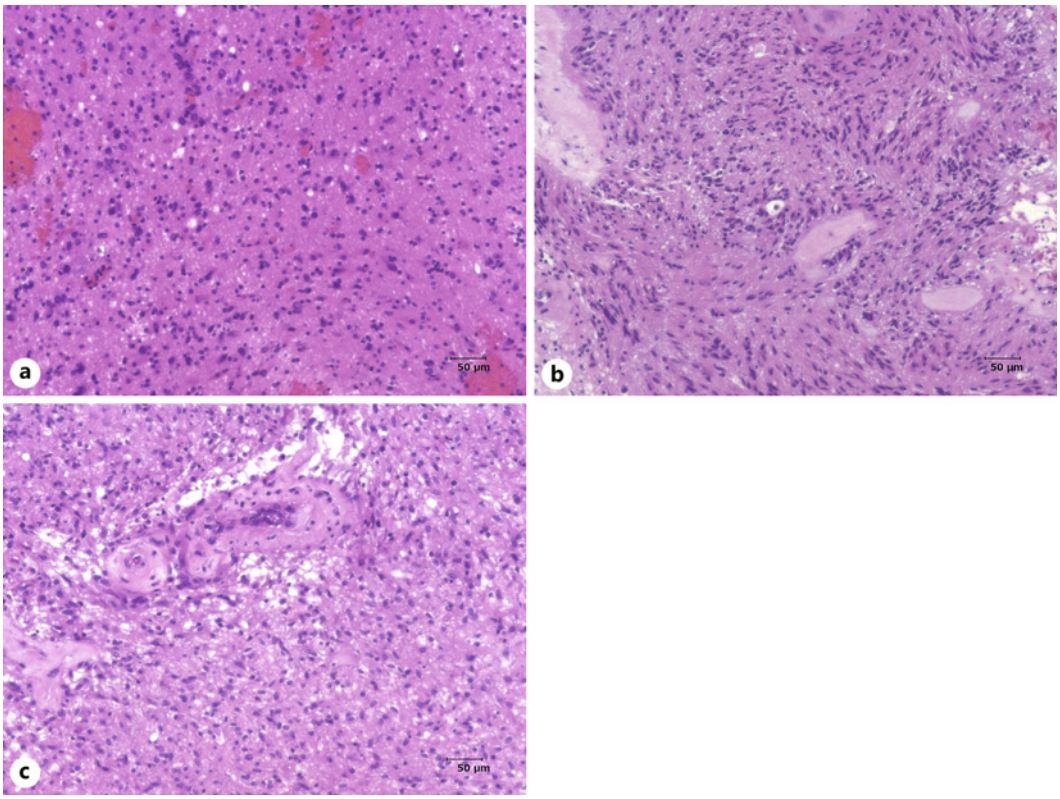

Fig. 2. Photomicrographs showing the pathological characteristics of the three lesions (hematoxylin and eosin stain, all at ×20 magnification). **a** Lesion (1). **b** Lesion (2). **c** Lesion (3). Lesions (2) and (3) contain atypical proliferating cells characterized by densely stained and enlarged nuclei. These areas are associated with areas of necrosis and increased microvascularization. Additionally, there is evidence of degeneration due to chemoradiotherapy, including fibrosis, ghost-like cellular changes, and calcifications. There are similar atypical proliferating cells in some regions of lesion (1).

Discussion

Multiple contrast-enhancing glioblastomas are known to have a worse prognosis than solitary glioblastomas, with a median survival of 6–10 months and progression-free survival of 4–14 months [1, 7]. These lesions are classified as multifocal or multicentric on the basis of the presence or absence of continuity in non-enhancing areas. The high signal intensity on FLAIR or T2WI surrounding contrast-enhanced lesions is believed to be attributable to infiltration of glioblastoma cells. Furthermore, analyzing genetic mutations in each lesion is considered useful for understanding the process of their development [8]. Many previous reports have described patients with two lesions, patients with three or more lesions being extremely rare [9, 10]. Therefore, the present report, which describes comprehensive analysis of three lesions, is of considerable interest.

Akimoto et al. [11] reported a patient with multifocal brain tumors in the right insular cortex and left corona radiata. They resected the right lesion and biopsied the left one. Interestingly, they found that the seemingly distinct tumors in separate locations had a genomic profile in common, indicating that both lesions were glioblastomas. The left tumor was resistant to treatment and was in contact with the lateral ventricle, leading to speculation that it was the primary tumor, whereas the right tumor was considered to be a secondary infiltrating lesion.

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Table 1. Results of genetic analysis of the three lesions

Genetic analysis	Lesion		
	(1)	(2)	(3)
IDH1-R132H	WT	WT	WT
IDH2-R172H	WT	WT	WT
BRAF-V600E	WT	WT	WT
H3F3A-K27M, G34 R/V	WT	WT	WT
HIST1H3B-K27M	WT	WT	WT
HIST1H3C-K27M	WT	WT	WT
TERT promoter	WT	C228T	C228T
MGMT promoter	Unmethylated	Unmethylated	Unmethylated
ATRX-lost	Retained	Retained	Retained
CDKN2A-deleted	Retained	Lost	Lost
CDKN2B-deleted	Retained	Lost	Lost
1p/19q	WT	WT	WT
+7/-10	WT	WT	WT
EGFR-Amp	WT	50 copies	55 copies
TP53	WT	WT	WT
PTEN	13 mutations	T405C, C720T	T405C, C720T
A1	C	–	–
A258	G	–	–
G397	A	–	–
A404	T	–	–
T405	–	C	C
G446	A	–	–
T531	C	–	–
T544	G	–	–
C556	G	–	–
A672	G	–	–
C700	T	–	–
A705	G	–	–
C720	T	T	T
C900	T	–	–

The upper section presents the presence or absence of mutations in each gene analyzed in this study. The lower section shows the mutations in PTEN, starting from the translation initiation codon (ATG), indicating which base number has undergone what kind of mutation. *ATRX*, alpha-thalassemia mental retardation X-linked; *BRAF*, V-Raf murine sarcoma viral oncogene homolog B1; *CDKN*, cyclin-dependent kinase inhibitor; *EGFR*-Amp, epidermal growth factor receptor amplification; *IDH*, isocitrate dehydrogenase; *MGMT*, O6-methylguanine-DNA methyltransferase; *PTEN*, phosphatase and tensin homolog deleted on chromosome 10; *TERT*, telomerase reverse transcriptase; *TP53*, tumor protein p53; WT, wild type; 1p/19q, the short arm of chromosome 1/the long arm of chromosome 19; +7/-10, gain of chromosome 7/loss of chromosome 10.

Agopyan-Miu et al. [12] reported a patient with synchronous supratentorial and infratentorial oligodendrogliomas. They resected the lesions sequentially and subjected them to genetic analysis. They found that both lesions exhibited *1p/19q* codeletion and *TERT* promoter mutation. However, the lesion above the tentorium had an *IDH1-R132G* mutation, whereas the lesion below the tentorium had an *IDH1-R132H* mutation. The different responses to treatment by the two lesions prompted additional biopsies and these revealed the discrepancy in *IDH1* mutations. Because chemotherapy had been administered between the genetic analyses of the two lesions, the authors speculated that a chemotherapy-resistant subclone had arisen from the initial tumors. Alternatively, the tumors could have arisen independently.

These reports highlight that, when multiple tumors appear to be completely separate on imaging, their genomic profiles may either match or differ, as demonstrated in these cases. We reoperated to excise all three of our patient's lesions because they were chemoradiation-resistant and we considered that there was potential for significant functional preservation based on the tumors' locations. Genetic analysis of our patients' lesions revealed mutations in *TERT* promoter C228T, *EGFR* amplification with similar copy numbers, and homozygous deletion of *CDKN2A/B* in lesions (2) and (3), but not in lesion (1). Both *IDH1-R132* and *IDH2-R172* were wild type and *MGMT* was unmethylated. Although alterations of BRAF V600E, TP53, gain of chromosome 7, and loss of chromosome 10 are common in glioblastoma, all 3 lesions were wild type. Interestingly, regarding *PTEN*, the *T405C* mutation observed in lesions (2) and (3) was not included in the 13-base mutation found in lesion (1). Although the lesions appeared to be separate on FLAIR imaging, consistent with the conventional notion of multicentric glioblastomas, their very similar genetic characteristics may indicate that the three lesion did not arise independently de novo, particularly lesions (2) and (3). Despite the absence of detectable continuity on imaging, we consider the more likely scenario is that lesion (2) originated from lesion (3) or vice versa. Furthermore, the results of PTEN analysis could be explained by either of the following two hypotheses. The first hypothesis is that all three lesions had the same origin, with lesion (2) giving rise to lesion (3) or vice versa, followed by one of them giving rise to lesion (1), where the *PTEN C720T* mutation occurred. Alternatively, it is also conceivable that lesion (1) was the origin of them, subsequently giving rise to lesions (2) and (3) (Fig. 3a). The second hypothesis is that lesions (2) and (3) share a common origin, while lesion (1) originated independently (Fig. 3b). We believe our patient's lesions were essentially multifocal rather than multicentric. Within the scope of our investigation, there were no previous reports showing multicentric and multifocal tumors coexisted in a single patient. Therefore, we believe that the former of the two hypotheses we proposed is more likely. The tumors could have been infiltrating the brain tissue diffusely, even in areas without high signal intensity on T2WI or FLAIR images. Additionally, these possibilities are consistent with reported autopsy findings of glioma cell infiltration distal to areas in which there were changes on imaging [13]. Diagnosing multifocality based solely on imaging findings can be challenging. If clinically feasible, obtaining tissue samples from each lesion in multiple glioblastomas is considered to hold certain significance. In the present case, despite the absence of continuity on imaging, we consider it likely that infiltration occurred along the superior longitudinal, middle longitudinal, or inferior fronto-occipital fasciculus. The lesions in this case were extensive, and to preserve brain function as much as possible, extraction considering white matter anatomy was not feasible. The focus was on removing the contrast-enhanced areas as extensively as possible. However, based on the results of this analysis, it is once again suggested that we should consistently consider white matter anatomy as well as signal changes on imaging in the resection of glioma [14, 15].

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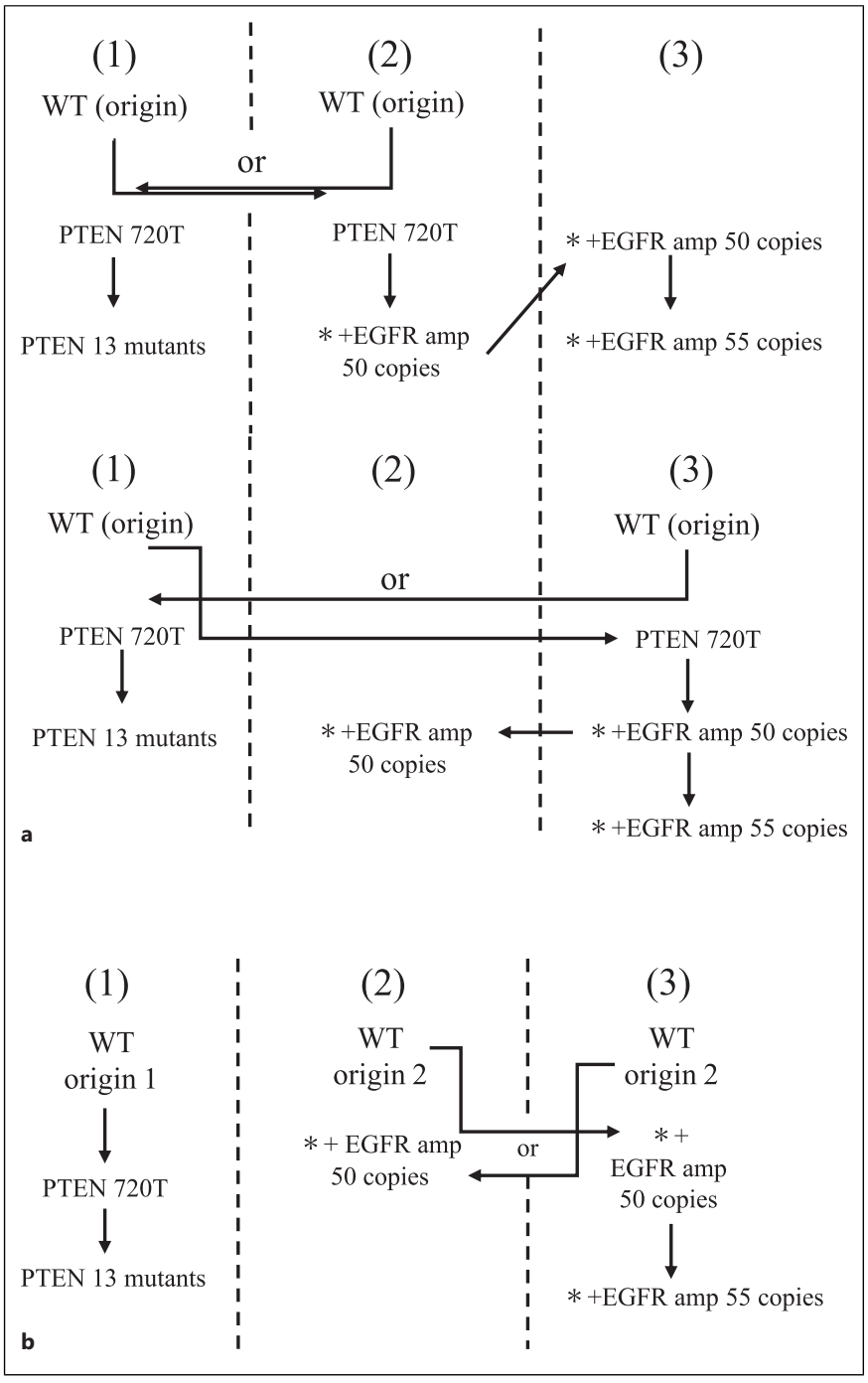

Fig. 3. Hypotheses based on genetic analysis regarding the sequence of progression of our patient's three lesions. **a** Hypothesis 1: assuming a common origin for the three lesions, it is conceivable that the mutation *PTEN C720T* led to growth of lesions (2) or (3), as well as lesion (1). **b** Hypothesis 2: this scenario posits that lesions (2) and (3) share a common origin, whereas lesion (1) independently originated from a distinct event. WT: wildtype, *EGFR* amp: *EGFR* amplification, *: *PTEN C720T*, T405C mutations, *TERT* promoter *C228T* mutation, homozygous deletion of *CDKN2A/B*.

One limitation of the present report is that we only have data on 1 patient. Ideally, a series of patients is needed to further investigate the possibilities we have raised.

Conclusions

We have here reported resection and genetic analysis of three noncontiguous glioblastomas. Presenting with three or more lesions is rare and there are very few reports of complete resection and analysis of so many lesions in a single patient. In our case, despite the lack of imaging continuity between the lesions, they showed some striking similarities in terms of genetic characteristics. Our findings suggest that glioblastomas may infiltrate areas without signal changes on T2WI or FLAIR imaging, reinforcing the possibility of early infiltration. Furthermore, genetic analysis of multiple glioblastomas is an important means of determining whether they are multifocal or multicentric.

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Statement of Ethics

We declare that written informed consent was obtained from the patient for publication of this case report and any accompanying images. Ethical approval is not required for this study in accordance with local or national guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization: K.K. and R.T.; investigation: K.K. and M.Y.; writing – original draft preparation: K.K.; writing – review and editing: R.T.; supervision, H.N., U.H., Y.K., R.U., and M.T.; all authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are included in the article. Further inquiries can be directed to the corresponding author.

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