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Molecular Case Studies

RESEARCH REPORT

Integrated tumor and germline whole-exome sequencing identifies mutations in MAPK and PI3K pathway genes in an adolescent with rosette-forming glio-neuronal tumor of the fourth ventricle

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Abstract The integration of genome-scale studies such as whole-exome sequencing (WES) into the clinical care of children with cancer has the potential to provide insight into the genetic basis of an individual's cancer with implications for clinical management. This report describes the results of clinical tumor and germline WES for a patient with a rare tumor diagnosis, rosette-forming glioneuronal tumor of the fourth ventricle (RGNT). Three pathogenic gene alterations with implications for clinical care were identified: somatic activating hotspot mutations in *FGFR1* (p.N546K) and *PIK3CA* (p.H1047R) and a germline pathogenic variant in *PTPN11* (p.N308S) diagnostic for Noonan syndrome. The molecular landscape of RGNT is not well-described, but these data are consistent with prior observations regarding the importance of the interconnected MAPK and PI3K/AKT/mTOR signaling pathways in this rare tumor. The co-occurrence of *FGFR1*, *PIK3CA*, and *PTPN11* alterations provides further evidence for consideration of RGNT as a distinct molecular entity from pediatric low-grade gliomas and suggests potential therapeutic strategies for this patient in the event of tumor recurrence as novel agents targeting these pathways enter pediatric clinical trials. Although RGNT has not been definitively linked with cancer predisposition syndromes, two prior cases have been reported in patients with RASopathies (Noonan syndrome and neurofibromatosis type 1 [NF1]), providing an additional link between these tumors and the mitogen-activated protein kinase (MAPK) signaling pathway. In summary, this case provides an example of the potential for genome-scale sequencing technologies to provide insight into the biology of rare tumors and yield both tumor and germline results of potential relevance to patient care.

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INTRODUCTION

Rosette-forming glioneuronal tumor of the fourth ventricle (RGNT) is a rare central nervous system (CNS) tumor that primarily affects older children and young adults. Although RGNTs have an indolent natural history and can be cured with complete resection, no proven therapies exist for the treatment of partially resected, progressive, or recurrent tumors (Ellezam et al. 2012; Gessi et al. 2014). RGNT was recently included in the World Health Organization (WHO) classification system as a Grade I glioneuronal tumor, with a characteristic biphasic histologic appearance consisting of neurocytic rosettes and a piloid astrocytic component (Louis et al. 2007; Rosenblum 2007; Adesina 2010), but little is known about the biologic basis of these tumors. Targeted molecular analyses in small cohorts of RGNT have revealed a lack of the recurrent *BRAF* and *IDH1/2* alterations found in low-grade gliomas (LGGs) (Bidinotto et al. 2015), with somatic mutations identified in *FGFR1* (Gessi et al. 2014) and *PIK3CA* (Ellezam et al. 2012) in a subset of cases (Gessi et al. 2011, 2012; Solis et al. 2011), linking the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signaling pathways to RGNT pathogenesis. No definitive link between RGNT and cancer predisposition syndromes is known, but two cases of RGNT (Sherman et al. 2009; Karafin et al. 2011) have been reported in children with the RASopathies Noonan syndrome and neurofibromatosis type 1 (NF1) (Scheithauer et al. 2009).

In this report, we describe the results of clinical tumor and germline whole-exome sequencing (WES) for a child with RGNT. This analysis revealed three key genetic alterations with potential implications for clinical care: somatic activating hotspot mutations in *FGFR1* and *PIK3CA* and a pathogenic germline *PTPN11* variant diagnostic for Noonan syndrome. The co-occurrence of these three mutations in a patient with RGNT confirms previous observations regarding the molecular pathways altered in this rare tumor and suggests possible therapeutic strategies in the event of tumor recurrence. More generally, this case provides an example of the diagnostic value of genome-scale testing for patients with rare tumors such as RGNT and highlights the importance of integrating both tumor and germline testing for childhood cancer patients (Zhang et al. 2015; Parsons et al. 2016).

RESULTS

Clinical Presentation

The patient is a 12-yr-old African–American female who presented to Texas Children's Cancer Center for evaluation of papilledema that was incidentally discovered on a yearly optometric examination. Persistent mild headaches, intermittent vomiting, and a mildly ataxic gait were reported in retrospect. The child had a complex medical and social history, including premature delivery between 32 and 36 wk of gestation and a maternal history of HIV infection and substance abuse. She was adopted shortly after birth and showed failure to thrive, developmental delay (speaking a few words at 2 yr of age and first walking at 2.5 yr), and spastic diplegic cerebral palsy. Her height and weight had been consistently measured below the fifth percentile for age since infancy. Structural cardiac anomalies (mild supravalvular pulmonary stenosis, small perimembranous ventricular septal defect, and coronary arterial dilation) were diagnosed in early childhood and medically managed without surgical intervention. The patient had not previously been evaluated by a geneticist.

Physical examination was notable for short stature (below fifth percentile). Examination of the head and neck demonstrated hypertelorism with downslanting palpebral fissures, a short nose with depressed nasal root, deep philtrum, and low-set ears. Auscultation revealed a grade 2/6 ejection murmur and systolic ejection click. No rashes, macules, or patches were

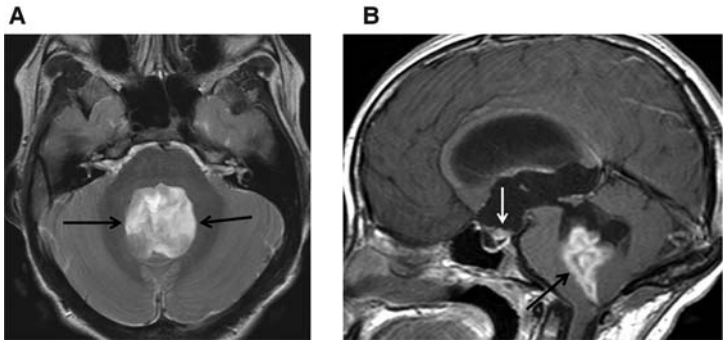

Figure 1. Brain MRI of mass at presentation. (*A*) Axial T2 and (*B*) post gadolinium T1 sagittal. The intraventricular tumor expands and obstructs the fourth ventricle, shows mixed T2 hyper-/hypointense signal (*A*, black arrows) and enhances heterogeneously (*B*, black arrow). There is also a small enhancing metastatic nodule at the inferior third ventricular recess (*B*, white arrow).

identified. On neurologic assessment, mild ataxia was noted, but examination of cranial nerves, coordination, sensation, muscle strength, and deep tendon reflexes was within normal limits.

Magnetic resonance imaging (MRI) of the brain and spine demonstrated a large complex T2 hyper-/hypointense lobular heterogeneously enhancing mass filling the expanded fourth ventricle and protruding through its outlets with associated obstructive hydrocephalus (Fig. 1A,B). A small enhancing metastatic nodule was noted in the inferior recess of the third ventricle (Fig. 1B). Post contrast spine MRI at presentation demonstrated T1 hyperintense substance within the terminal thecal sac, reflecting hemorrhage and/or drop metastasis; this subsequently improved on follow-up scans. A suboccipital craniotomy was performed and near total resection of the primary tumor was achieved. The patient did not receive adjuvant chemotherapy or radiation therapy. Following recovery from surgery, she underwent inpatient physical and occupational therapy and multidisciplinary management of her comorbid conditions.

Pathologic review revealed a RGNT with mixed architecture consisting of rosette-forming neurocytic cells with synaptophysin-positive cores (Fig. 2A,B), sheets of oligo-like cells with delicate capillary network (Fig. 2C), and piloid cells with gliofibrillary processes (Fig. 2D). An infarct-type necrosis with peri-infarct arcade of vascular proliferation, as well as regional calcification, was noted. Routine clinical molecular testing was negative for somatic alterations in *BRAF* (V600E point mutation and *KIAA1549-BRAF* fusion) and hotspot mutations in *IDH1* and *IDH2* by pyrosequencing.

The patient is now 16 mo out from neurosurgical resection, with stable residual tumor noted on follow-up MRI studies. Neuro-ophthalmologic examination has also been stable with right internuclear ophthalmoplegia. She has made a gradual overall recovery with physical and occupational therapy and is followed by the cardiology and genetics services.

Family History

The patient has five full and three half biologic siblings ranging in age between 3 and 23 yr, all in good health and without clinical features of Noonan syndrome. A family history of cancer in adulthood was reported in two maternal uncles (throat cancer; pancreatic cancer) and the maternal grandfather (colorectal cancer). Multiple paternal relatives reportedly died of cancers of unknown types. Additional details of the family history were limited by the patient's history of adoption.

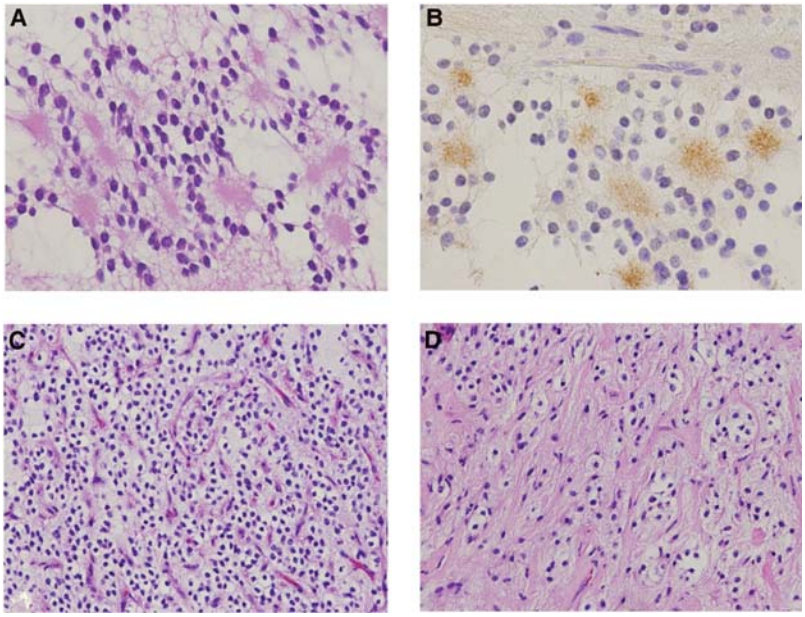

Figure 2. Immunohistopathology demonstrates (*A*) neurocytic rosettes with (*B*) synaptophysin-positive cores, (*C*) sheets of oligo-like neurocytes with associated delicate vascular network, and (*D*) regions with intermixed piloid gliofibrillary architecture. The histologic features are those of a rosette-forming glioneuronal tumor. Magnification: (*A*,*B*) 400×; (*C*,*D*) 200×.

Genomic Analysis

The patient was enrolled in the Baylor College of Medicine institutional review board (IRB)-approved BASIC^3 (Baylor Advancing Sequencing into Childhood Cancer Care) study, a National Human Genome Research Institute (NHGRI)- and National Cancer Institute (NCI)-funded Clinical Sequencing Exploratory Research project seeking to investigate the impact of WES on the care of pediatric oncology patients (Scollon et al. 2014). As part of this study, WES in a College of American Pathologists (CAP)- and Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory was performed on paired frozen tumor and blood samples as previously described (Parsons et al. 2016).

Analysis of the tumor WES data after subtraction of germline variants observed in the blood sample revealed four somatic (tumor-specific) mutations (Table 1), including mutations in the *FGFR1* and *PIK3CA* oncogenes. The exon 12 missense *FGFR1* mutation identified (c.1638C>A; p.N546K) occurs within the kinase domain of the protein and is the most frequent somatic *FGFR1* mutation reported to date, having been found in both low- and

Table 1. Somatic mutations identified by tumor whole-exome sequencing

Gene	Genomic coordinates (hg19)	HGVS cDNA	HGVS protein	Variant type	COSMIC ID	Variant allele fraction
FGFR1	Chr 8: 38274849	NM_023110: c.1638C>A	p.N546K	Missense	COSM19176	0.29
PIK3CA	Chr 3: 178952085	NM_006218: c.3140A>G	p.H1047R	Missense	COSM775	0.33
PPP1R1A	Chr 12: 54975791	NM_006741: c.372_373 delinsCA	p.E124_S125 delinsDT	Dinucleotide substitution	N/A	0.38
RNF216	Chr 7: 5662795	NM_207116: c.2297G>A	p.R766H	Missense	N/A	0.21

HGVS, Human Genome Variation Society; COSMIC, Catalogue of Somatic Mutations in Cancer; N/A, not applicable.

Table 2. Pathogenic germline variants in disease genes related to clinical phenotype

Gene	Genomic coordinates (hg19)	HGVS cDNA	HGVS protein	Variant type	SIFT/PolyPhen-2 predicted effect	dbSNP/ dbVarID	Genotype
PTPN11	Chr 12: 112915524	NM_002834: c.923A>G	p.N308S	Missense	Tolerated/benign	rs121918455	Heterozygous

HGVS, Human Genome Variation Society; SIFT, Sorting Tolerant from Intolerant; dbSNP, Database for Short Genetic Variations.

high-grade gliomas and glioneuronal tumors (Gessi et al. 2014; Forbes et al. 2015). It has been shown to activate MAPK and PI3K/AKT/mTOR signaling pathways (Turner and Grose 2010; Zhang et al. 2013b). The c.3140A>G missense mutation (p.H1047R) identified in exon 21 of *PIK3CA* is the most frequently observed *PIK3CA* hotspot alteration in human cancers, including high-grade gliomas and glioneuronal tumors (Ellezam et al. 2012), and has also been demonstrated to result in constitutive activation of the PI3K/AKT/mTOR pathway (Bader et al. 2005; Engelman 2009; Wu et al. 2014; Thorpe et al. 2015). Tumor WES also revealed novel somatic mutations in *PPP1R1A* and *RNF216*, genes that have been reported to be rarely mutated in cancer (Forbes et al. 2015) and are not known to contribute to cancer pathogenesis.

Germline WES revealed a c.923A>G (p.N308S) *PTPN11* pathogenic variant (Table 2), a well-described alteration in patients with Noonan syndrome (Tartaglia et al. 2002). The germline WES report also included nine variants of uncertain significance in genes related to cancer or intellectual disability (Supplemental Table S1) and seven pathogenic variants in genes associated with rare autosomal-recessive Mendelian disorders (Supplemental Table S2). Parental blood samples were unavailable for analysis.

DISCUSSION

Our patient showed clinical features of Noonan syndrome, a RASopathy characterized by distinctive facial features more prominent in infancy and childhood, short stature, pectus abnormality, cryptorchidism, congenital heart defects, and increased risk of bleeding (Romano et al. 2010; Niemeyer 2014). A genetic diagnosis of Noonan syndrome had not previously been made until germline WES identified a known pathogenic variant in *PTPN11* (c.923A>G, p.N308S) (Tartaglia et al. 2002). Approximately half of Noonan syndrome cases can be attributed to pathogenic variants in the *PTPN11* gene (Tartaglia et al. 2002), which encodes the protein tyrosine phosphatase SHP2, a cell-signaling molecule in the MAPK signaling pathway (Fig. 3; Keilhack et al. 2005; Niemeyer 2014). Notably, SIFT and PolyPhen-2 algorithms predicted this alteration as tolerated or benign, highlighting the limitations of existing prediction models for variant pathogenicity and the continued need for a thorough clinical assessment of patients harboring variants.

Patients with Noonan syndrome require multidisciplinary medical care for management of a wide range of associated comorbidities. Although patients with a *PTPN11* pathogenic variant are at increased risk of developing a spectrum of cancers, most commonly hematologic malignancies such as juvenile myelomonocytic leukemia (Romano et al. 2010), no specific surveillance program exists (Jongmans et al. 2011). Less commonly, CNS tumors including oligodendroglioma and other low-grade glial tumors have been associated with *PTPN11* Noonan syndrome (Smpokou et al. 2015), including a single case of RGNT (Sherman et al. 2009). A second case of RGNT in a patient with a clinical diagnosis of Noonan syndrome, but unknown genetic etiology has also been described (Karafin et al. 2011).

The somatic mutations identified in our patient with RGNT suggest potential targets for molecular therapeutics in the event of tumor recurrence. Although clinical data for this rare

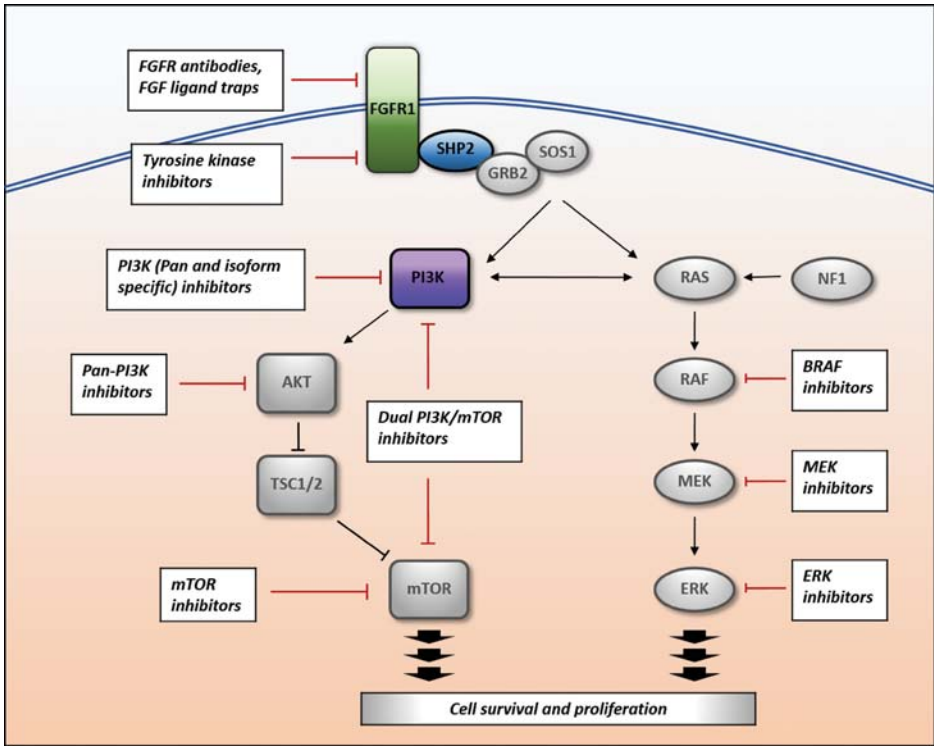

Figure 3. Overlapping RAS/MAPK and PIK3CA/AKT/mTOR signaling pathways and potential therapeutic targets. Pathogenic variants identified in this patient are *FGFR1*, *PTPN11*, and *PIK3CA*. FGFR, fibroblast growth factor receptor; FGF, fibroblast growth factor; PI3K, phosphoinositide-3 kinase; mTOR, mammalian target of rapamycin; NF1, neurofibromatosis type 1; BRAF, B-Raf proto-oncogene, serine/threonine kinase; MEK, mitogen-activated protein kinase/ERK kinase; ERK, extracellular-signal-regulated kinase.

tumor are limited, surgical resection and adjuvant radiation therapy would be considered a standard approach (Zhang et al. 2013a). However, should these modalities be contraindicated or insufficient, the presence of activating somatic *FGFR1* and *PIK3CA* mutations raises the possibility of targeted inhibition of the MAPK and PI3K/AKT/mTOR signaling pathways as an enticing albeit unproven intervention (Fig. 3). Activating *FGFR1* mutations, amplifications, and translocations have been reported in a variety of adult and pediatric cancers (Liang et al. 2013) and FGFR inhibitors are in clinical trial development for adult malignancies (Kim et al. 2011; Andre et al. 2013; Angevin et al. 2013; Soria et al. 2014; Cabanillas et al. 2015; Schlumberger et al. 2016) but not yet tested in children. Similarly, mutations and copy number alterations in a number of genes in the PI3K/AKT/mTOR pathway (most prominently *PIK3CA*, *PTEN*, *TSC1*, and *TSC2*) are recurrent events in numerous cancer types (Samuels and Ericson 2006; Thorpe et al. 2015). Agents targeting multiple nodes in the PI3K/AKT/mTOR pathway are in clinical development, including PI3K inhibitors, AKT inhibitors, mTOR inhibitors, TORC1/2 inhibitors, and PI3K/mTOR inhibitors (Weigelt and Downward 2012; Rodon et al. 2013; Fruman and Rommel 2014) and have begun to be evaluated in pediatric cancer patients. Aberrations of the PI3K/AKT/mTOR pathway have been suggested as a negative prognostic factor in the small number of RGNT cases described in the literature; of four tumors with somatic *PIK3CA* mutations, two recurred (Ellezam et al. 2012; Gessi et al. 2014). Somatic *PIK3CA* alterations have been associated with clinically aggressive features in LGGs, such as in pilocytic astrocytomas with uniquely anaplastic histology (Rodriguez et al. 2011). Given the small number of

RGNTs subjected to genetic analysis, the prognostic significance of these specific mutations in RGNT is currently unclear.

Although comprehensive data regarding the mutational landscape of RGNT are lacking, results from the few cases sequenced to date provide clues into the genetic and biological basis of these tumors. RGNTs share histologic similarity to pediatric LGGs, tumors defined by genetic alterations in the MAPK signaling pathway (Zhang et al. 2013b), and our data confirm previous reports of frequent MAPK pathway alterations in RGNT. However, the genes mutated in LGGs and RGNTs appear somewhat distinct: for example, missense mutations in *FGFR1* appear frequently in RGNTs (Gessi et al. 2014) but are rare events in pediatric LGGs (Zhang et al. 2013b), whereas the characteristic *BRAF* fusion seen in pilocytic astrocytomas has not been detected in RGNTs (Gessi et al. 2012). Concurrent *FGFR1* and *PTPN11* mutations, as found in our patient, have been identified in rare *BRAF* wild-type midline pilocytic astrocytomas and may represent an alternate mechanism of MAPK pathway activation or potentially have a potentiating effect on tumorigenesis (Zhang et al. 2013b).

Interestingly, concurrent somatic mutations were identified in *FGFR1* and *PIK3CA*, which code for gene products involved in distinct but closely communicating molecular signaling pathways. We report variant allele fractions of 29% (*FGFR1*) and 33% (*PIK3CA*) in this case, implying that a majority of sequenced cells (58% and 66%, respectively) carry each mutation (presuming heterozygous mutations in these oncogenes) and that the mutations therefore co-occur in tumor cells as opposed to being separate subclonal events. The identification of mutations in both MAPK and PI3K/AKT/mTOR signaling (Fig. 3) distinguishes RGNTs from the "single pathway" MAPK-driven LGGs. Interestingly, concurrent mutations in these same pathways have been reported in a variety of other cancer types (Janku et al. 2011), including a recurrent RGNT in an adult patient (Gessi et al. 2014). Previous targeted sequencing studies have revealed *PIK3CA* mutations, which are common in high-grade gliomas but not LGGs, in three of four RGNTs analyzed (Ellezam et al. 2012; Cachia et al. 2014). Functional studies describing the interplay of these alterations would be of biologic and therapeutic interest.

The identification of both germline and somatic mutations with potential implications for clinical care in this case highlights the potential benefit of integrating both tumor and germline testing in the evaluation of childhood cancer patients. Although most clinical tumor sequencing (primarily consisting of mutation panels including selected cancer genes and variants) is performed without concurrent analysis of a matched normal tissue sample, recent studies have highlighted the importance of paired tumor/germline analysis to decrease false-positive somatic mutation calls and distinguish germline variants from somatic mutations (Jones et al. 2015; Raymond et al. 2016). Given an observed frequency of pathogenic germline cancer susceptibility variants in 8%–10% of childhood cancer patients (Mody et al. 2015; Zhang et al. 2015; Parsons et al. 2016), clarification of whether individual mutations are somatic or germline is of particular relevance in the pediatric setting. Our patient provides a pertinent example: the specific germline *PTPN11* (p.N308S) pathogenic variant identified in this child with Noonan syndrome has been previously reported as a somatic event in several cases of acute lymphoblastoid leukemia (Zhang et al. 2011; Roberts et al. 2014).

In summary, this case of a child with RGNT and Noonan syndrome demonstrates the potential of genome-scale sequencing technologies such as WES to provide insight into the biology of rare tumors and yield both tumor and germline results of potential relevance for patient care. The identification of three pathogenic mutations (*FGFR1*, *PIK3CA*, and *PTPN11*) converging on MAPK and PI3K/AKT/mTOR signaling provides further evidence for the importance of these pathways in RGNT and offers possible therapeutic strategies in the event of tumor recurrence. Clinical trials evaluating agents that target these molecular pathways will be necessary to demonstrate the benefit of these drugs and genotype-directed precision oncology strategies for childhood cancer patients.

METHODS

WES of peripheral blood and tumor biopsy samples was performed in the CLIA-certified Genetics Laboratories at Baylor College of Medicine including library construction, exome capture by VCRome version 2.1 (target size 35.45 Mb) and 2 × 100-bp paired-end sequencing on an Illumina HiSeq instrument as previously described (Yang et al. 2013; Parsons et al. 2016). The tumor/germline matched pair was sequenced on a single lane of a HiSeq 2500 with mean coverage of 197× (blood) and 223× (tumor) and a target base coverage of 20× at 98% (blood and tumor). Germline and tumor WES reports were completed in 14 and 20 wk, respectively. The reported *FGFR1*, *PIK3CA*, and *PTPN11* variants were confirmed by Sanger sequencing.

ADDITIONAL INFORMATION

Data Deposition and Access

Tumor and germline WES data will be deposited to the National Center for Biotechnology Information (NCBI) Database of Genotypes and Phenotypes (dbGaP; http://www.ncbi.nlm.nih.gov/gap) with the remainder of the BASIC^3 patient cohort data upon study completion, under accession number phs001026.v1.p1. The tumor and germline variants have been submitted into ClinVar (Landrum et al. 2014) (http://www.ncbi.nlm.nih.gov/clinvar/) under accession numbers SCV000292258–SCV000292262.

Ethics Statement

The BASIC^3 study protocol (H-30755) was approved by the Baylor College of Medicine IRB, which is also the IRB for Texas Children's Hospital, the study's clinical site. Written informed consent for study enrollment was obtained by a trained study project manager or genetic counselor as previously described (Scollon et al. 2014).

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Author Contributions

Patient clinical care was provided by F.Y.L., A.J., S.B., and A.M.A. Study design, participant recruitment, consent, and support were provided by F.Y.L., K.B., A.B., S.S., R.R.-M., M.M.C., A.R., S.E.P., and D.W.P. Sequence data analysis and interpretation were performed by R.P., A.R., S.E.P., and D.W.P., F.Y.L., K.B., A.R., S.E.P., D.W.P. wrote the initial draft of the manuscript. All authors contributed to reviewing and editing the final draft.

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Competing Interest Statement

The authors have declared no competing interest.

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