Alterations of *BRAF* and *HIPK2* loci predominate in sporadic pilocytic astrocytoma

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ABSTRACT

Objective: Independent studies have previously demonstrated that both the *HIPK2* and *BRAF* genes are amplified and rearranged, respectively, in pilocytic astrocytomas (PAs). The purpose of this study was to further investigate the frequency of *BRAF* and *HIPK2* alterations in PAs, the concordance of these events, and their relationship to clinical phenotype.

Methods: We performed extensive characterization by array-based copy number assessment (aCGH), *HIPK2* copy number analysis, and *BRAF* rearrangement and mutation analysis in a set of 79 PAs, including 9 tumors from patients with neurofibromatosis type 1 (NF1).

Results: We identified 1 of 3 previously identified *BRAF* rearrangements in 42/70 sporadic PAs. An additional 2 tumors with no rearrangement also exhibited *BRAF* mutation, including a novel 3-base insertion. As predicted from the genomic organization at this locus, 22/36 tumors with *BRAF* rearrangement also exhibited corresponding *HIPK2* amplification. However, 14/36 tumors with *BRAF* rearrangement had no detectable *HIPK2* gene amplification and 6/20 tumors demonstrated *HIPK2* amplification without apparent *BRAF* rearrangement or mutation. Only 12/70 PAs lacked detectable *BRAF* or *HIPK2* alterations. Importantly, none of the 9 PA tumors from NF1 patients exhibited *BRAF* rearrangement or mutation.

Conclusions: *BRAF* rearrangement represents the most common genetic alteration in sporadic, but not neurofibromatosis type 1–associated, pilocytic astrocytomas (PAs). These findings implicate BRAF in the pathogenesis of these common low-grade astrocytomas in children, and suggest that PAs arise either from *NF1* inactivation or *BRAF* gain of function. **Neurology® 2009;73:1526–1531**

GLOSSARY

aCGH = array comparative genomic hybridization; **GBM** = glioblastoma multiforme; **NF1** = neurofibromatosis type 1; **PA** = pilocytic astrocytoma.

Pilocytic astrocytomas (PAs) represent one of the most common brain tumors in the pediatric population.[1] These glial neoplasms are classified by the World Health Organization as grade I astrocytomas, characterized by biphasic architecture, low proliferative indices, microglial infiltration, and overall indolent clinical behavior.[2] Fifteen percent of PAs occur in the context of the inherited cancer syndrome, neurofibromatosis type 1 (NF1),[3,4] and these NF1-associated gliomas exhibit biallelic inactivation of the *NF1* gene.[5,6] In contrast, sporadic pilocytic astrocytomas retain *NF1* gene expression, suggesting that other genetic events must be responsible for the genesis of these common childhood brain tumors.[6,7]

Previous genetic studies of sporadic PAs have demonstrated gains involving chromosomes 7 and 8[8-10]; however, the causative genes residing on these chromosomes that might be responsible for PA formation remain elusive. Recently, we and others have employed high-density gene expression and gene copy number-based microarray methods to identify potential genes in this region. Our initial

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analyses found that the homeobox-interacting protein kinase-2 (*HIPK2*) gene on chromosome 7q[11] and matrilin-2 (MATN2)[12] on chromosome 8p were increased in expression in sporadic PAs, and that the *HIPK2* gene locus itself was frequently amplified in these tumors. While both of these genes represent compelling candidates for a "PA-initiating" genetic change, *HIPK2* and MATN2 expression was increased in both sporadic and NF1-associated PA. This observation suggests that these genes might be involved in PA growth, but are unlikely to be responsible for sporadic PA tumorigenesis.

On chromosome 7q, near the *HIPK2* locus, resides the *BRAF* gene, which has been linked to numerous other cancers.[13-15] Recent studies by several groups have identified mutations and rearrangements of the *BRAF* gene in low-grade gliomas,[16-18] prompting speculation that this gene might substitute for *NF1* gene inactivation in the genesis of sporadic PA. In this report, we describe the spectrum of *BRAF* alterations in the largest series of PAs to date, and show that *BRAF* genetic changes are observed in nearly two-thirds of sporadic PAs, but are rarely seen in high-grade gliomas. Moreover, *BRAF* alterations were not seen in PAs from patients with NF1, supporting the contention that this specific genetic alteration may drive sporadic PA tumorigenesis.

METHODS

Specimen sources. Frozen tissue and derived nucleic acid specimens for this study were collected and utilized under IRB approved protocols (HRPO 99-0573 and 04-0980). Extraction of RNA and DNA was carried out according to the vendor's standard protocol using the RNeasy and DNeasy kits, respectively (Qiagen, Valencia, CA). RNA quality was assessed by Agilent Bioanalyzer, and quantification of both RNA and DNA was assessed with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington DE).

Array CGH and analysis. Affymetrix GeneChip Human Mapping 250K NSP and 250K STY arrays (Affymetrix, Santa Clara, CA) were processed by the Siteman Cancer Center Multiplexed Gene Analysis Facility, following the manufacturer's standard protocol. Affymetrix GTYPE v4.1 software was used to generate Affymetrix CEL files and to call SNP genotypes using the BRLMM algorithm. Copy number analysis for Affymetrix GeneChip Human Mapping 250K NSP and 250K STY arrays was performed using dChip,[19] Partek Genomic Suite (PGS v6.07), Affymetrix Genotyping Console 2.1 (GC), and CNAG (v3.0).[20-22] SNPs were mapped to the NCBI genome build 36. Thirty-seven nonmalignant DNA samples from independent subjects were used as a "normal genome" reference set.

First, unpaired analysis was performed using dChip to calculate copy number at each SNP and to create segment data as previously described.[11] A region was called "amplified" if copy number was ≥ 2.5 and "deleted" if copy number was ≤ 1.5. Second, unpaired analysis was performed using PGS, and copy number at each SNP was calculated. Copy number data at each SNP locus were converted to segment level data to detect regions of amplifications and deletions using the HMM algorithm (default parameters) implemented in PGS. A region was considered "amplified" if copy number was ≥ 2.5 and "deleted" if copy number was ≤ 1.5. Third, unpaired analysis was performed using GC, and copy number at each SNP marker was calculated. Copy number at each SNP locus was converted to segment level data to detect regions of amplifications and deletions using the Segment Reporting Tool (default parameters) implemented in GC. A region was called "amplified" if copy number was >2.0 and "deleted" if copy number was <2.0. Finally, unpaired analysis was performed using CNAG (default parameters). A region was called "amplified" if copy number was >2.0 and "deleted" if copy number was <2.0. CNAG reports the results at segment level for build 35; therefore, reported regions were converted to build 36 using the LiftOver tool.[23]

Amplified segments from chromosome 7 were selected for overlap analysis. The segment level data from dChip, PGS, GC, and CNAG were compared to identify overlapping and recurrent segments (i.e., present in more than 1 sample and predicted by 2 or more algorithms).

***HIPK2* quantitative PCR.** Quantitative PCR (qPCR) analysis was employed to identify *HIPK2* amplification as previously described.[11] Primers and TaqMan probes for *HIPK2* and the internal reference gene, *DOCK10*, were designed with Primer Express v1.5 (Applied Biosystems, Foster City, CA). Assays were performed on an ABI PRISM 7900HT system.

***BRAF* rearrangement assay.** Using previously reported protocols,[18] BRAF rearrangement was assessed by RT-PCR. RNA was reverse-transcribed to cDNA using Oligo(dT)-primers and SuperScript reverse transcriptase II (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. PCR was performed using conditions and primers previously described to identify 3 types of KIAA1549-BRAF fusion products (16_9, 16_11, and 15_9).

***BRAF* sequencing.** All sequencing was performed by the Laboratory for Clinical Genomics at the Washington University School of Medicine. Primers (table e-1 on the *Neurology*® Web site at www.neurology.org) were designed using the Primer Design Pipeline tool developed by the Siteman Cancer Center Bioinformatics Core. PCR and sequencing employed Amplitaq® Gold DNA Polymerase and ABI BigDye V3.1 (Applied Biosystems, Foster City, CA). For amplicon generation, cycling conditions were initial denaturation at 95°C for 10 m, 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 90 s, a 7-minute extension step at 72°C, followed by a hold at 10°C until the final purification step. PCR products were then purified using ExoSAP-IT (USB, Cleveland, OH) for sequencing using Edge Biosystems Performa V3 96-Well Short Plates injected on a 3730xl DNA Analyzer using POP-7 polymer, and analyzed using 3730/3730xl DNA Analyzer Data Collection Software v3.0 (Applied Biosystems). The total volume injected was 15 μL of purified sequencing product. Mutation detection analysis was performed using Mutation Profiling Pipeline and its graphical user interface Mutation Viewer developed by the Siteman Cancer Center Bioinformatics Core.

Immunohistochemistry. Immunohistochemistry was performed on 2 independent tissue microarrays containing sporadic

Table 1 *BRAF* rearrangement and *HIPK2* amplification status in 70 pilocytic astrocytoma cases

	HIPK2 amplification		
BRAF rearrangement	*HIPK2* +	*HIPK2*–	Total cases
16_9	17	9	32*
16_11	3	3	6
15_9	2	2	4
Total rearrangements	22	14	42
No rearrangements	6	14	28*

*Several cases analyzed for *BRAF* were not analyzed for corresponding *HIPK2* amplification.

and NF1-associated PAs using 2 different BRAF antibodies (ab33899 and ab59354; Abcam, Cambridge, MA) according to established protocols in our laboratory.[24] Normal human brain was used as an internal positive control for immunostaining of BRAF+ neurons. Tumors with greater than 10% immunoreactive cells were scored as positive.

RESULTS AND DISCUSSION Previously, we demonstrated that 40%–60% of PAs had amplification of the *HIPK2* gene and that *HIPK2* overexpression stimulated cell growth.[11] While the extent of *HIPK2* amplification in our PA tumors did not extend to the *BRAF* gene, others have demonstrated that the *BRAF* gene, in ~2 MB proximity to the *HIPK2* locus on chromosome 7q, is frequently rearranged and/or mutated in PA.[16-18] In these reports, *BRAF* rearrangements were observed in 19/25 (76%), 29/44 (65%), and 31/53 (58%) of PAs. Consistent with a functional role for BRAF in PA growth, *BRAF* alteration was associated with increased MEK signaling, and either MEK pharmacologic inhibition or silencing of *BRAF* in PA tumor cells resulted in reduced cell growth in vitro.[16,17]

To characterize further the frequency of *BRAF* rearrangements in PA and to correlate this genetic change with the presence of *HIPK2* amplification, we performed a simultaneous analysis of *HIPK2* amplification and *BRAF* rearrangement in 79 PAs, including 9 cases from patients diagnosed with NF1. For comparison, we also examined 2 cases of grade II astrocytoma and 38 cases of glioblastoma multiforme (GBM). In a subset of these PAs, we were also able to examine genome copy number across the entire *BRAF–HIPK2* locus, using array comparative genomic hybridization (aCGH) data. Table e-2 provides full details of the individual PA cases analyzed.

As shown in table 1, 42/70 (60%) of the sporadic PAs analyzed had 1 of the 3 previously described *BRAF* rearrangements. The overall frequency and relative ratios of each rearrangement type (70% of rearrangements involving exons 1–16 and 9–18) are comparable to those found by others.[16-18] However, using 2 tissue microarrays (a total of 120 individual PA tumors), we did not detect increased BRAF protein expression in PA tumors using antibodies that recognize either the amino or carboxyl terminal domain of the protein (data not shown), suggesting these *BRAF* alterations most likely affect the function of the chimera, but do not increase protein levels.

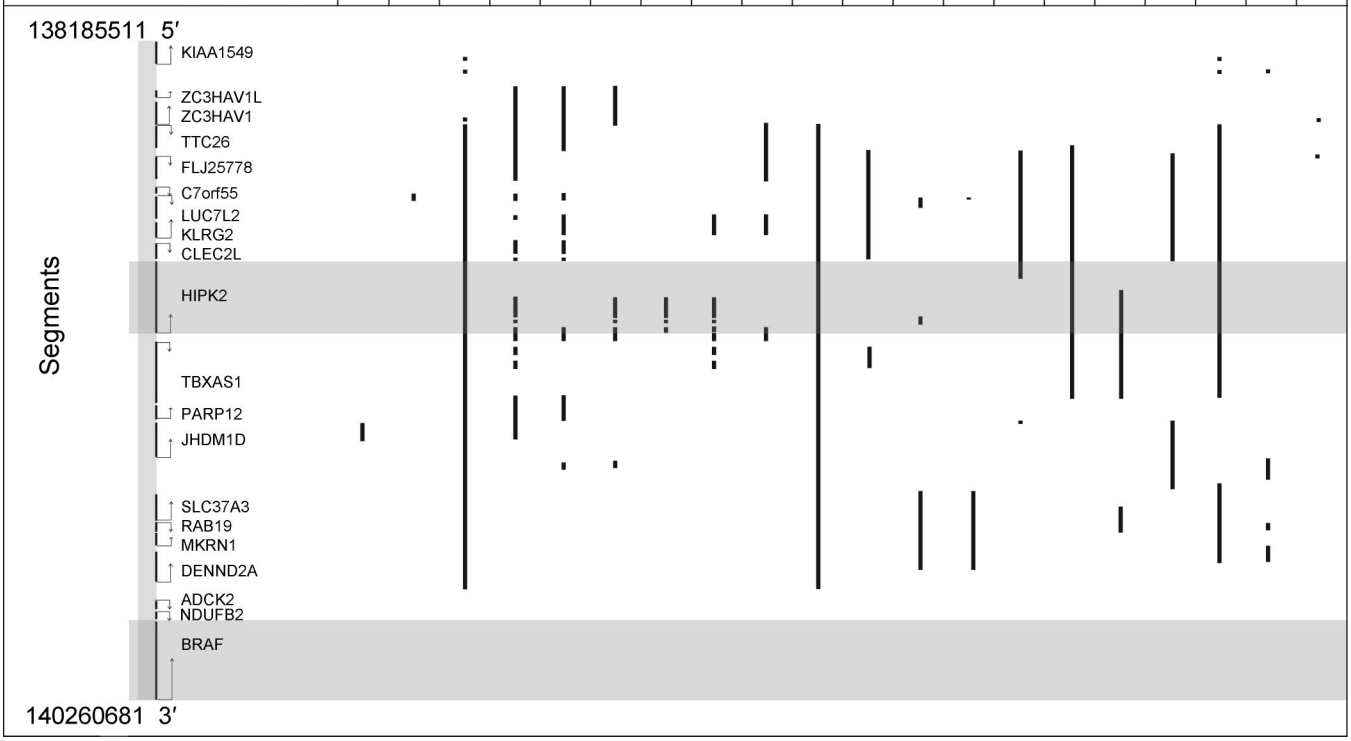
Rearrangement between *BRAF* and *KIA1549* results in a tandem duplication of the intervening genomic segment, which includes *HIPK2,* a gene which we previously found to be amplified in 40%–60% of sporadic PAs.[11] Not surprisingly, we found that 22/36 (61%) cases had concordant *BRAF* rearrangement and *HIPK2* amplification. Of the remaining 14 tumors with *BRAF* rearrangement, no *HIPK2* gene amplification was detected, possibly because of a lack of sensitivity for detecting subtle, quantitative copy number changes. More interestingly, we conversely identified 6/20 (30%) cases which clearly had *HIPK2* gene amplification, but no corresponding *BRAF* rearrangement. This could be the result of novel *BRAF* rearrangements that amplify the internal *HIPK2* locus, and are not detected by the 3 primer sets used in our and previously described assays. More provocatively, it could suggest independent contributions of the *BRAF* and *HIPK2* genes to the PA phenotype.

To understand more fully the genomic landscape of PAs at this locus, we compared whole genome aCGH copy number assessment performed on 20 PAs with the presence or absence of both *BRAF* rearrangement and *HIPK2* amplification, as assessed by quantitative PCR. As shown in figure 1, there is no clear relationship between the type of *BRAF* rearrangement and the extent of quantitative amplification in the region. Furthermore, we identified 2 tumors with neither *BRAF* rearrangement nor corresponding DNA amplification in this region of chromosome 7q. These data raise the possibility that further genomic heterogeneity exists at this locus, which may influence the molecular phenotype of these tumors.

Although the majority of sporadic PAs arise in the posterior fossa (table 2), we found a significant difference in the frequency of *BRAF* rearrangements in tumors arising outside of this anatomic region. Almost 70% of tumors arising in the posterior fossa (cerebellum, brainstem, fourth ventricle) harbored a *BRAF* rearrangement, whereas only 1/11 (9%) PAs arising in the optic nerve or cerebral hemispheres exhibited *BRAF* alteration. To further support the hypothesis that *BRAF* rearrangement is specific to the PA phenotype, we also examined other, more aggressive glial tumors. We examined 2 cases of grade II astrocytomas and 38 cases of GBM. Neither of the grade II

Figure 1 Comparison of quantitative array comparative genomic hybridization (aCGH) analysis and *HIPK2* amplification/*BRAF* rearrangement status in 20 sporadic pilocytic astrocytoma tumors

Patient ID		963	791	33953	719	1831	4118	13060	1163	1125	33954	33962	33965	33966	33971	701199	701202	701206	33955	33956*	33959*
BRAF fusion	15_9	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	16_9	-	-	-	Y	Y	Y	Y	-	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-
	16_11	-	Y	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HIPK2 Amp (qPCR)		-	Y	NE	Y	Y	Y	Y	Y	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE



The area intervening between the reported *BRAF* and KIAA1549 rearranged loci is shown. Gray bars indicate regions of *HIPK2* and *BRAF* genes. Vertical lines demonstrate the extent of amplification in each tumor sample as determined by aCGH. Above, the *BRAF* fusion type and *HIPK2* amplification status is noted. *Samples with no detectable molecular alteration at either gene locus.

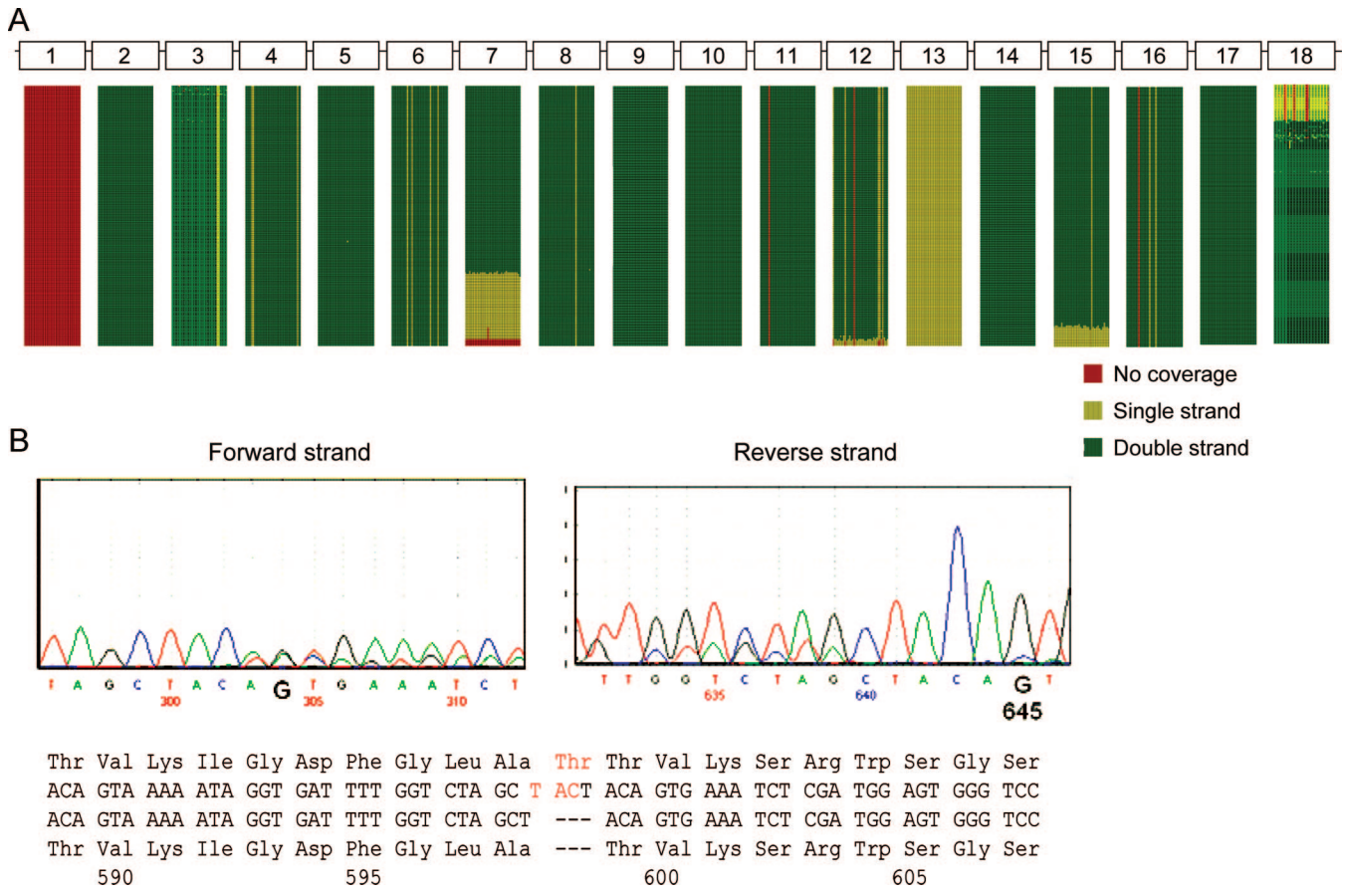
astrocytomas and only 1/38 GBMs exhibited *BRAF* rearrangement. Together, these data suggest that *BRAF* rearrangement represents a relatively specific PA molecular alteration and that other genomic changes may predominate in supratentorial PA.

Table 2 *BRAF* rearrangement in sporadic pilocytic astrocytomas based on anatomic site

Tumor site	Total	*BRAF* fusion+	
Brainstem	19	12 (63%)	
Cerebellum	38	28 (74%)	
4th Ventricle	2	1 (50%)	$p < 0.05$
Cerebral hemisphere	6	1 (17%)	
Optic nerve	2	0	
Midbrain	2	0	

Although we identified frequent *BRAF* rearrangements in this study, 28 (40%) cases still had no detectable genomic alterations. Since the *BRAF* gene is frequently mutated in a number of tumor types, we performed comprehensive resequencing of the *BRAF* coding region in 27 PAs with no detectable rearrangements, as well as in 6 PAs with 1 of the 3 detected *BRAF* rearrangements. As shown in figure 2A, we obtained high-quality, double-stranded sequence for the majority of *BRAF* gene exons (with the notable exception of exon 1) in all 33 cases. None of the 6 cases with *BRAF* rearrangement demonstrated an additional mutation, while 2/27 PAs had a detectable mutation. One tumor demonstrated a previously documented V600E single nucleotide substitution, whereas a second tumor harbored a novel 3-nucleotide insertion (figure 2B), which inserts an additional in-frame threonine residue at position

Figure 2 Sequencing of *BRAF* in pilocytic astrocytomas (PA)



(A) Coverage plot showing extent of double stranded sequence coverage of *BRAF* exons in 33 PA samples. (B) Three-nucleotide insertion detected in *BRAF* at amino acid residue 599. Sequence traces from forward and reverse strand are shown, along with resulting sequence and predicted reading frame.

599, and thereby also converts position 600 from a valine to a glutamine residue. The fact that this mutation occurs within the catalytic domain in close proximity to Valine-600, which is important for BRAF function, suggests that this mutation may lead to dysregulated BRAF activity as well. Future studies are planned to address this important question. Finally, despite this intensive characterization of the entire *BRAF* locus, 12 of 70 tumors in this cohort (17%) still lacked identifiable *BRAF* or *HIPK2* molecular alterations, implicating other genes in the pathogenesis of sporadic PA.

Although the majority of PAs arise sporadically, 15% of PAs arise in the context of the NF1-inherited tumor predisposition syndrome as a result of *NF1* gene mutation. In contrast to sporadic PA, no *BRAF* rearrangements were identified in the 9 PAs from NF1 patients. This finding suggests that *BRAF* might be a unique driver in sporadic PA that stimulates glial progenitor cell growth through the RAS pathway, similar to RAS hyperactivation secondary to biallelic *NF1* loss. This is consistent with previous studies from us and others identifying rare oncogenic *KRAS* mutations in sporadic PA.[24,25] Future mechanistic studies will be required to determine whether *BRAF* activation in permissive glial progenitor types is sufficient for low-grade gliomagenesis.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Jinsheng Yu.

DISCLOSURE

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REFERENCES

1. Central Brain Tumor Registry of the United States. Primary Brain Tumors in the United States Statistical Report 2007–2008. CBTRUS: Hinsdale, IL; 2008.
2. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. WHO Classification of Tumours of the Central Nervous System. IARC: Lyon; 2007.
3. Listernick R, Darling C, Greenwald M, Strauss L, Charrow J. Optic pathway tumors in children: the effect of neurofibromatosis type 1 on clinical manifestations and natural history. J Pediatr 1995;127:718–722.
4. Listernick R, Louis DN, Packer RJ, Gutmann DH. Optic pathway gliomas in children with neurofibromatosis 1: consensus statement from the NF1 optic pathway: Glioma Task Force. Ann Neurol 1997;41:143–149.
5. Gutmann DH, Donahoe J, Brown T, James CD, Perry A. Loss of neurofibromatosis 1 (NF1) gene expression in NF1-associated pilocytic astrocytomas. Neuropathol Appl Neurobiol 2000;26:361–367.
6. Kluwe L, Hagel C, Tatagiba M, et al. Loss of NF1 alleles distinguish sporadic from NF1-associated pilocytic astrocytomas. J Neuropathol Exp Neurol 2001;60:917–920.
7. Wimmer K, Eckart M, Meyer-Puttlitz B, Fonatsch C, Pietsch T. Mutational and expression analysis of the NF1 gene argues against a role as tumor suppressor in sporadic pilocytic astrocytomas. J Neuropathol Exp Neurol 2002;61:896–902.
8. Sanoudou D, Tingby O, Ferguson-Smith MA, et al. Analysis of pilocytic astrocytoma by comparative genomic hybridization. Br J Cancer 2000;82:1218–1222.
9. White FV, Anthony DC, Yunis EJ, et al. Nonrandom chromosomal gains in pilocytic astrocytomas of childhood. Hum Pathol 1995;26:979–986.
10. Zattara-Cannoni H, Gambarelli D, Lena G, et al. Are juvenile pilocytic astrocytomas benign tumors? A cytogenetic study in 24 cases. Cancer Genet Cytogenet 1998;104:157–160.
11. Deshmukh H, Yeh TH, Yu J, et al. High-resolution, dual-platform aCGH analysis reveals frequent HIPK2 amplification and increased expression in pilocytic astrocytomas. Oncogene 2008;27:4745–4751.
12. Sharma MK, Watson MA, Lyman M, et al. Matrilin-2 expression distinguishes clinically relevant subsets of pilocytic astrocytoma. Neurology 2006;66:127–130.
13. Michaloglou C, Vredeveld LC, Mooi WJ, Peeper DS. BRAF (E600) in benign and malignant human tumours. Oncogene 2008;27:877–895.
14. Lanzilotta SG, Grammatica L, Paradiso A, Simone G. BRAF in papillary thyroid carcinoma. Cell Oncol 2997; 29:269–277.
15. Dhomen N, Marais R. New insight into BRAF mutations in cancer. Curr Opin Genet Dev 2007;17:31–39.
16. Pfister S, Janzarik WG, Remke M, et al. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. J Clin Invest 2008;118: 1739–1749.
17. Bar EE, Lin A, Tihan T, Burger PC, Eberhart CG. Frequent gains at chromosome 7q34 involving BRAF in pilocytic astrocytoma. J Neuropathol Exp Neurol 2008;67: 878–887.
18. Jones DT, Kocialkowski S, Liu L, et al. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 2008;68:8673–8677.
19. Lin M, Wei LJ, Sellers WR, Lieberfarb M, Wong WH, Li C. dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. Bioinformatics 2004;20:1233–1240.
20. Partek Inc. Partek® Genomics Suite™. Partek Inc.; 2008.
21. Affymetrix R Genotyping Console TM Software. Available at: www.affymetrix.com/products_services/software/specific/genotyping_console_software.affx. Accessed March 12, 2009.
22. Nannya Y, Sanada M, Nakazaki K, et al. A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. Cancer Res 2005;65:6071–6079.
23. Lift Genome Annotations. Available at: genome.ucsc.edu/cgi-bin/hgLiftOver. Accessed March 12, 2009.
24. Sharma MK, Zehnbauer BA, Watson MA, Gutmann DH. RAS pathway activation and an oncogenic RAS mutation in sporadic pilocytic astrocytoma. Neurology 2005;65: 1335–1336.
25. Janzarik WG, Kratz CP, Loges NT, et al. Further evidence for a somatic KRAS mutation in a pilocytic astrocytoma. Neuropediatrics 2997;38:61–63.