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Clinical Study

BRAF V600E mutation in neocortical posterior temporal epileptogenic gangliogliomas

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ABSTRACT

The aim of this study was to verify the presence of *BRAF* mutations in a series of six patients affected by drug-resistant focal epilepsy associated with neocortical posterior temporal gangliogliomas (GG) who were subjected to lesionectomy between June 2008 and November 2013. GG are an increasingly recognized cause of epilepsy and represent the most common tumor in young patients undergoing surgery for intractable focal epilepsy. *BRAF* mutations have been identified in up to 50% of GG. Interestingly, these six patients shared a specific anatomical posterior temporal site. In all patients, histological examination confirmed the diagnosis of GG, and two were also associated with a focal cortical dysplasia (FCD) type IIa. *BRAF* mutations were found in four out of six GG (66.6%). Furthermore, dysplastic tissue of Patient 2 showed a concomitant *BRAF* V600E mutation. All patients but one (83.3%) achieved Engel Class Ia seizure control. The patient carrying a concomitant *BRAF* mutation in GG and FCD fell into Engel Class II. Further analyses will be required in order to better understand the meaning of *BRAF* mutations in epilepsy-associated tumors and FCD and their possible role as a prognostic seizure outcome and tumor behavior marker.

1. Introduction

Gangliogliomas (GG) are an increasingly recognized cause of epilepsy and represent the most common type of long-term epilepsy-associated tumor (LEAT) in young patients undergoing surgery for chronic intractable focal epilepsy [1–3]. Typical characteristics of LEAT include a young age of onset of symptoms (usually with epilepsy as the primary and often only neurological symptom), slow growth, a neocortical location and usually a temporal lobe location.

GG and dysembryoplastic neuroepithelial tumors may have intrinsic epileptogenic properties due to their neuronal and glial components [1,2,4]. However, the epileptological mechanisms associated with these lesions are not yet completely understood and are still under investigation.

Recently, *BRAF* mutations have been identified in up to 50% of GG [5,6] and in the associated focal cortical dysplasia (FCD) [7]. As a member of the RAF family of serine/threonine kinases, *BRAF* is a key mediator of the mitogen-activated protein kinase signaling pathway (also known as the RAF-MEK-ERK pathway) [7]. *BRAF* is involved in a wide variety of cellular functions including cell proliferation, cell cycle arrest, terminal differentiation and apoptosis [8]. The *BRAF* gene is activated by oncogenic mutations. Most *BRAF* mutations are missense mutations at amino acid position 600 resulting in an exchange of valine for glutamate (referred to as *BRAF* V600E) [7].

The aim of this study was to verify the presence of *BRAF* mutations in a homogeneous series of patients suffering from chronic intractable focal epilepsy associated with neocortical posterior temporal GG.

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2. Material and methods

From the database of the Epilepsy Surgery Centre at the Institute of Neurological Sciences Bellaria Hospital, Bologna we retrieved data for 36 patients operated on for pharmacoresistant temporal lobe epilepsy associated with low grade tumors, 30 of whom had temporo-mesial LEAT and tailored surgery, and had already been involved in recent studies [7,9].

In this study, we analyzed six consecutive patients affected by neocortical lateral posterior temporal GG and treated with pure lesionectomy who were operated on between June 2008 and November 2013. Interestingly, these six patients shared a specific anatomical posterior temporal site. A detailed clinical history was taken and all patients underwent neurophysiological presurgical assessment by means of non-invasive long-term video electroencephalogram monitoring for seizure recording. MRI, CT scan and neuropsychological evaluation were carried out in all patients. Postsurgical seizure outcome was defined according to Engel's classification at 2 years follow-up.

For histological examination, specimens were fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections of 4 μm were serially cut and underwent hematoxylin and eosin, Nissl, Kluver, and reticulin staining. All patients were histologically classified according to the World Health Organization classification of tumors of the central nervous system [10] and the recently published criteria for FCD diagnosis [11].

The areas identified as dysplastic were carefully evaluated with CD34, MAP2, p53, Ki67, and IDH1 antisera in order to rule out the possibility of tumor infiltration misdiagnosed as dysplastic tissue.

All specimens were investigated for *BRAF* mutational status. The *BRAF* gene was separately analyzed in all GG and in the two tumor-associated FCD specimens. In order to assess the *BRAF* molecular status, DNA was extracted from formalin-fixed paraffin-embedded material scraped under microscope guidance from five 10 μm thick sections, as previously described [12]. Sequencing was performed using the 454 GS-Junior next generation sequencer (Roche Diagnostic, Mannheim, Germany) according to established protocols and results were analyzed using Amplicon Variant Analyzer software (http://www.454.com/; Roche Diagnostic). Next generation sequencing of target DNA sequences not only allows the identification of mutations but also gives the percentage of mutated amplicons in the analyzed sample.

3. Results

The mean age of the six patients (four males, two females) at surgery was 26.1 years (standard deviation [SD] 17.1). Mean age of seizure onset was 9.8 years (SD 5.5) and mean duration of epilepsy was 16.3 years (SD 18.7). MRI showed features compatible with a glioneuronal tumor in all patients (Fig. 1). In five patients, GG were located in the postero-lateral third of the right middle temporal gyrus (Fig. 1b–f), in one patient the GG was in the posterior part of the right fusiform gyrus (Fig. 1a). All patients were right handed.

In all patients, the surgical procedure consisted of a lesionectomy assisted by neuronavigation and intraoperative sonography. The postoperative course was uneventful in all patients.

Clinico-pathological characteristics of the patients are summarized in Table 1, molecular findings are reported in Table 2.

All patients but one (83.3%) achieved Engel Class Ia seizure control, one was Engel Class II. In all patients, histological examination revealed a GG and in two (Patient 2 and 6) it was associated with an FCD type IIa. *BRAF* mutations were found in four out of six GG (66.6%). Dysplastic tissue of Patient 2 harbored a *BRAF* V600E mutation.

4. Discussion

GG consist of a mixture of dysplastic neurons and neoplastic astroglial cells and they are most commonly located in the temporal lobe, in particular, in the temporo-mesial site. There are some suggestions that the anatomical location of these tumors (mesial *versus* lateral) may influence the extension and complexity of the epileptogenic zone, thus, affecting the choice of the most effective surgical strategy. In fact, some evidence indicates that lesionectomy is more effective in GG with temporo-lateral and extratemporal location, whereas a tailored surgery, including the hippocampus, provides better postsurgical seizure outcome in temporo-mesial GG [13–16].

The histological composition, together with the expression of stem cell markers (such as CD34 and nestin) and the association with cortical dysplasia, have attracted considerable interest with respect to the origin of these lesions [2]. Furthermore, the association of GG with FCD also suggests a possible evolutionary oncogenic progression from precursor cells or dysplastic cells, either during embryological cortical development or during postnatal life [17,18].

In our experience, MRI is not always able to detect FCD associated with a GG [19] and in the present series the areas of FCD associated with the GG were discovered only at pathological examination.

Recently, *BRAF* V600E mutations have been detected in GG, pleomorphic xanthoastrocytomas, desmoplastic infantile gangliogliomas [5,12,20–22] and in general in LEAT [7]. The *BRAF* gene is activated mostly by missense mutations at amino acid position 600 (*BRAF* V600E) [17]. Small molecule inhibitors that target the *BRAF* V600E mutation have recently been developed [23]. It has been observed that mutant *BRAF* protein in GG is predominantly expressed by neuronal tumor cells [24]. Our results confirm the presence of the *BRAF* V600E mutation in four out of six patients (66.6%). Furthermore, dysplastic tissue of Patient 2 harbored the *BRAF* V600E mutation.

All patients but one (83.3%) achieved Engel Class Ia seizure control. The patient carrying a concomitant *BRAF* mutation in GG and FCD (Patient 2) achieved only Engel Class II. Furthermore, in our previous experience, *BRAF* V600E mutation is strongly associated with temporo-mesial LEAT [7], in agreement with the literature [24,25]. In the present series, we also found the *BRAF* V600E mutation in lateral posterior temporal site GG. Interestingly, *BRAF* mutations are present not only in LEAT but also in the FCD accompanying LEAT [7]. These data are intriguing since it has been postulated that the origin of LEAT and FCD is from a common precursor that undergoes abnormal glioneuronal development, although this is not yet proven.

A recent study revealed that expression of *BRAF* V600E is associated with a worse postoperative seizure outcome in glioneuronal tumors, [26] probably due to lack of removal of FCD in the adjacent cortex. Interestingly, in the present series, the patient with the worst outcome presented concomitant GG and FCD, both of which are characterized by *BRAF* mutations [7].

Therefore, molecular or immunohistochemical detection of *BRAF* V600E mutation may be a valuable tool in the diagnosis of glioneuronal lesions [26]. In fact, this alteration is frequently present in LEAT, while it is observed only in low percentages of diffuse gliomas [26,27].

Furthermore, regarding clinical behavior, there is some evidence that *BRAF* V600E mutated GG exhibit shorter recurrence-free intervals compared to *BRAF* V600E-negative tumors [28]. Finally, the highest incidence of *BRAF* V600E mutations has previously been reported in young patients [24] and in our series, the oldest patient had a wild type *BRAF* gene.