J Neuropathol Exp Neurol
Vol. 75, No. 4, April 2016, pp. 306–315
doi: 10.1093/jnen/nlw001

ORIGINAL ARTICLE

Whole Chromosome 7 Gain Predicts Higher Risk of Recurrence in Pediatric Pilocytic Astrocytomas Independently From KIAA1549-BRAF Fusion Status

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Abstract

The most frequent genetic alteration identified in pediatric pilocytic astrocytomas and pilomyxoid variant is the *KIAA1549-BRAF* fusion, which typically results from a 2.0 Mb tandem duplication in chromosome band 7q34. Less frequent abnormalities include fusion genes, *BRAF, FGFR, KRAS*, and *NF1* point mutations, and whole chromosome gains. To correlate genetic alterations with clinical course data, we retrospectively analyzed the tumors with pilocytic and pilomyxoid histology of a cohort of 116 pediatric patients, aged 5 months to 23 years. Gross total resection was associated with a decreased risk of recurrence ($p = 0.001$), supporting previous findings that complete tumor excision correlates with long-term and disease-free survival. We found no significant association between recurrence rate and the presence of the *KIAA1549-BRAF* fusion or *BRAF* mutation ($p = 0.167$). Interestingly, gain of whole chromosome 7 (WC7) was associated with a 4.7-fold increased risk of tumor recurrence, even after adjusting for surgical status ($p = 0.025$), and other genetic alterations. Using fluorescence in situ hybridization, we demonstrated that when WC7 gain accompanies the *KIAA1549-BRAF* fusion, the fusion likely arises first. This study highlights the utility of genetic studies for risk assessment of pilocytic and pilomyxoid astrocytomas, which may impact treatment selections.

Key Words: BRAF, KIAA1549, Pediatric brain tumor, Pilocytic astrocytoma, Pilomyxoid astrocytoma, Single-nucleotide polymorphism (SNP) array.

INTRODUCTION

Pilocytic astrocytoma (PA) and the pilomyxoid variant (PMA) are the most common types of brain tumor in children and adolescents (1). Although these are typically considered indolent tumors (2), patients with PAs that are not amenable to surgical resection can have significant long-term morbidity including neurocognitive deficits, neuroendocrine deficiencies, and visual impairment. The tumors are most commonly found in the cerebellum, optic pathways, and third ventricular/hypothalamic region but they can be located anywhere along the neural axis (2, 3). On neuroimaging, PAs classically appear as a large cyst in the cerebellum with an enhancing, circumscribed tumor nodule; however, a wide spectrum of appearances is recognized, including cystic, solid, or a mix of cystic and solid components (4). The extent of surgical resection is the clinical factor most associated with improved long-term and disease-free survival. Therefore, children with PAs not amenable to complete resection usually require adjuvant chemotherapy, often requiring multiple different regimens over the span of several years in order to control growth (3).

PMAs, which usually occur in the hypothalamic/chiasmatic region of younger patients (median age, 10 months), are thought to represent a more aggressive variant of PAs, due to a higher risk of cerebrospinal spread and recurrence (5, 6); however, there is great variation in the biological behavior of PMAs and not all are more aggressive tumors. Moreover, the greater likelihood of recurrence may be related to the location of the tumor and the difficulty of achieving a complete surgical resection rather than its intrinsic biologic properties. Interestingly, PMAs have been documented to mature to classic PAs and tumors with intermediate features are recognized (7). Indeed, there seems to be a histologic continuum, rendering it difficult for neuropathologists to differentiate the 2 variants.

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This work was supported by the Damon Runyon Cancer Research Foundation Hyundai Hope on Wheels Bear Necessities Pediatric Cancer Foundation R01NS085336 NCI/NINDS (AJW). NIH/NIGMS Medical Genetics Research Training Grant (T32-GM008638) (JJR).

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The authors have no duality or conflicts of interest to declare.

Supplementary Data can be found at http://www.jnen.oxfordjournals.org.

Less than a decade ago, our group and others identified the first genetic alteration underlying the majority of sporadic PAs and PMAs (8–10). This alteration is a 2.0 Mb internal tandem duplication in chromosome band 7q34 that results in a *KIAA1549-BRAF* fusion protein, which then leads to the constitutive activation of mitogen-activated protein kinase (MAPK) signaling. The frequency of this alteration generally ranges from 50%–70% of PAs (10, 11). Additional MAPK somatic genetic alterations, including other *BRAF* fusion partners, *RAF1* fusions, *BRAF* and *KRAS* point mutations, and whole chromosome gains have been reported (12–17). Prior to these findings, dysregulation of the RAS/RAF (ie, MAPK) signaling pathway was observed in PAs associated with the neurofibromatosis type 1 (NF1) syndrome. Up to 10% of PAs are associated with NF1 and children with NF1 carry an increased risk of developing PAs in the hypothalamic/optic tract pathways (18).

There are conflicting reports as to whether the *KIAA1549-BRAF* fusion correlates with outcome. Hawkins et al demonstrated improved progression-free survival in patients with *KIAA1549-BRAF* fusion-positive PAs and PMAs. However, their study population was restricted to patients with noncerebellar tumors that had been incompletely resected (19). A more recent study also reported a better outcome of PAs with *KIAA1549-BRAF* fusion in mixed adult and pediatric populations (20). Other studies comprising a more diverse patient population, in terms of location and clinical status, failed to demonstrate that *KIAA1549-BRAF* fusion status predicts response to therapy or risk of recurrence (8, 14, 21–23). This observation is not necessarily surprising given the high prevalence of the *KIAA1549-BRAF* fusion in PAs and PMAs.

In the present study, we sought to identify other genetic factors that might correlate with outcome in pediatric PAs and PMAs. We performed a retrospective analysis of patients with PA and/or PMA resected at The Children's Hospital of Philadelphia (CHOP) in Philadelphia, Pennsylvania, between 1998 and 2014. The majority of tumors were diagnosed as PA according to World Health Organization (WHO) 2007 criteria (2), with classic biphasic architecture, followed by monophasic, compact, or microcystic growth patterns. A minority of cases was in the pilomyxoid spectrum (7). Clinical follow-up data were correlated with molecular and cytogenetic techniques, including single-nucleotide polymorphism (SNP) array, Sanger sequencing, reverse transcription-PCR (RT-PCR), and fluorescence in situ hybridization (FISH).

MATERIALS AND METHODS

Patients

This retrospective study included 116 patients with CNS PA and PMA diagnosed at CHOP, January 1998 to August 2014. For 6 cases, the pathologic and genetic data were available for both the primary and the recurrent tumor; therefore, the total number of specimens examined was 122. The study was conducted in compliance with local and federal human protection guidelines and institutional review board regulations. Selection criteria for inclusion in the study were the following: pathologic diagnosis of PA and/or PMA confirmed by the neuropathologist (MS) and the availability of genetic results by at least 1 of a variety of methods: SNP array, Sanger sequencing, RT-PCR, and FISH. All of these analyses were performed on fresh-frozen material. The extent of resection was assessed as gross total resection (GTR), subtotal resection (STR), or biopsy based on the surgical or radiographic information extracted from the patient charts. In addition, information was recorded regarding comorbidity and underlying disorder such as NF1, postoperative adjuvant therapies, and long-term disabilities associated with the disease.

Pathological Review

For all 122 specimens, formalin-fixed and paraffin-embedded microscopic sections stained with H&E (hematoxylin and eosin) were available for review. In the majority of cases, additional immunohistochemical stains included glial fibrillary acid protein, MIB-1 (Ki-67), and neurofilament protein or synaptophysin. All cases selected by the neuropathologist (MS) conformed to WHO 2007 diagnostic criteria for PA/PMA. Other low-grade glial tumors such as diffuse fibrillary astrocytoma (grade II), pleomorphic xanthoastrocytoma, ependymoma, oligodendroglial tumor, or glioneuronal tumor such as ganglioglioma/gangliocytoma were excluded.

SNP Array Analysis

SNP array analysis was performed on an Illumina (San Diego, CA) high-density platform, as previously described (17). This assay allows detection of chromosomal gains and losses in at least 20% of the tumor (assay sensitivity).

RT-PCR Analysis

RT-PCR analysis was performed as previously described (12).

FISH

FISH was performed on touch imprints from frozen tissue and analyzed as previously described (17). The probes targeted the centromere of chromosome 7 (D7Z1) and the *LUC7L2* gene on chromosome 7q34, as previously described (10).

BRAF Sequencing Analysis

BRAF sequencing was performed according to a previously described method and included analysis of exon 15, with or without simultaneous analysis of exon 11 (17).

Statistical Analysis

Patient demographics and clinical characteristics were analyzed with descriptive statistics such as frequencies and percent. Associations between clinical and genetic variables and tumor recurrence were initially explored using frequency tables and the Fisher exact test. Significant variables were then entered into a multivariate logistic regression to estimate adjusted associations with tumor recurrence. Adjusted odds

ratios and their 95% confidence intervals from the logistic regression are presented.

RESULTS

Our single institution retrospective analysis included 116 patients and 122 tumor specimens. For 6 cases, the pathologic and genetic data were available for both the primary and the recurrent tumor: case 29 (08-190 and 12-222); case 32 (10-020 and 14-023); case 36 (10-056 and 12-148); case 42 (10-254 and 10-254B); case 51 (11-274 and 11-274B); and case 93 (13-259 and 259B). Clinicopathological results are summarized in Supplementary Data 1.

Histopathological Characterization of Tumors

Review of tumor histology revealed 74 classic PAs (Fig. 1A). Rosenthal fibers and eosinophilic granular bodies varied from abundant to rare. Thirty-four tumors were monophasic, dominated by either microcystic features or compact, piloid growth (Fig. 1B, C). The remaining 14 specimens showed either classic pilomyxoid features with bipolar cells in a myxoid/mucoid background accompanied by variable angiocentric structures (Fig. 1D) or only focal pilomyxoid features (7). With the exception of a single case, identical histological features were observed in both primary and recurrent tumors. In case 29, the initial tumor (08-190) was monophasic with microcystic features, whereas the recurrence (12-222) appeared compact. Tumor 11-468 demonstrated oligodendroglioma-like cytology and 3 tumors, 99-173, 06-247, and 12-058, showed prominent calcifications. There were no tumors with anaplastic features such as cytologic atypia, brisk mitotic activity, and/or pseudopalisading necrosis.

Clinical Description of Cases

The 116 cases included 46 female (40%) and 70 male (60%) patients, ranging in age from 5 months to 23 years. The majority of patients, 61 (53%), were between 3 and 11 years of age; 21 (18%) were 5 months to 2 years; and 34 (29%) were older than 12 years. The average age at presentation was 8 years. As expected for PA, the most frequent location was the posterior fossa (65%) with direct cerebellum involvement. One patient presented with a tumor that extended from the midbrain to the third ventricle. Three tumors involved only the tectum. One tumor arose in the medulla, another in the midbrain, and 4 in the fourth ventricle. Of the supratentorial tumors, 54% arose in the suprasellar/hypothalamic region and 30% involved the optic chiasm. GTR was achieved in 63 cases (54%); STR, representing both subtotal and partial resection, in 41 cases (35%), and 12 cases were biopsy only (Table). Long-term morbidities

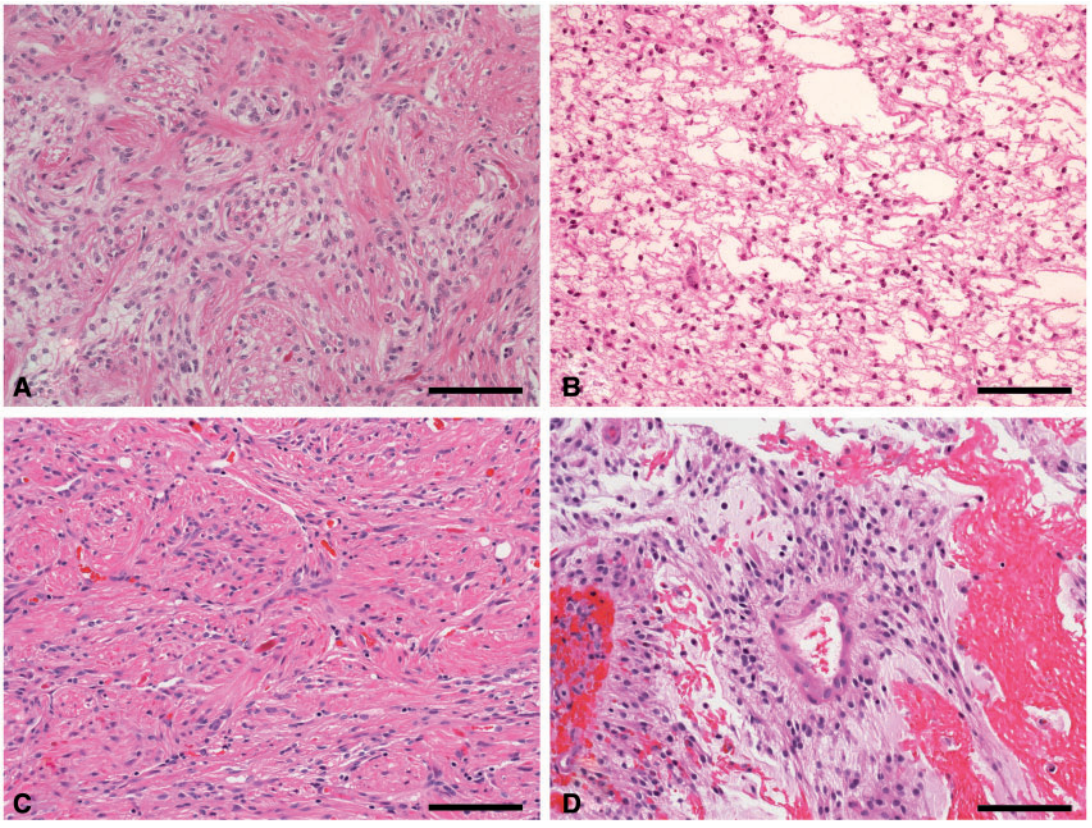

FIGURE 1. Representative images of histologic features in pilocytic astrocytoma and pilomyxoid astrocytoma. **(A)** Classic biphasic pattern. **(B)** Monophasic microcystic/spongy pattern. **(C)** Monophasic compact pattern with Rosenthal fibers. **(D)** Pilomyxoid variant with angiocentric orientation of tumor cells. **(A–D)** Hematoxylin and eosin stain. Scale bar = 50 μm; original magnification = x200.

were not assessed; therefore, information on neurocognitive outcomes or neuroendocrine deficiencies was not included.

Assessment of Tumor Progression and/or Recurrences

Sufficient follow-up information was available for all cases. Event-free survival at the end of the study period in August 2014 was 73%. Time to first progression and/or recurrence ranged from 1 month to 12 years from diagnosis and averaged approximately 3 years. The majority of cases requiring adjuvant treatment for tumor progression and/or recurrence was documented in the setting of STR or biopsy only (Table). As reported in other series (24), GTR was associated with lower recurrence risk ($p < 0.001$, Table). We found no significant association between age and frequency of tumor recurrence (data not shown). Of note, 4 patients had a clinical history of NF1 (patients 44, 70, 90, and 91); and 1 patient (patient 96) had a history of acute lymphoblastic leukemia treated with chemotherapy alone (ie, no radiation).

Identification of Genomic Biomarkers

A total of 122 specimens from 116 patients were analyzed in the Cancer Cytogenetics Laboratory at CHOP. Genetic studies were performed on the primary tumors from 101 patients (101 tumors), the recurrent/residual tumors from 9 patients (9 tumors), and both the primary and recurrent/residual tumors from 6 patients (12 tumors). Molecular studies included at least one of the following tests: SNP array, FISH, RT-PCR, and *BRAF* exon 11 and/or 15 sequencing. Due to insufficient material, not all tests could be performed on every specimen. The full genetic results were either previously reported (10, 17) or appear in Supplementary Data 2.

The most frequently identified genetic alteration, detected by SNP array, FISH, or RT-PCR, was a 2.0 Mb gain in chromosome 7 band 7q34 (Fig. 2A, arrow). This duplication resulting in the *KIAA1549-BRAF* fusion gene was identified in 86 of 116 patients (74%) (8–10). Gain of whole chromosome 7 (WC7) was the second most common genetic abnormality, present in tumors from 17 patients (out of a total of 104) analyzed by SNP array (16.5%) (Fig. 2A, B). Another tumor demonstrated a gain in the long arm of chromosome 7 (7q21.11qter) (Fig. 2C). Based on SNP array calculations, when the tandem duplication was present, chromosome 7 was observed in approximately 3 to 5 copies, with an additional gain at 7q34 (25). Gains of other chromosomes, with or without gain of chromosome 7, were present in tumors from 26 of 104 patients (25%) (Fig. 3A, B). In addition to the gain of chromosome 7, other commonly gained chromosomes included 5, 6, 11, 15, and 20. The last genetic alterations in our cohort were *BRAF* exon 15 mutations, which were present in 6 of 103 (6%) tumors. Five tumors showed the BRAF V600E mutation, while another tumor had a mutation involving the preceding codon (p.Thr599dup). Genetic results analyzed by more than 1 method were concordant in all tumors. Of note, patients with 2 tumors are presented only once in the calculations.

In order to identify co-occurrence of the most common recurrent abnormalities, genetic data from all 122 tumors were examined (Fig. 3B). The *KIAA1549-BRAF* fusion was the single most common alteration, but co-occurred with WC7 gain, other chromosomal gains, or both chromosome 7

TABLE. Summary of Results

	All patients	Recurrence (%)	Unadjusted p value	Adjusted OR (95% CI)**	Adjusted p value**
Surgical Status					
Biopsy only	12	5 (41.6%)*	<0.001	Reference	Reference
Subtotal resection	41	22 (53.6%)		1.21 (0.30–4.81)	0.787
Gross total resection	63	4 (6.3%)		0.07 (0.01–0.38)	0.001
Total	116	31			
BRAF Status					
Exon 15, mutation	6	1 (16.6%)	0.167		
Fusion	86	20 (23.2%)			
Negative	24	10 (41.6%)			
WC7 GAIN					
Yes	18	9 (50%)	0.029	4.71 (1.21–8.29)	0.025
No	86	21 (24.4%)		Reference	Reference
WC OTHER GAIN					
Yes	25	8 (32%)	0.689		
No	79	22 (27.8%)			
WC GAINS COMBINED					
No gain	73	18 (24.6%)	0.112		
WC other alone	13	3 (23.1%)			
WC7 alone	6	4 (66.6%)			
WC7 and WC other	12	5 (41.6%)			

*percent shows the row percent (percent of patients having a recurrence or not).
**Adjusted OR and p value are from a multivariate logistic regression of recurrence including both surgical status and WC7 gain simultaneously as risk factors. CI, confidence interval; OR, odds ratio; WC, whole chromosome; WC7, whole chromosome 7.

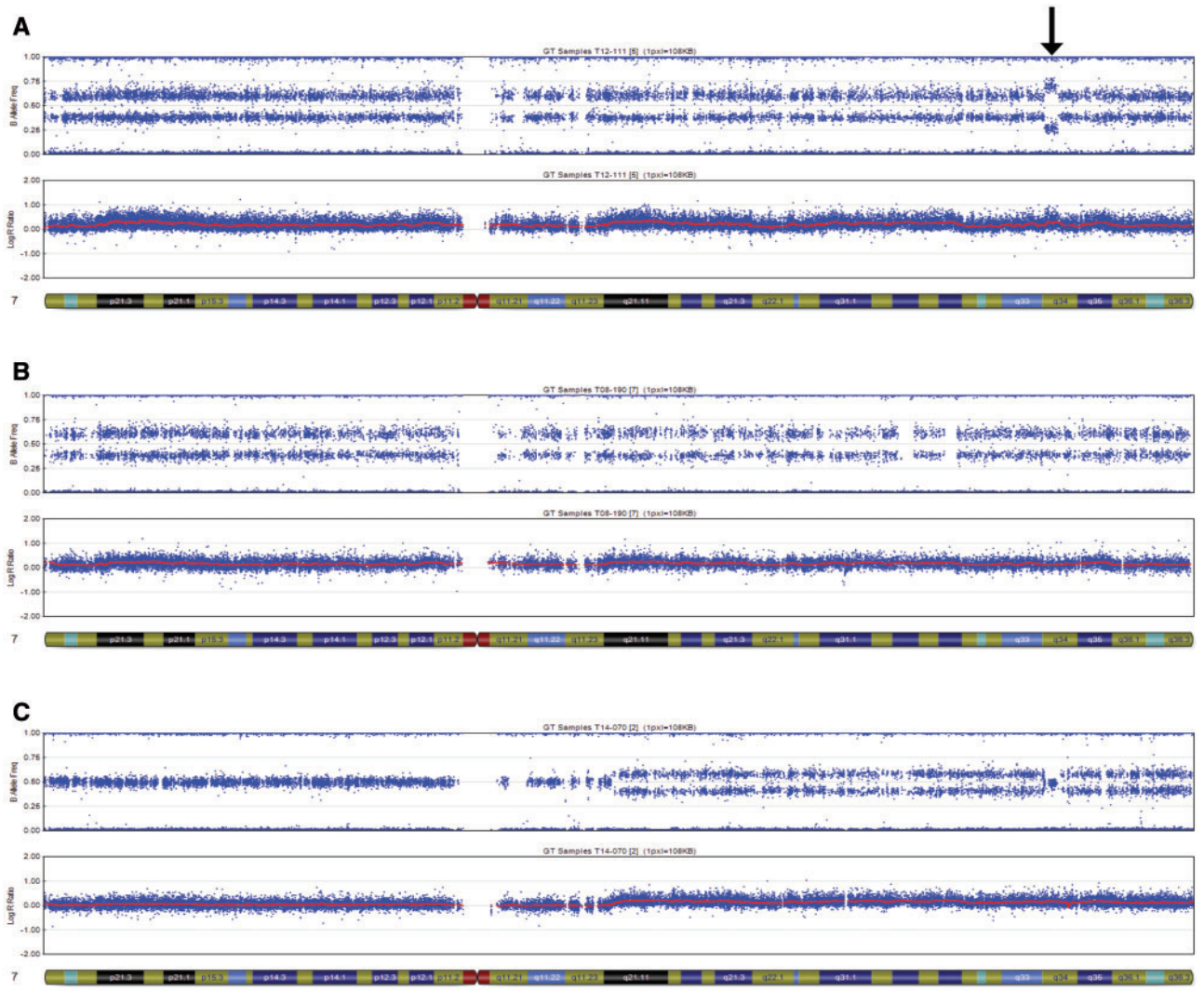

FIGURE 2. Representative SNP array images of WC7 gains observed in pilocytic astrocytoma. **(A)** 7q34 duplication (arrow) with WC7 gain. **(B)** WC7 gain without 7q34 duplication. **(C)** Gain of 7q21.11qter with a 7q34 duplication. SNP, single-nucleotide polymorphism; WC7, whole chromosome 7.

and other gains in 27.5% tumors (20 of 73). WC7 gains were most commonly seen in conjunction with the *KIAA1549-BRAF* fusion and/or gains of other chromosomes. Only 2 tumors demonstrated gain of WC7 alone, without the fusion or other chromosomal gains. Similarly, other WC gains (not chromosome 7) were more often observed in the context of WC7 gain and the *KIAA1549-BRAF* fusion. All 3 genetic alterations occurred together in 8 of 122 tumors (6.5%).

Primary and recurrent tumors were analyzed from 6 patients (patients 29, 32, 36, 42, 51, and 93). The *KIAA1549-BRAF* fusion status did not change between primary and recurrent tumors; 3 tumors demonstrated the fusion in both initial and recurrent lesions (patients 32, 36, and 93), while other examples (patients 29, 42, and 51) lacked the fusion (Supplementary Data 1 and 2). The single case with a *BRAF* exon 15 mutation (patient 29, tumor 08-190) retained the mutation in the recurrent tumor (tumor 12-222). SNP array analysis of 4 primary and recurrent tumors demonstrated a change in whole chromosome gains between the primary and recurrent tumor in patients 42 and 29. No significant abnormalities were identified in the initial lesion (tumor 10-254) from case 42, whereas the recurrent tumor (tumor 10-254B) demonstrated a WC12 gain (Fig. 4A–F). See below and the "Retention of WC7 Gain and *BRAF* Mutation Between Primary and Recurrent Tumor" section for results of case 42.

WC7 Gain Is Significantly Associated With Tumor Recurrence

We compared clinical and genetic data from all patients in an effort to identify significant correlations. A significant association was observed between WC7 gain and tumor recurrence ($p = 0.029$, chi-square test), irrespective of additional genetic abnormalities (Table). A total of 18 specimens demonstrated gain of chromosome 7 (not including duplicate tumors from the same patient). Of these 18 patients, 9 had

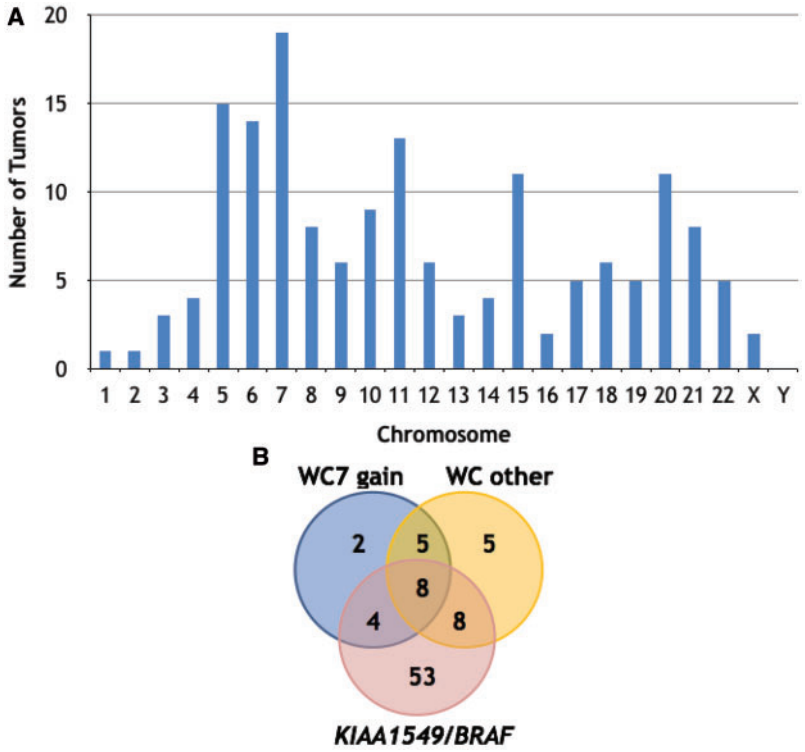

FIGURE 3. Distribution of recurrent genetic alterations identified in tumors that underwent SNP array analysis. **(A)** Total number of occurrences of chromosomal gains observed for each WC. Partial chromosome gains are not included. Gained material is present in at least 3 copies detectable by SNP array analysis. **(B)** Venn diagram displaying the overlapping distribution of the 3 most common genetic alterations observed in this cohort of pilocytic astrocytomas: *KIAA1549-BRAF* fusion, WC7 gain, and WC other (not 7) gain. SNP, single-nucleotide polymorphism, WC, whole chromosome; WC7, whole chromosome 7.

tumor recurrences (50%), in contrast to 21 of 86 (24%) without WC7 gain. The correlation between WC7 gain and recurrence remained significant after adjusting for surgical status ($p = 0.025$) (Table). The odds of tumor recurrence with WC7 gain are 4.7 times greater than for patients lacking a WC7 gain (Table).

Given that WC7 gain is associated with a more than 4-fold risk of tumor recurrence, we compared *BRAF* genetic alterations in WC7-gained tumors of recurrent and nonrecurrent tumors. As noted, 9 tumors harboring WC7 gain recurred, including 6 with the *KIAA1549-BRAF* fusion, 1 with a *BRAF* exon 15 mutation, and 2 without chromosome 7 alterations. A similar distribution of genetic alterations was seen in 9 tumors with WC7 gain and no recurrence; 6 showed the *KIAA1549-BRAF* fusion; 2 had a *BRAF* exon 15 mutation; and 1 tumor had no alterations on chromosome 7. Although these results indicate that *BRAF* alterations are not more frequent in patients with recurrence in tumors with WC7 gain ($p = 0.716$, data not shown), additional evidence suggests that these tumors appear preferentially to gain or retain mutant chromosome 7 (see "*KIAA1549-BRAF* Alteration Occurs Before WC7 Gain" and "Retention of WC7 Gain and *BRAF* Mutation Between Primary and Recurrent Tumor," below).

In addition to WC7 gain, many tumors demonstrated gains of other chromosomes. When comparing 4 groups defined by both WC7 and other WC gains; WC7 gain without other WC gain; other WC gain without WC7 gain; or no chromosomal gains, there was no increased risk of tumor recurrence in any of the 4 groups. However, there was a trend towards higher recurrence rate in tumors with sole WC7 gain and WC7 gain associated with other WC gains (67% and 42% recurrence, respectively). A trend towards lower recurrence was seen in groups defined by "other WC gains" and those lacking chromosomal gains (23% and 25% recurrence, respectively) (Table). Tumor recurrence was not significantly associated with the presence of either the *KIAA1549-BRAF* fusion, *BRAF* exon 15 mutation, or other WC gains when each alteration was considered irrespective of additional genetic changes in the tumor (Table).

KIAA1549-BRAF Alteration Occurs Before WC7 Gain

In tumors with WC7 gain and the 7q34 tandem duplication, we sought to determine whether alterations occurred within the same clone, which genetic alteration occurred first, and if the extra chromosome 7 contained the fusion. Depending on the order of occurrence, there are multiple possible combinations of tumor alterations, including initial WC7 gain followed by fusion formation on 1 of the chromosomes 7, or initial fusion formation followed by gain of either the fusion-positive or fusion-negative chromosome 7. FISH analyses were performed on touch imprints from tumor 12-111 with

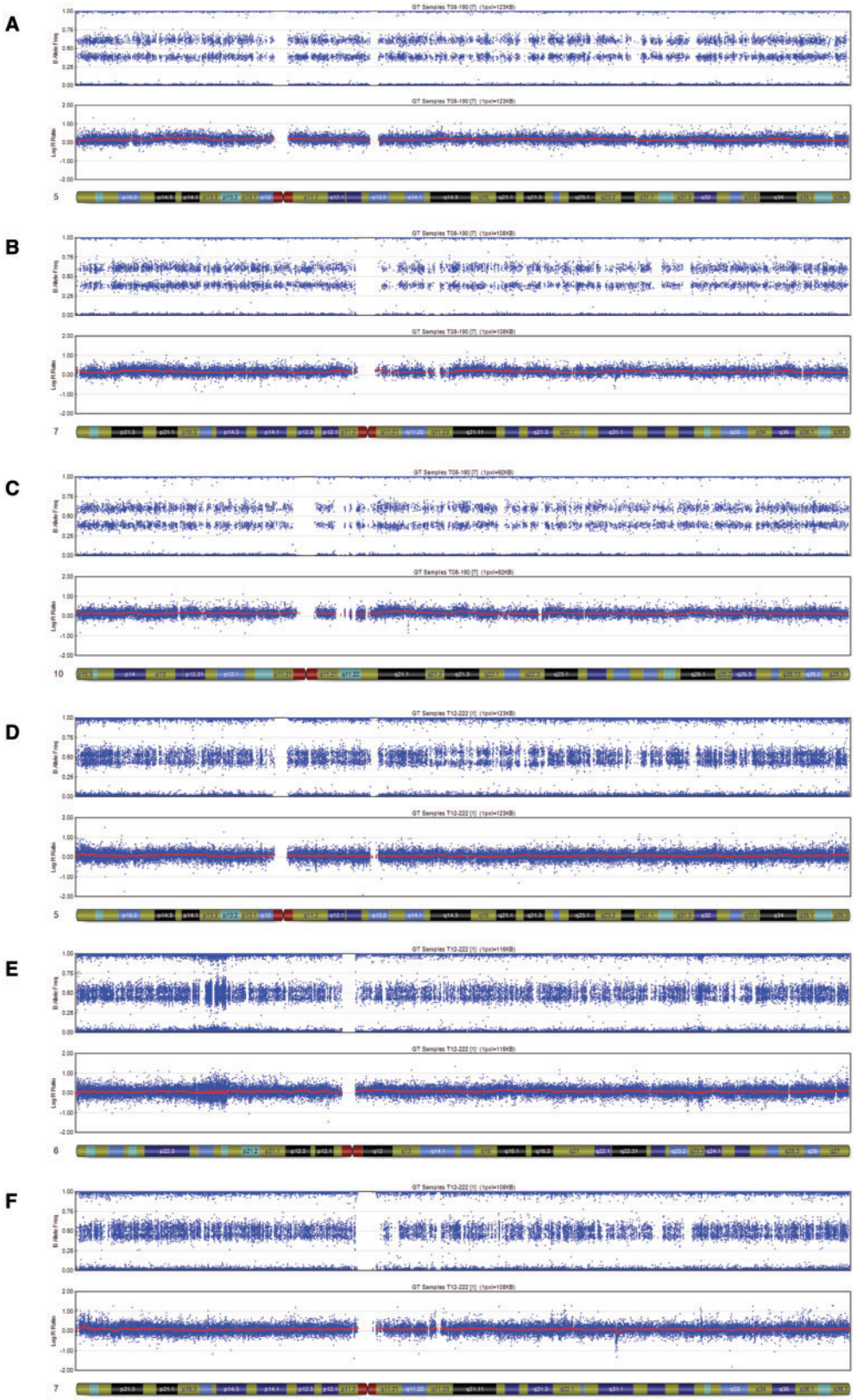

FIGURE 4. SNP array images of genetic alterations identified in a primary (tumor 08-190) **(A–C)** and recurrent tumor (12-222) **(D–F)** from the same patient. The primary tumor demonstrates gains of chromosomes 5 **(A)**, 7 **(B)**, and 10 **(C)**, whereas in the recurrent tumor, chromosomes 5 **(D)**, 6 **(E)**, and 7 **(F)** are gained. SNP, single-nucleotide polymorphism.

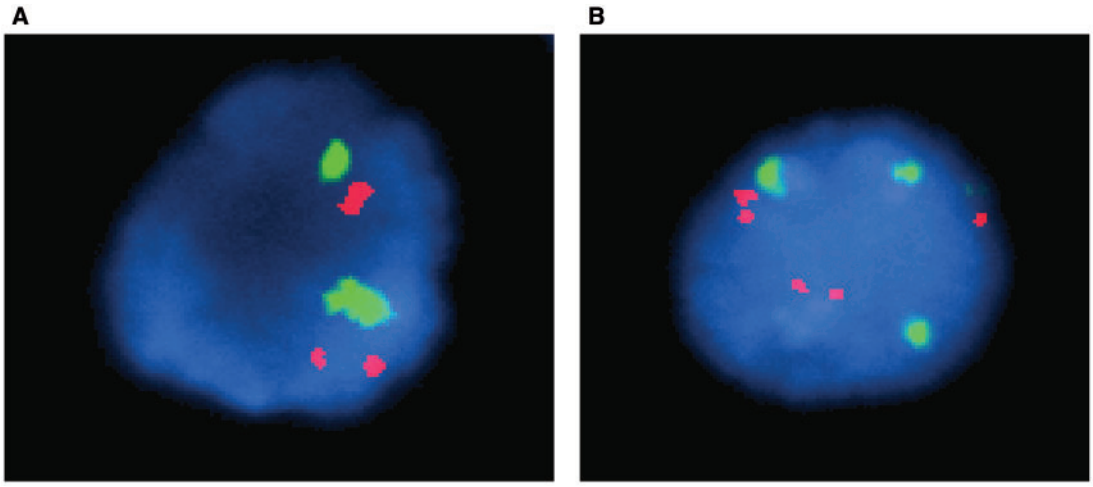

FIGURE 5. Representative cells from FISH analysis of tumor 12-111. The FISH probe labeled in green is targeted to the centromere of chromosome 7 (D7Z1); the probe labeled in red is targeted to the *LUC7L2* gene, which is included in the 7q34 tandem duplication. DAPI is used to stain the cell nucleus. **(A)** A cell demonstrating 2 D7Z1 and 3 *LUC7L2* signals. Two of the *LUC7L2* signals are located close to each other, appearing as a doublet, representing 1 copy of the 7q34 tandem duplication. **(B)** A cell demonstrating 3 D7Z1 and 5 *LUC7L2* signals. The location of 4 of the *LUC7L2* signals is consistent with the presence of 2 7q34 duplications in this cell. FISH, fluorescence in situ hybridization; DAPI, 4′,6-diamidino-2-phenylindole.

probes for the centromere of chromosome 7 (D7Z1) and the *LUC7L2* gene, located in chromosome band 7q34. Representative cells are shown (Fig. 5). Figure 5A illustrates a cell with 2 chromosome 7 centromeres and 3 copies of band 7q34, indicating that the fusion and WC7 gain occur within the same cells and that the fusion arose before the WC7 gain. Figure 5B displays 3 signals for chromosome 7 centromere and 5 signals for band 7q34, indicating that the chromosome 7 harboring the 7q34 fusion was gained. The red probe signals appearing in close proximity is characteristic of tandem duplication.

Retention of WC7 Gain and *BRAF* Mutation Between Primary and Recurrent Tumor

Retention of chromosome 7 harboring a *BRAF* mutation was observed in a recurrent tumor (patient 29, specimen 12-222) from a patient whose tumor was previously reported by our group (17). Both the primary and recurrent tumor harbored a mutation in *BRAF* exon 15: c.1794_1796dupTAC (p.Thr599dup) (Supplementary Data 2). In addition, the primary tumor demonstrated gains of chromosomes 5, 7, and 10 (Fig. 4A–C). The recurrent tumor showed gains of chromosomes 5 and 7, but lost the chromosome 10 gain and instead showed a gain of chromosome 6 (Fig. 4D–F).

Incidence of Whole Chromosome Gains in Older Patients

When analyzing the population in terms of age, WC7 gain was more frequent in patients aged 16 years and older (3/8, 37.5%) when compared to patients younger than 16 (15/96, 15.6%), but this was not significant ($p = 0.116$). Similarly, other WC gains were observed more frequently in patients 16 years and older (3/8, 37.5%) vs patients younger than 16 (22/96, 22.9%), but this association was also not significant ($p = 0.353$).

DISCUSSION

In this retrospective study, we correlated clinical and genomic data in 116 patients with PA and PMA, representing the largest such cohort to date. Retrospective genomic analysis with combined cytogenetic and molecular methodologies was compared to clinical data, including age, sex, treatment, and relevant medical history. In addition, the likelihood of tumor recurrence was correlated with the genetic findings. We confirm that the *KIAA1549-BRAF* fusion does not increase the risk of recurrence (19). However, the novel finding of this study is that after adjusting for surgical status, the odds of recurrence for tumors that harbor WC7 gain is 4.7 times greater than for chromosome 7 diploid tumors.

After adjusting for other factors that have been shown to affect prognosis including tumor burden, location, and subtype as well as Karnofsky performance score, GTR is the most important determinant of outcome. Consistent with this observation, we found that the risk of recurrence is significantly lower in patients with GTR in comparison to STR and/or biopsy only (23, 26, 27). The majority of patients with STR in our cohort underwent adjuvant chemotherapy, and in a subset of cases, when local control could not be achieved, radiation therapy as well (28–31).

Our retrospective cohort is representative of the pediatric PA/PMA population in terms of tumor location with the majority of tumors occurring in the cerebellum followed by the supratentorial compartment, including the optic pathway and hypothalamus (32). Histologically, most tumors displayed a biphasic pattern with only a minority (those arising in the hypothalamic region) with pilomyxoid features (5).

Genetic alterations in this cohort were concordant with previous findings for PA/PMAs. Our study demonstrated that 74% of PAs show a 7q34 tandem duplication and *KIAA1549-BRAF* fusion (8–10, 20, 33). *BRAF* mutation (nonfusion) rates of 6% in PA were also seen in our population (10, 14, 15, 20, 33). Similarly, whole chromosome gains, including chromosome 7 and other commonly identified gains of chromosomes 5, 6, 7, 11, 15, and 20, were previously identified in PAs, including earlier reports from our group and others (9, 10, 13, 17, 34). Two of these previous studies identified an association between an increase in chromosomal copy number with an increase in patient age (13, 34). Although our study results trended toward the same association, we did not observe statistical significance; this is likely because of the small sample size of patients older than 16 years of age.

Aneuploidy, specifically chromosomal gains, is well documented in cancer. WC7 gains have been identified in tumors of the prostate, breast, and lung (35–37). In addition to PA, WC7 gain has been found in gangliogliomas, anaplastic astrocytomas, ependymomas, and glioblastomas (17, 38–40). We demonstrate that PAs with WC7 gain show a 4.7 times increased risk of tumor recurrence. Similarly, tumors with WC7 gain were more commonly identified in more advanced stage cancers (35–37, 40, 41).

Wessels et al reported that in adult patients (age greater than 18 years), WC7 gain in grade II astrocytomas was correlated with shorter survival times. Of note, higher frequency WC7 gain (>20% cells) correlates with shorter survival in the tumor (41). In patients with glioblastomas, WC7 gain alone correlates more closely with significantly shorter overall survival than *EGFR* amplification (40). Outside of the brain, advanced stage prostate tumors were shown to have an increased frequency of trisomy 7 compared to early stage tumors and normal tissues. In addition, metastatic lesions from prostate tumors harbor a higher frequency of WC7 gain, suggesting that WC7 gain is linked to tumor progression (35). In breast cancer, the percentage of cells with WC7 gain positively correlates with the number of metastatic lymph nodes. Moreover, a higher percentage of cells with WC7 gain correlates with shorter disease-free survival and overall survival in breast cancer patients (36). In contrast, polysomy 7 is associated with longer overall survival in non-small cell lung cancer patients treated with gefitinib (37). Taken together, these reports and our present study highlight the prognostic value of WC7 gain in a variety of cancers. Whether WC7 gain is a better predictor of clinical outcome is likely cancer type-specific and dependent on other factors including treatment and the presence or absence of additional genetic alterations.

Although the present study shows that WC7 gain is a genomic prognostic marker, its exact role in tumor recurrence remains unclear. Several groups have reported a correlation between *KIAA154-BRAF* status and progression-free and overall survival (19, 20). We and others have shown that the *KIAA1549-BRAF* fusion status does not predict tumor recurrence (8, 14, 19, 21–23, 34). In support of this observation, we found an equal distribution of the fusion in tumors that recurred and those that did not. The difference in results between the present study and the report from Baker et al (20) may be ascribed to the number of cases and differences in the patient cohort, such as inclusion of patients over the age of 20, exclusion of PMA histology, and follow-up time. A similar distribution of *BRAF* point mutations was seen among tumors with WC7 gain in our study, although the numbers were quite small. Even though the *KIAA1549-BRAF* fusion is not linked to recurrence, little doubt remains that the fusion and other MAPK alterations play an important role in tumor initiating events in PAs. Whole genome sequencing studies have reported that 62% of PAs display a single genetic alteration, which inevitably affects the MAPK pathway, most commonly the *KIAA1549-BRAF* fusion (15, 16). The fact that the *KIAA1549-BRAF* fusion is typically the sole abnormality in PA and that most PAs do not recur, supports the conclusion that other genetic or epigenetic alterations drive tumor recurrence.

While *BRAF* alterations on chromosome 7 are not linked to tumor recurrence, our study has shown that in 1 of our tumors, the chromosome 7 harboring the *KIAA1549-BRAF* fusion was the chromosome 7 gained. Increased fusion gene expression levels due to increased DNA copy number may provide an explanation for recurrence in tumors with WC7 gain. Further expression studies are needed to support this hypothesis. Additionally, other alterations that often accompany WC7 gain should be considered as contributing to the pathogenesis of tumor recurrence. In line with the hypothesis that co-occurring genetic alterations may contribute to the effects of WC7 gain, the pattern of other WC gains in PA appears to be nonrandom with a preference for gain of chromosomes 5, 6, 11, 15, and 20 and WC7 (13, 34). In a recent study by Fontebasso et al, these aneuploidy PAs exhibited differential expression of over 500 genes when compared to euploid tumors (34). Differentially expressed genes include those in pathways such as cell cycle regulation and CNS development, which could contribute to the tumor formation of aneuploidy tumors and possibly to the increased risk of recurrence of tumors harboring WC7 gain.

In this study, WC7 gain was linked to increased risk of tumor recurrence. It remains to be seen whether this observation correlates with overall survival. Although the *KIAA1549-BRAF* fusion is a useful diagnostic biomarker, we were unable to demonstrate it as having a prognostic value.

ACKNOWLEDGMENTS

The authors thank Dr Elisabeth J. Rushing for editorial assistance and Luanne Wainwright, Laura Tooke, and Tracy Busse for technical assistance.

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