



# Germline *BRCA2* mutations detected in pediatric sequencing studies impact parents' evaluation and care

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**Abstract** There has been no indication to test for *BRCA1/2* in children (with the rare exception of Fanconi anemia) as screening begins in adult years and there is a potential to induce anxiety related to adult-onset cancers. However, in the setting of pediatric cancer, with increasing utility and frequency of companion tumor-normal sequencing without regard for phenotype and with *BRCA1/2* included in tumor profiling panels, germline mutations in *BRCA1/2* and other DNA damage repair genes have been found. When mutations in these genes are revealed, there are implications for immediate family members. Here we present two children in whom *BRCA2* mutations identified through tumor sequencing prompted parental genetic testing and medical action. These cases illustrate the potential importance of including a matched normal DNA sample when performing tumor profiling of pediatric cancer patients to ensure optimal care.

## CASE PRESENTATION

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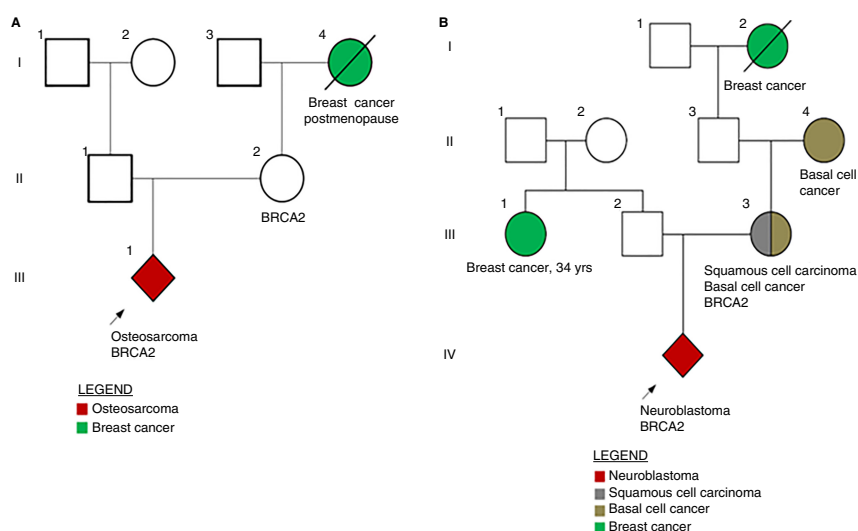
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### Patient 1

An 11-yr-old boy with localized osteosarcoma in the left proximal humerus presented with intermittent pain in the affected arm and underwent a definitive resection of a biopsy-proven osteosarcoma. Mutation panel sequencing of the tumor was performed by Foundation Medicine that revealed a *BRCA2* Q2354\* (Frampton et al. 2013). Given the potential implications of a germline *BRCA2* mutation for family members, parental blood samples were drawn, revealing the patient's 40-yr-old mother was found to carry the *BRCA2* mutation. This finding led to her decision for a risk-reductive mastectomy and bilateral salpingo oophorectomy. Although the patient's grandmother had a history of breast cancer, her age of onset did not raise suspicion for a predisposition to cancer or indicate germline testing at the time of her diagnosis (Fig. 1A).

### Patient 2

A 2 yr-old boy with global developmental delay presented with a 3- to 4-wk history of respiratory symptoms. Chest computed tomography (CT) revealed a 9.3 cm × 8.7 cm mediastinal mass. An iodine-123-meta-iodobenzylguanidine (MIBG) scan showed uptake compatible with neuroblastoma that was histologically confirmed.



**Figure 1.** Children identified harboring *BRCA2* mutations prompting maternal testing and care. (A) Patient 1. (B) Patient 2.

Foundation Medicine tumor sequencing revealed a *BRCA2* p.Y2215fs\* mutation. In-house paired tumor-normal sequencing, MSK-IMPACT was also performed utilizing a control germline blood sample for tumor variant calling. Of note, the practice at Memorial Sloan Kettering Cancer Center (MSKCC) is to not report germline mutations detected by MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) unless specific consent to return germline results has been provided. In this instance, MSK-IMPACT testing did not report the *BRCA2* p. Y2215fs mutation; prompting the suspicion that the *BRCA2* mutation reported by Foundation Medicine was constitutional. The boy's family history was notable for his mother having been diagnosed with squamous cell carcinoma at age 34 yr, his maternal grandmother diagnosed with basal cell carcinoma at age 40 yr, his maternal great grandmother diagnosed with breast cancer in her 50s, and a paternal aunt diagnosed with breast cancer at age 34 yr (Fig. 1B). Parental testing revealed the child's mother harbored the medically actionable *BRCA2* p.Y2215fs\* mutation, which subsequently led her to undergo magnetic resonance imaging (MRI) and mammography breast screening and to seek consultation with breast and gynecologic specialists.

## TECHNICAL ANALYSIS

In Patient 1, the *BRCA2* p.Q2354\* germline mutation identified through tumor testing (performed by Foundation Medicine) and, in Patient 2, the *BRCA2* p. Y2215fs\* germline mutation identified by tumor testing (performed by Foundation Medicine and MSKCC IMPACT testing) were confirmed by targeted site-specific testing at the commercial laboratory GeneDx (Frampton et al. 2013; Cheng et al. 2015; Susswein et al. 2016).

## VARIANT INTERPRETATION

The Q2354\* variant in *BRCA2* is entered five times in ClinVar and annotated as pathogenic (Landrum et al. 2016). The variant is linked with Database for Short Genetic Variations (dbSNP): 80358936 and the National Center for Biotechnology Information (NCBI) 1000

Genomes browser rs80358936. This specific truncation mutation is not annotated by the Evidenced-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA), the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA), or the Breast Cancer Information Core (BIC) (Genomes Project et al. 2010; Spurdle et al. 2012). ENIGMA describes the variant as predicted to encode a truncated nonfunctional protein. Moreover, the mutation is reported in the literature as occurring in a Spanish patient with breast cancer, and the commercial laboratory InVitaE has asserted this variant as pathogenic (Duran et al. 2003; Hirsch et al. 2004).

The p.Y2215fs\* variant in *BRCA2* is predicted to encode a truncated nonfunctional protein and termination mutations at this codon (Y2215\*) are annotated three times as pathogenic in ClinVar (Landrum et al. 2016). Termination variants at this codon are linked with dbSNP: 80358892, and the NCBI 1000 Genomes browser rs80358892 and the BIC have asserted termination variants at this location as pathogenic (Genomes Project et al. 2010). However, this specific truncation mutation is not annotated in the CIMBA (Spurdle et al. 2012; Rebbeck et al. 2016).

## SUMMARY

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Here we present two childhood cancer patients, for whom the results of tumor sequencing prompted parental testing for germline *BRCA2* mutations, leading to genetic counseling and medical action in both mothers harboring the germline *BRCA2* variants.

*BRCA1* and *BRCA2* are proven autosomal dominant breast, ovarian, pancreatic, and prostate cancer predisposition genes (Neuhausen et al. 1996; Kauff et al. 2001; Pritchard et al. 2016). Pediatric phenotypes are known to result when a child inherits biallelic mutations in *BRCA2*, and rarely in *BRCA1* (i.e., the recessive disorder Fanconi anemia) (D'Andrea and Grompe 1997, 2003; Howlett et al. 2002; Sawyer et al. 2015).

In pediatrics, the only indication to test for *BRCA1/2* has been in the context of Fanconi anemia given the potential to induce anxiety related to adult-onset cancers (Green et al. 2013; Sawyer et al. 2015). Recently, with increasing companion tumor-normal sequencing performed, without regard for phenotype and with *BRCA1/2* included in tumor profiling panels, germline mutations in these and other DNA damage genes have been detected (Table 1; Dite et al. 2010; Walsh et al. 2014; Mody et al. 2015; Zhang et al. 2015; Parsons et al. 2016; Schrader et al. 2016). Pathogenic cancer predisposition gene mutations were found in 95/1120 (8.5%) pediatric cancer patients participating in the Pediatric Cancer Genome Project (PCGP), as compared to 1.1% of persons in the 1000 Genomes Project and 0.6% of the participants in the autism study (Daly et al.; Downing et al. 2012; Genomes Project et al. 2015). However, given that a true incidence study comparing pediatric cancer patients with healthy controls has yet to occur and a uniform sequencing approach has not been deployed in all pediatric studies to date, it remains to be determined if heterozygous mutations in *BRCA2* detected in pediatric cancer patients are driver or passenger events (Offit et al. 2003; Zhang et al. 2015). Notably, the second most commonly mutated gene in the PCGP was *BRCA2* ( $n = 6$ ), and *BRCA2* mutations may be enriched in children cancer survivors compared with controls (Zhang et al. 2015; Wang et al. 2017).

Regardless, when mutations in these genes are revealed, there are implications for immediate family members. Standard of care dictates that testing for *BRCA1/2* mutations commences at age 25 yr in at-risk individuals with known familial mutations or family histories described as per the National Comprehensive Cancer Network (NCCN) guidelines and in some instances younger if this information will impact family planning. This standard is based on the premise that *BRCA1/2* mutations underpin cancers that arise in adulthood and the clinical benefits of this knowledge result from screening and risk-reductive surgeries

**Table 1.** Review of germline BRCA1/2 mutations detected in pediatric sequencing studies

Tumor type	Gene	Chr	Location	HGVS DNA ref	HGVS protein	Variant type	Germline platform	Allele frequency (normal)	Target coverage	Reference
Osteosarcoma	BRCA2	Chr 13	32354913	G	p.Q2354*	Nonsense	Sanger	0.46	500x	This study
Neuroblastoma	BRCA2	Chr 13	32340999	C	p.Y2215fs	Frameshift	Sanger	0.48	500x	This study
Neuroblastoma	BRCA1	Chr 17	43124028	G	p.E23fs	Frameshift	WES	0.43	272x	Parsons et al. 2016
Anaplastic medulloblastoma	BRCA1	Chr 17	43094833	GT	p.V233fs	Frameshift	WES	0.5	272x	Parsons et al. 2016
Ewing sarcoma	BRCA2	Chr 17	32332757	G	p.D427fs	Frameshift	WES	0.6	272x	Parsons et al. 2016
Acute lymphoblastic leukemia	BRCA1	Chr 17	41245200	AT	p.I783fs	Frameshift	WES	0.43	>30x	Zhang et al. 2015
Acute lymphoblastic leukemia	BRCA2	Chr 13	32945092	G	p.W2830_E20splice	Splice	WES	0.51	>30x	Zhang et al. 2015
Acute lymphoblastic leukemia	BRCA2	Chr 13	32968863	C	p.Y3098*	Nonsense	WES	0.51	>30x	Zhang et al. 2015
Medulloblastoma	BRCA2	Chr 13	32929213	C	p.P2408fs	Frameshift	WGS	0.21	>30x	Zhang et al. 2015
Medulloblastoma	BRCA2	Chr 13	32953932	T	p.L3000*	Nonsense	WGS	0.5	>30x	Zhang et al. 2015
Neuroblastoma	BRCA2	Chr 13	32945092	G	p.W2830_E20splice	Splice	WES	0.43	>30x	Zhang et al. 2015
Rhabdomyosarcoma	BRCA2	Chr 13	32911681	G	p.Q1063_S1064fs	Frameshift	WGS	0.37	>30x	Zhang et al. 2015

HGVS, Human Genome Variation Society.

occurring in adulthood (Brooks et al. 2006). However, results from recent tumor-normal sequencing studies in pediatrics give pause and raise questions regarding the benefit of testing siblings of children diagnosed with cancer harboring *BRCA* mutations (Table 1).

In view of recent pediatric sequencing studies detecting children with germline *BRCA1/2* mutations across solid and liquid tumors, and the cases described here, pediatric oncologists should be linked with avenues to adult care for the potential screening, risk-reductive interventions (such as surgery), and family planning options currently available for parents of children carrying *BRCA1/2* mutations. For our patients, detection of previously unidentified heritable mutations prompted medical action and provided potentially lifesaving information.

It is important to note that numerous mutational platforms exist wherein tumors are profiled without corresponding normal DNA samples, rendering it difficult to assign certain mutations as somatic or germline. Moreover, in some institutions, germline mutations are subtracted from reporting if consent has not been obtained and may not be realized. These cases illustrate the importance that a corresponding normal DNA be reported to ensure accurate profiling and optimal care.

## ADDITIONAL INFORMATION

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### Data Deposition and Access

Both variants described in this manuscript were deposited in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>) and were assigned the submission numbers SCV000564127 and SCV000564128 (Landrum et al. 2016).

### Ethics Statement

Patients reported here provided written consent permitting use of molecular and phenotypic description under MSKCC's institutional review board (IRB)-approved studies #06-107 and #12-245.

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### Author Contributions

M.F.W. and P.M. conceived the study. M.F.W., J.K., M.H., and K.O. performed clinical interpretation of the germline variants. All authors reviewed and drafted the manuscript.

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### Competing Interest Statement

The authors have declared no competing interest.

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