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Desmoplastic Infantile Ganglioglioma/Astrocytoma (DIG/DIA) Are Distinct Entities with Frequent BRAFV600 Mutations

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Abstract

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Desmoplastic infantile ganglioglioma (DIG) and desmoplastic infantile astrocytoma (DIA) are extremely rare tumors that typically arise in infancy; however, these entities have not been well characterized in terms of genetic alterations or clinical outcomes. Here, through a multi-institutional collaboration, the largest cohort of DIG/DIA to date is examined using advanced laboratory and data processing techniques. Targeted DNA exome sequencing and DNA methylation profiling were performed on tumor specimens obtained from different patients ($n = 8$) diagnosed histologically as DIG/DIGA. Two of these cases clustered with other tumor entities, and were excluded from analysis. The remaining 16 cases were confirmed to be DIG/DIA by histology and by DNA methylation profiling. Somatic *BRAF* gene mutations were discovered in 7 instances (43.8%); 4 were $BRAF^{V600E}$ mutations, and 3 were $BRAF^{V600D}$ mutations. Three instances of malignant transformation were found, and sequencing of the recurrence demonstrated a new *TP53* mutation in one case, new *ATRX* deletion in one case, and in the third case, the original tumor harbored an *EML4–ALK* fusion, also present at recurrence. DIG/DIA are distinct pathologic entities that frequently harbor $BRAF^{V600}$ mutations. Complete surgical resection is the ideal treatment, and overall prognosis is excellent. While, the small sample size and incomplete surgical records limit a definitive conclusion about the risk of tumor recurrence, the risk appears quite low. In rare cases with wild-type *BRAF*, malignant progression can be observed, frequently with the acquisition of other genetic alterations.

Implications: DIG/DIA are a distinct molecular entity, with a subset frequently harboring either $BRAF^{V600E}$ or $BRAF^{V600D}$ mutations.

Introduction

The World Health Organization's classification of central nervous system (CNS) neoplasms categorizes desmoplastic infantile astrocytoma (DIA) and desmoplastic infantile ganglioglioma (DIG) together as one diagnosis, among grade I "neuronal and mixed neuronal-glial" entities (1). In 1978, Friede reported a desmoplastic glial tumor of the medulla with leptomeningeal metastases, proposing the terms "gliofibroma" or "desmoplastic glioma" (2). DIA was introduced by Taratuto, describing 6 infants with "superficial cerebral astrocytoma attached to dura" (3). Vandenberg reported 11 infants with DIG in 1987, noting mixed glial and neuronal histology (4). The typically large size, nodular contrast enhancement pattern, cellular pleomorphism and atypia, frequent mitoses, and undifferentiated small-cell component, all conjure the visage of an aggressive malignancy. Yet, complete resection is expected to yield an excellent prognosis (5). DIG/DIA most frequently occur in patients under 24 months of age, and accounts for a significant proportion of intracranial neoplasms seen in the first year of life (6, 7). Mitotic figures, primitive-appearing cells, and cellular pleomorphism are common histologic features of DIGs and DIAs. Yet, such high-grade characteristics have repeatedly failed to correlate with worse clinical outcomes in DIGs and DIAs, regardless of location (8, 9).

DIG/DIAs commonly present in the periphery of the supratentorial compartment, consisting of a large cystic component with a contrast-enhancing solid nodule attached to the overlying dura. Even without a significant cystic portion at presentation, this can develop over time (Fig. 1A; ref. 10). Rarely, DIG/DIA presents as an intraventricular lesion (Fig. 1B), or a mostly solid sellar/hypothalamic mass (Fig. 1C). Histologically, desmoplasia is prominent

within dense stroma, with fibroblastic and neuroepithelial elements. Neoplastic cells are limited to the solid nodule and adjacent leptomeninges (5, 10–13). Neuroepithelial elements vary in proportions of astrocytic and neuronal cells, and as the names suggest, presence of neuronal differentiation distinguishes DIG from DIA. DIGs, but not DIAs, commonly involve rests of primitive ganglion cells suggesting anaplasia, giving them an appearance such that the term “desmoplastic neuroblastoma” was previously used to described this entity (14). Presence of primitive neuronal components does not seem to imply more aggressive biology in DIG/DIA (9, 15–17). Craniospinal seeding or metastasis (Fig. 1D) has been reported (15–19), as have few instances of malignant transformation (20–22).

A common origin of DIG/DIA has been suggested, as has a relationship with PXA and GG (23–28). The argument for divergence along a common ancestral lineage accounting for the similarities and differences between these tumor types has been made entirely on histopathologic findings thus far (29). PXA shares many features with DIA in particular—both are frequently desmoplastic and involve the leptomeninges, are often cystic, and have a generally benign prognosis. PXA tends to present at an older age, and the pleomorphic, xanthomatous appearance of its astrocytes differentiate the two tumor types. Vandenberg notes a basal lamina seen in DIG/DIA that is also typical of PXA (9).

Little in the way of genetic and molecular characterization of DIG/DIA has been published, and no clonal chromosomal abnormalities have been found in DIA or DIG (22, 30–33). While various nonclonal aberrations have been reported, no specific locus has consistently appeared. Gessi and colleagues, performed a genome-wide DNA copy number analysis in combination with a multiplex analysis of candidate genes in 4 DIAs and 10 DIGs, observing inconsistent focal recurrent genomic losses in chromosome regions 5q13.3, 21q22.11, and 10q21.3 in both DIA and DIG (34). Principal component analysis did not show any significant differences; however, in 6 of these cases, gain of genomic material at 7q31, which corresponds to the *MET* gene, was found. A total of 7 cases of $BRAF^{V600E}$ mutations have been found previously in DIG/DIA (34–38). Hypermethylated alleles in the $P14^{ARF}$ gene were seen in one case (32). Lonnrot and colleagues, identified *MYCN* amplifications in 2 cases and *EGFR* amplifications in 3 cases (39). Several negative *TP53* analyses in patients with DIG/DIA have been performed (7, 31, 32, 39, 40), with 1 case report of a *TP53* SNV found in both a primary DIG and in its subsequent malignant transformation (22).

With this study, we aimed to more precisely understand the genetic underpinnings of DIG and DIA among glial–neuronal CNS tumors.

Materials and Methods

SCH cohort

Institutional review board approval was obtained prior to retrieval of pathologic tissue samples from Seattle Children’s Hospital (SCH) and contributing sites. All tissue samples were re-reviewed by independent neuropathology faculty of the Department of Pathology at SCH for diagnosis, tumor content, and adequacy for DNA extraction. UW-OncoPlex assay version 5, a targeted, massively parallel gene sequencing assay that detects mutations in 262 cancer-related genes (http://tests.labmed.washington.edu/UW-OncoPlex, last accessed May

5, 2017), was performed as described previously (41). The test uses next-generation “deep” sequencing to detect mutations including single nucleotide variants (SNV), small insertions and deletions (InDel), copy number alterations including gene amplifications, and selected gene fusions.

Briefly, DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) solid tumor tissue samples using the Gentra Puregene DNA Isolation Kit (catalog #158489; Qiagen). H&E-stained slides were reviewed before DNA extraction for all FFPE samples, and when feasible, macrodissection of tumor-containing regions was performed to enrich tumor cellularity. Tumor cellularity was estimated by review of H&E-stained slides. DNA sequencing was performed on a HiSeq2500 sequencing system (Illumina) with 2 × 101 bp, paired-end reads, and on a NextSeq 500 (Illumina) with 2 × 150 bp, paired end reads according to the manufacturer’s instructions. The average coverage across the capture region was 500×, minimum gene-level average coverage was set to 50×, with genes below this threshold reported as failed. The minimum acceptable average coverage for the entire panel was set at 150×, and the minimum library complexity (the fraction of unique DNA fragments sequenced) was set at 20%.

HD cohort

Tissue samples were retrieved with Ethics Committee approval from the archives of the Department of Neuropathology of the German Cancer Research Center/Heidelberg University (HD), and the N.N. Burdenko Neurosurgical Institute (Moscow, Russia). All included cases were reviewed by faculty in the Department of Neuropathology at HD. DNA from FFPE tissue was extracted on the Promega Maxwell device (Promega) following manufacturer’s instructions. Targeted exome sequencing was performed as described previously (42).

DNA methylation analysis

DNA was extracted from tumors and analyzed for genome-wide DNA methylation patterns using the Illumina Infinium Methylation EPIC BeadChip (850k) array. Processing of DNA methylation data was performed with custom approaches as described previously (43, 44). Analysis of tumor subgroups was performed using a t-SNE-based approach taking the 5,000 most variably methylated probes (45).

Results and Discussion

Individual molecular and genetic characterization of pediatric tumors may yield unexpected avenues for treatment

Ten primary tumor samples from SCH were examined using targeted DNA exome sequencing. In 5 tumors (50%), we found no genetic aberrations involving the 262 genes included in UW-OncoPlex. Within the other five tumors, we discovered 2 (20%) *BRAF*^{V600E} mutations, 2 (20%) *BRAF*^{V600D} mutations, and one *EML4-ALK* fusion (Table 1A). No other gene fusions were detected in either *BRAF*-mutant or wild-type tumors, including the *KIAA1549–BRAF* fusion. *CDKN2A* (p16) was intact at the DNA level in all

of our tested samples. *CDK4* and *CDK6* are also at a normal copy number state. Detailed clinical and sequencing information are listed in the Supplementary Data.

A replication cohort of 8 samples histologically diagnosed as DIG/DIA was collected at HD. Analysis of DNA methylation profiles through a molecular classification platform (www.molecularneuropathology.org) indicated that 2 of these cases clustered with PA, which were therefore excluded from subsequent analysis. Targeted DNA exome sequencing in the remaining 6 cases revealed two V600E and one V600D mutations (Table 1B). Of note, the 2 excluded cases did not harbor *BRAF* gene mutations. The clinical implications of our findings, in terms of potential therapeutic targets, argue for incorporation of broad molecular and genetic characterization in the diagnostic process for pediatric CNS tumors. This is particularly true of lower grade tumors likely driven by few oncogenic aberrations, and in patients for whom radiation sparing is paramount.

BRAF mutations are common in DIG/DIA

Seven of 16 (43.8%) patients in our series harbored somatic nonsynonymous single nucleotide variant substitutions at codon 600 of the *BRAF* gene. Four were the canonical V600E valine-to-glutamic acid; interestingly, 3 of 4 were found in histologically diagnosed DIAs. The other 3 were exceptionally rare V600D valine-to-aspartic acid point mutations, which account for less than 1% of all V600 *BRAF* mutations (http://cancer.sanger.ac.uk, last accessed January 8, 2018; refs. 46, 47). All three $BRAF^{V600D}$ mutations occurred in patients with DIG diagnosed at less than 1 year of age. *BRAF* mutations were thus discovered in 4 of 12 (25%) DIGs, but in 3 of 4 (75%) DIAs. None of the patients harboring *BRAF* mutations experienced recurrence.

Prior to our study, eight instances of *BRAF* mutations had been described in DIG/DIA, seven V600E substitutions, and one V600D (48). Dougherty and colleagues, reported a $BRAF^{V600E}$ mutation in 1 of 2 cases of DIG (35). Karabagli reported finding a $BRAF^{V600E}$ mutation in a skull base DIA (37). Prabowo and colleagues, found $BRAF^{V600E}$ mutations in 2 of 4 DIGs (38). Gessi and colleagues, discovered a single case of a $BRAF^{V600E}$ mutation in a DIA, among 14 DIG/DIAs (34). Koelsche and colleagues, found 2 cases of $BRAF^{V600E}$ mutations among 18 cases of DIG/DIA, 1 in a DIG and 1 in a suprasellar DIA (36). Of note, in several of these series, VE1 IHC staining was performed as screening, and molecular analysis was then performed only on positively staining cases to confirm or disprove the presence of the mutation. As a screen for $BRAF^{V600E}$ mutations, VE1 staining accurately identified these mutations in only 67%–75% of DIG (36, 38); moreover, it does not appear to detect other $BRAF^{V600}$ mutations, including the V600D mutation.

BRAF mutations have been sparsely seen among adult low-grade glial–neuronal tumors (49–51). TCGA low-grade glioma (LGG) analysis revealed only two *BRAF* mutations among 283 patients, including one V600E mutation and one D594G mutation, while the UCSF LGG analysis included one V600E mutation in 23 patients. (http://www.cbioportal.org, last accessed January 8, 2017; refs. 52, 53) Certain typically pediatric tumors are far more likely to show *BRAF* alterations; however, $BRAF^{V600E}$ mutations have been found in an estimated 66% of pleomorphic xanthoastrocytomas (PXA; ref. 42), 18%–58% of gangliogliomas (38, 42, 54), 30% of dysembryoplastic neuroepithelial tumors

(DNET; ref. 38), and 9% of pilocytic astrocytomas (42). Bergthold and colleagues, found that *BRAF*^{V600E} mutations clustered among supratentorial pediatric LGG, a group comprised primarily of gangliogliomas, diffuse astrocytoma, DNET, and a small proportion of pilocytic astrocytomas, also demonstrating that this cluster was significantly enriched for a significant number of gene sets that together suggested a neuronal signature (55). Furthermore, they did not detect any alteration in gene expression profile among *BRAF*-altered tumors when compared with wild-type *BRAF* in LGG.

The effect of these two types of *BRAF* mutations on RAS/MAPK function is thought to be equivalent; however, implications for treatment and diagnostic options are potentially confounding. While a small number of targeted molecular therapies have demonstrated efficacy in tumors harboring V600E and V600K mutations, V600D mutations have not been specifically evaluated in a clinical setting. However, preclinical studies suggest that *BRAF*^{V600D} mutations are likely to be sensitive to vemurafenib (56) and dabrafenib (57), and as alternative proven options are lacking, *BRAF* inhibitors are likely to play an important role in the management of patients with V600D mutations, even in infants (58).

Nosology of DIG and DIA is distinct, and likely involves a primitive precursor capable of glial and neuronal differentiation

To further examine the relationship between *BRAF*^{V600}-mutant and wild-type DIG/DIA and other pediatric glioneuronal tumors, including other entities that harbor *BRAF*^{V600E} mutations, we performed a global DNA methylation analysis using Illumina Infinium (850k) arrays. This analysis included 9 primary DIG/DIA from SCH, 6 primary DIG/DIA from the HD series, and reference samples of other entities collected in HD (11 GG *BRAF*^{V600E} mutant, 12 PXA *BRAF*^{V600E} mutant, 7 PA *BRAF*^{V600E} mutant, and 12 PA with other MAPK pathway alterations). Similarities in methylation profiles were visualized using a *t*-distributed stochastic neighbor embedding (TSNE) approach, as described previously (59).

DIG/DIA clearly formed a distinct molecular group regardless of *BRAF* mutation status (Fig. 2). No overlap with other *BRAF*^{V600E}-enriched pediatric brain tumor subtypes (gangliogliomas and PXA) was observed, demonstrating that DIG/DIA are molecularly distinct from these other entities. Of note, no discernible separation was observed between DIG and DIA samples, indicating that these may rather be morphologic variants of a single molecular entity.

On an autopsy of a patient with suprasellar/intraventricular DIG, Komori and colleagues, noted that, within the optic nerves, tumor infiltrates consisted of neuroepithelial cells of varying levels of differentiation, only occasional staining for GFAP or neurofilament, and unassociated with desmoplasia (60). Tumor astrocytes, primitive-appearing neuroepithelial cells, and Schwann-like cells were all intimately coexistent within the reticulin-rich basal lamina typical of DIG/DIA, as has been repeatedly observed in DIG/DIA (9, 28, 60). While astrocytes are neural-tube derivatives, Schwann cells originate from the neural crest, findings such as these indicate a more primitive derivation, perhaps related to the neural plate, to be capable of neuronal, astrocytic, and Schwannian differentiation.

Koelsche and colleagues, found *BRAF*^{V600E} mutations in 41 of 71 (58%) of gangliogliomas, further localizing the mutant *BRAF*^{V600E} protein product predominantly to the neuronal compartments within these tumors using VE1 IHC, indicating that *BRAF* mutations occur in cells that have the capacity to differentiate into ganglionic cells (54). A positive association has been found between VE1 positivity and synaptophysin-positive clusters, while a separate study made the similar observation of colocalization of *BRAF*^{V600E} mutation with the neuronal markers synaptophysin and NeuN (38). In addition, *BRAF*^{V600E} mutation was found to be tightly associated with pS6 (a marker for mTOR activation) and CD34 immunoreactivity within only the neuronal components of glial–neuronal tumors, but not within the glial components, where pS6 and VE1 staining was sparse. Although the number of tumors studied in this fashion is too small to draw conclusions regarding nosology, the frequent observation of both neural tube and neural crest derivatives within these tumors suggests a derivation more primitive than both. Case-by-case correlation between the BRAF mutation allele frequency and ganglion cell content would provide crucial understanding of the derivation of this tumor.

Acquired genetic alterations drive malignant transformation of DIG and DIA

Four (40%) patients with SCH experienced tumor recurrence/progression; 3 (30%) suffered malignant transformation of their tumors, and so UW-Oncoplex next-generation sequencing and DNA methylation profiling were performed on the recurrent tumors as well. One patient (Fig. 3A) presented at 4 months of age with a DIG negative for somatic variants on sequencing of the native tumor, underwent subtotal resection (STR) and received carboplatin and vincristine according to the Children's Oncology Group (COG) 9952 protocol for growth of the residual tumor almost immediately after surgery. The tumor further progressed 2 years later, at which time she again underwent STR, and sequencing of this sample showed a new frameshift insertion affecting *TP53*. The patient then underwent gross total resection for tumor progression 1 year later, and sequencing of this tumor held the same *TP53* mutation. She survived over 3 years from her presentation, before dying from her disease. Somatic *TP53* gene mutations are some of the most frequently observed in human cancers, including malignant CNS tumors (61).

Another patient (Fig. 3B), who presented at 7 months of age with a DIA negative for somatic variants on sequencing of the native tumor, underwent STR. Because of leptomeningeal involvement at presentation, she received COG 99703 protocol chemotherapy with a 25% dose reduction for age. Over 10 years passed before local recurrence was discovered, for which she again underwent STR of the recurrent tumor. Sequencing of this tumor showed a frameshift deletion affecting *ATRX*, as well as a nonsynonymous single-nucleotide substitution in *BCORL1*. Histopathology revealed high-grade malignant transformation of the native tumor, and 5,400 cGy of focal fractionated radiotherapy was administered. She continues to be followed, now 13 years after her initial presentation.

The *ATRX* gene is a core component of a chromatin remodeling complex active in telomere function, the alteration of which results in alternative lengthening of telomeres, a presumed precursor to genomic instability, and a recognized contributor to gliomagenesis (62). The

additional finding of a *BCORL1* point mutation is of uncertain significance. Both *ATRX* and *BCORL1* are on the X chromosome, and *BCOR* and *BCORL1* alterations are known to be involved in acute myelogenous leukemia (63). They are also rarely observed in CNS malignancies, including medulloblastoma, glioblastoma, and supratentorial CNS embryonal tumors (59, 64, 65). While 1 case of concurrent *ATRX* and *BCOR* mutations was discovered in a pediatric malignant glioma, *ATRX* has not been consistently described to coincide with *BCOR* (*L1*) specifically (65).

A 3rd patient (Fig. 3C) presented at 8 months of age with a DIG, and underwent GTR. Sequencing of this native tumor revealed an *EML4–ALK* gene fusion event. Tumor recurrence within 4 months was treated according to COG 9952 regimen A protocol with carboplatin and vincristine. She subsequently developed acute lower extremity paresis and bulky leptomeningeal disease progression, and sequencing of this sample showed the same *EML4–ALK* gene fusion. Of note, the native tumor sequencing also showed a premature stop mutation in *CREBBP*, which was not detected on the recurrent tumor. She was treated at this time according to CCG 99703 protocol. At her most recent follow-up, the patient was 7 years removed from her initial presentation, with complete response to treatment, and no signs of disease recurrence.

Conclusion

DIG/DIA are a distinct molecular entity, without overlap with other $BRAF^{V600E}$-enriched pediatric brain tumor subtypes (gangliogliomas and PXA). We have identified a subset of DIG/DIAs harboring *BRAF* mutations with approximately a 43.8% frequency. While $BRAF^{V600D}$ mutations have, to this point, proven exceedingly rare in primary CNS tumors, our cohorts revealed 3 among only 16 total DIG/DIAs. No other oncogenic mutation was consistently identified in the wild-type or mutant *BRAF* DIG/DIAs. $BRAF^{V600E}$ mutations were seen in 3 of the 4 DIAs, whereas the $BRAF^{V600D}$ mutations were all found in DIGs, perhaps suggesting a tendency for the former to arise in cells of astrocytic, rather than neuronal, lineage.

Malignant transformation of these tumors might be more common than previously recognized. Malignant transformation was identified in 33.3% of the patients followed long-term at SCH, with 2 of these 3 patients acquiring a new oncogenic mutation, not present in the native tumor, when the malignant tumor was sequenced. Each incidence of malignant transformation was driven by an identifiable genetic aberration other than a *BRAF* mutation.

Maximal safe resection remains the mainstay of treatment for these tumors. The need for chemotherapy and radiation in cases where complete resection is unable to be obtained does not appear to predict a higher mortality rate, and these remain optional in cases of tumor progression. However, in a small percentage of *BRAF* wild-type cases, we identified malignant transformation with the acquisition of other genetic alterations. Our findings in this regard serve to highlight the need to test all DIG/DIA for *BRAF* and other gene mutations including gene fusions, which might allow for the use of targeted molecular therapies, or dictate the need for chemotherapy or radiation in refractory or recurrent cases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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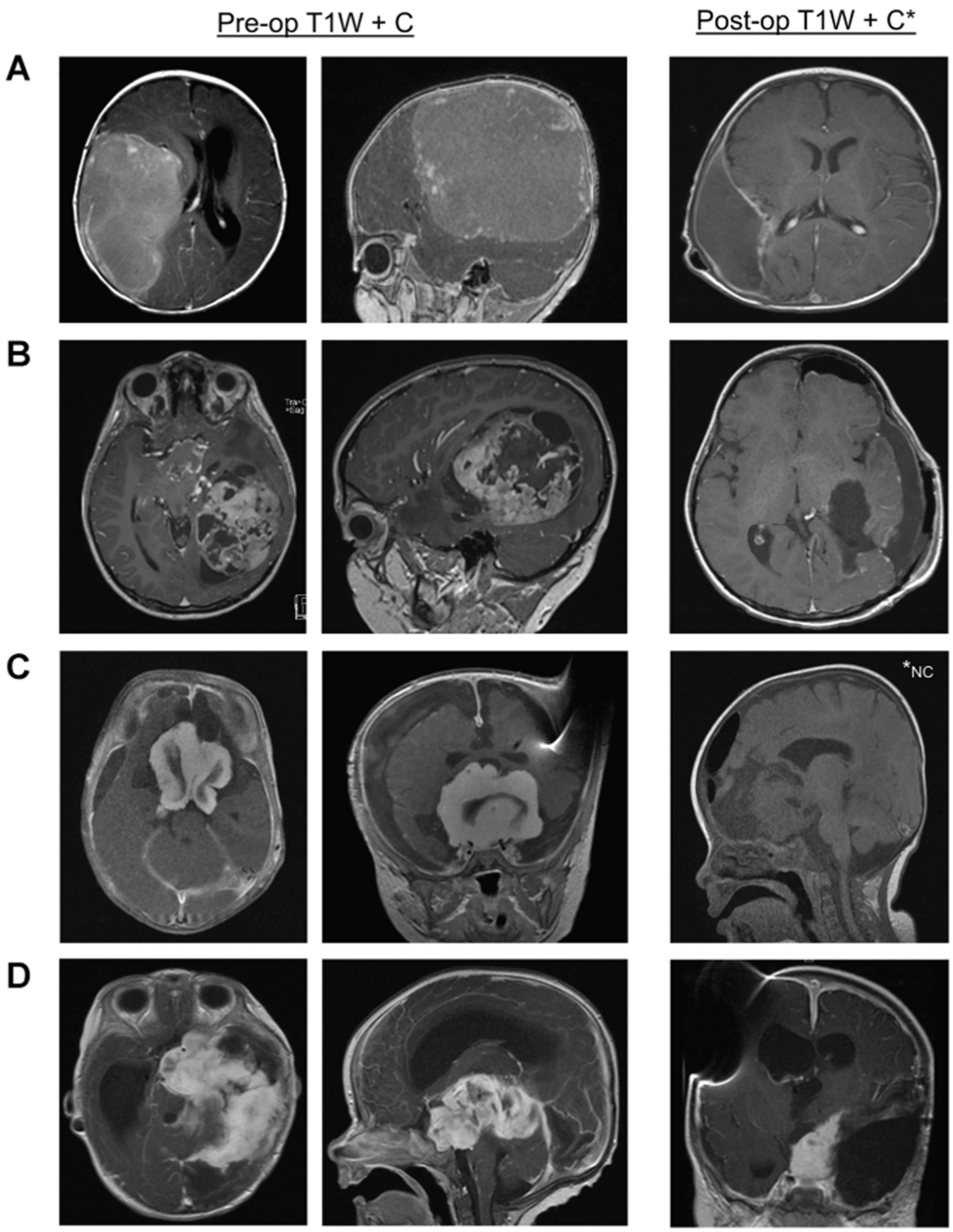

Figure 1:

A, Solid dura-based DIG. **B**, Multicystic intraventricular DIG. **C**, Suprasellar DIA with leptomeningeal metastases. **D**, Suprasellar and cisternal DIA

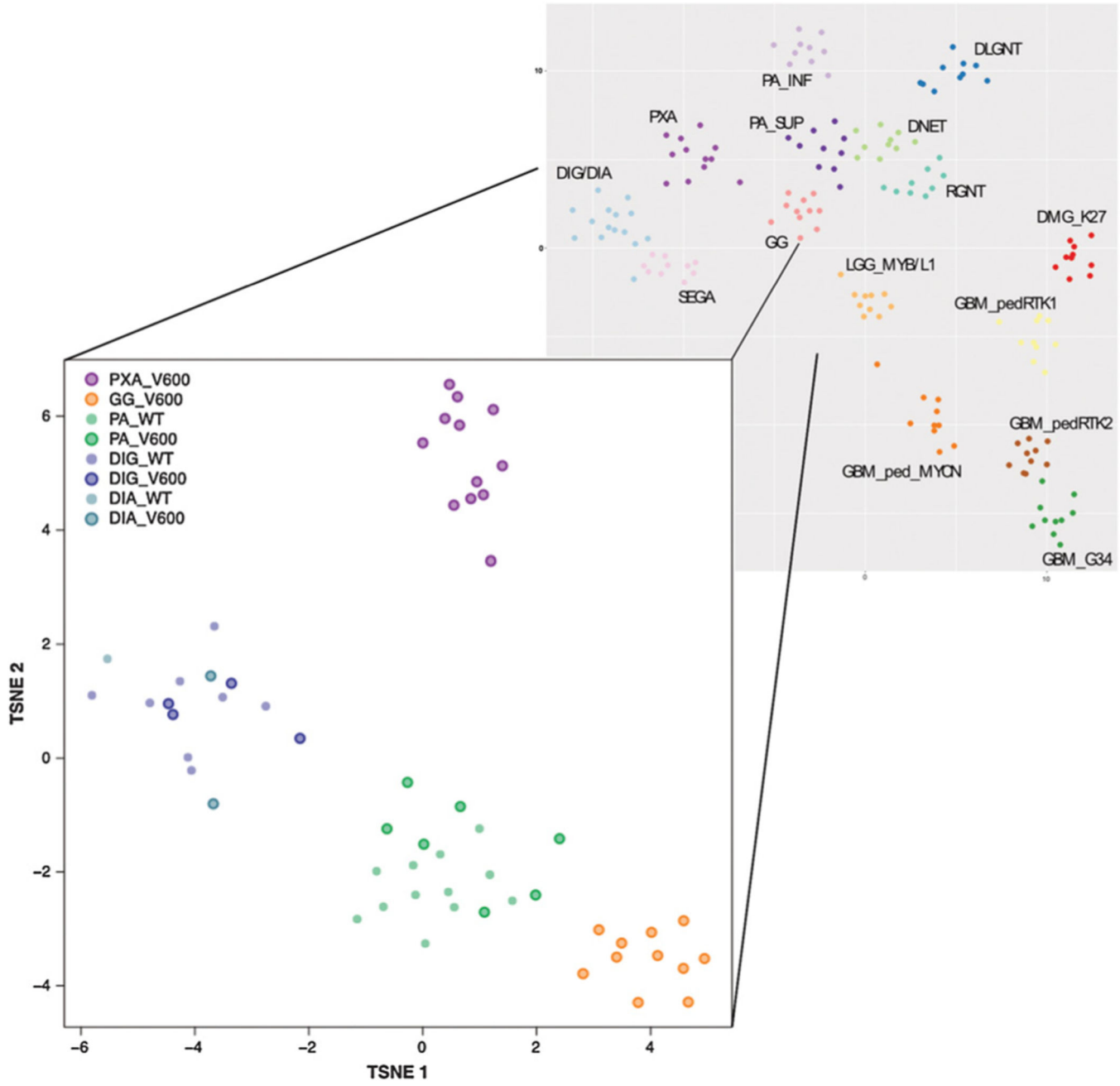

Figure 2:

DNA methylation profiling of DIG/DIA, relative to other pediatric glioneuronal tumors frequently harboring *BRAF* gene mutations. This *t*-distributed stochastic neighbor embedding plot represents DNA methylation assay data of the 5,000 most variable CpG sites across a larger cohort of pediatric glioneuronal CNS tumors. PXA with $BRAF^{V600E}$ mutations and GG with $BRAF^{V600E}$ mutations are clearly distinct from DIG/DIA. PA forms two other distinct groups, with $BRAF^{V600E}$ mutant and wild-type cases intermixed. Similarly, DIG/DIA form a distinct group with the *BRAF* mutant and wild-type cases intermixed, with no clear differentiation between DIA and DIG. These data suggest that

DIG/DIA is one distinct pathologic entity, with a significant proportion of cases harboring *BRAF* mutations.

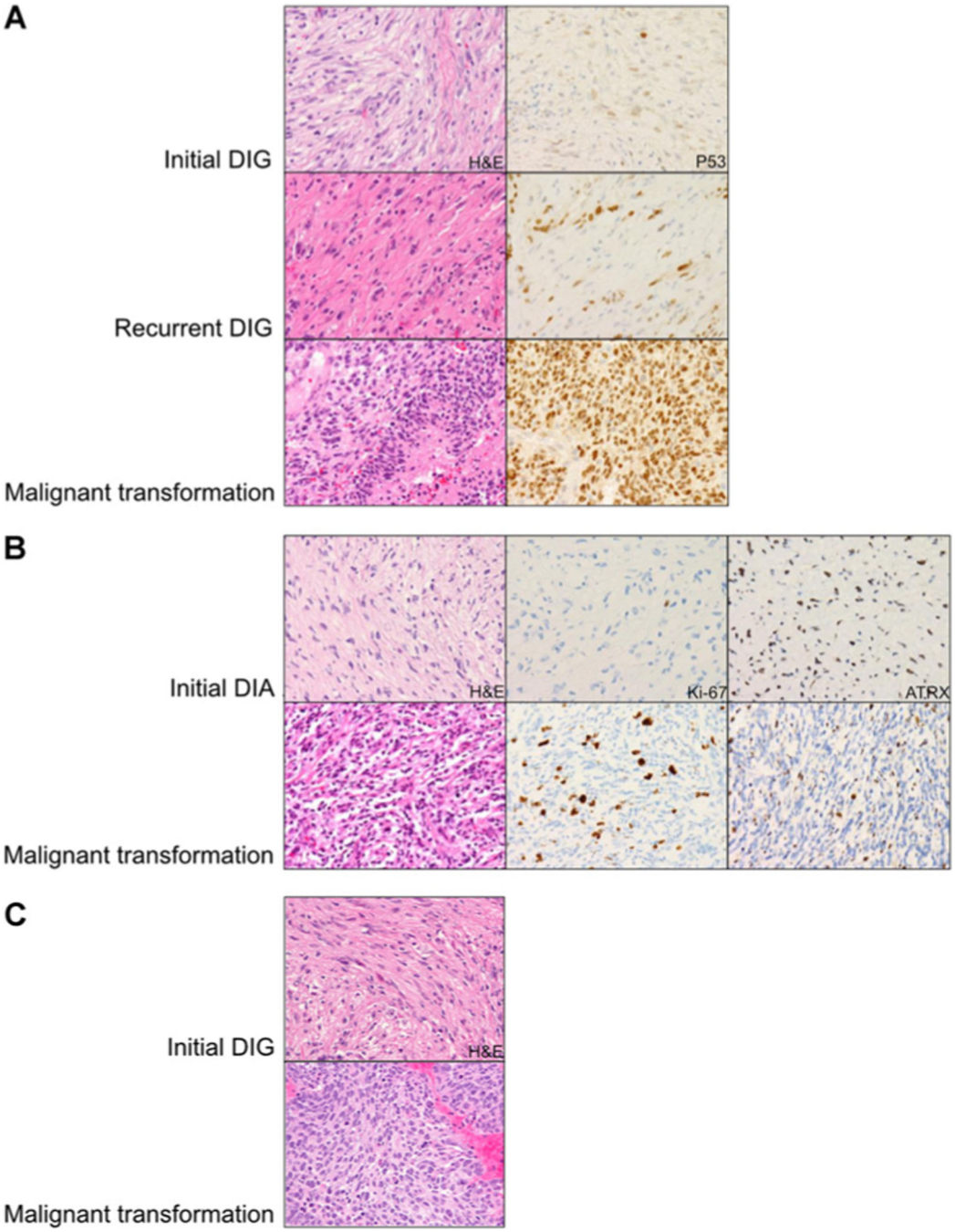

Figure 3.

A, Malignant transformation driven by *TP53* mutation. **B**, Malignant transformation driven by *ATRX* mutation. **C**, Malignant transformation driven by *EML4–ALK* fusion.