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## Loss of heterozygosity does not occur in BRCA1/2 mutant pediatric solid and central nervous system tumors

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### Abstract

Utilization of tumor-only sequencing has expanded in pediatric cancer patients, which can lead to identification of pathogenic variants in genes that may be germline and/or have uncertain relevance to the tumor in question, such as the homologous recombination (HR) pathway genes BRCA1/2. We identified patients with pathogenic BRCA1/2 mutations from somatic tumor sequencing and performed additional germline sequencing to assess for the presence of loss of heterozygosity (LOH). Of seven patients identified, four (57.1%) mutations were found in the germline and none had associated LOH. Our data suggests that BRCA1/2 mutations identified in this context are likely incidental findings.

### Keywords

pediatric oncology; BRCA; pediatric oncology; cancer; genomics; homologous recombination

### 1. Introduction

Somatic tumor sequencing has led to a vast increase in our understanding of pediatric cancer pathogenesis, and in many cases offers clinically significant information related to diagnosis, prognosis and treatment selection.<sup>1-4</sup> While pediatric cancers compared to adult cancers have lower mutational rates and harbor less frequent targetable kinase alterations (such as EGFR or HER2 in adult lung or breast cancer, respectively), they tend to be enriched in

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targetable gene fusions such as NTRK (infantile fibrosarcoma, other solid and CNS tumors) and BRAF (low grade glioma).<sup>5-7</sup> Additionally, paired sequencing of germline can reveal information with wide-ranging clinical implications as pediatric cancer patients have been found to have a high rate of mutations in cancer susceptibility genes which can inform treatment decisions for the patient and guide surveillance recommendations for the patient and their family.<sup>8,9</sup>

Homologous recombination (HR) is a high-fidelity repair mechanism that is critical in repairing DNA double-stranding breaks.<sup>10</sup> Germline genomic alterations of HR pathway components, mainly BRCA1/2, have been associated with predisposition to adult-onset breast and ovarian cancers which develop when the other BRCA1/2 allele is lost in the tumor (second hit).<sup>11,12</sup> The resulting tumors are BRCA1/2 deficient which leads to sensitivity to PARP inhibitors through a “synthetic lethality” interaction, which has been exploited with encouraging results in BRCA1/2 mutated breast, ovarian, and prostate cancer patients.<sup>13-15</sup> Biallelic inactivation of BRCA1/2 is associated with abnormal DNA repair which leads to a mutational signature known as “signature 3” or “BRCAness”.<sup>16</sup> Germline pathogenic BRCA1/2 mutations have been reported in several pediatric sequencing studies, with rates variably reported higher than the baseline population and some reports suggest a higher than expected incidence of pediatric cancers in families with germline BRCA1/2 mutations.<sup>8,9,17,18</sup> However, no studies have systematically evaluated whether pediatric cancers with germline or somatic BRCA1/2 mutations have loss of heterozygosity or signature 3 characteristics. This is an important unanswered question as it would be informative with regards to tumor pathogenesis and activity of PARP inhibitors.

## 2. Methods

### 2.1. Patients and Samples

This study was approved by the Dana-Farber Cancer Institute (DFCI; Boston, MA) Institutional Review Board. Patients were identified for inclusion in the study by querying a database of 278 pediatric patients with solid and CNS malignancies who had samples subjected to tumor only sequencing with a targeted next-generation sequencing assay, OncoPanel, as a participant in the PROFILE Cancer Research Study.<sup>19</sup> Patients were included in this study if sufficient tumor DNA or material was available for re-sequencing and if germline DNA was available.

### 2.2. Next Generation Sequencing

Molecular profiling of tumor and germline DNA was achieved via massively parallel sequencing with the OncoPanel V3 platform as previously described.<sup>20</sup> Briefly, samples were sequenced on an Illumina HiSeq 2500 with 2x100 paired end reads. Sequence reads were aligned to reference sequence b37 edition from the Human Genome Reference Consortium using bwa, duplicate reads were removed using Picard (version 1.90, <http://broadinstitute.github.io/picard/>) and indel sites were locally realigned using Genome Analysis Toolkit (GATK, version 1.6-5-g557da77).<sup>21</sup> Single nucleotide variants were called using MuTect v1.1.4, insertions and deletions were called using GATK Indelocator, and variants that were also present in the matched germline were removed.<sup>22</sup> MMR deficiency

using OncoPanel data was determined using the clinically validated threshold of at least 2.5 single base pair insertions or deletion mutations per megabase sequenced, occurring in mononucleotide repeat regions of four or more nucleotides.<sup>23</sup> Loss of heterozygosity (LOH) and copy-neutral LOH assessment were determined by analyzing genome-wide copy number calls and allelic frequencies using FACETS (Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing).<sup>24</sup> Mutational signature analysis was performed using SigMA (Signature Multivariate Analysis).<sup>25</sup> BRCA1/2 mutations were evaluated for pathogenicity using the ClinVar database and in one case of a previously unreported variant using functional prediction models PolyPhen and SIFT and comparison to other reported pathogenic variants.<sup>26–28</sup> Only patients with BRCA1/2 pathogenic or likely pathogenic mutations were included.

### 3. Results

#### 3.1. Study Population and Clinical Characteristics

From the PROFILE database (n=278 at the time of initial query), 7 patients (2.5%) were identified with a likely pathogenic or pathogenic mutation in either BRCA1 or BRCA2. All patients had sufficient tumor tissue and available germline DNA to undergo targeted somatic and germline sequencing. Patients with BRCA1/2 pathogenic mutations had glioblastoma (1), low-grade glioma (1), ganglioglioma (1), Wilm's tumor (2) and hepatocellular carcinoma (2). Clinical characteristics of the seven patients in the study cohort are summarized in Supplementary Table 1.

#### 3.2. Identified pathogenic BRCA1/2 variants

Of the 7 patients with BRCA1/2 mutations there were 4 patients with germline mutations for a rate of germline BRCA1/2 mutations of 1.4% in the 278 patient study population (Table 1). One patient had a BRCA1 germline mutation (0.4%) and three had a germline BRCA2 mutation (1.1%). There were three somatic mutations (rate of 1.1% in the study population); one in BRCA1 and two in BRCA2. All pathogenic BRCA1/2 mutations except for the somatic BRCA1 mutation were nonsense or frameshift mutations. Of note, one patient with a high-grade glioma (referred to as P1 hereafter) had a high tumor mutational burden (TMB, 302 mutations per megabase) with a mutational signature that was indicative of underlying mismatch repair (MMR) deficiency. No other patients were found to be MMR deficient. Analysis of P1's germline sequencing results uncovered a homozygous PMS2 missense mutation (c.133A>G, p.N45D). This amino acid change occurs at a highly conserved residue, and is predicted to affect protein function using in-silico modeling (PolyPhen-2).<sup>26</sup> It has not been identified in cancer databases such as PeCan, COSMIC, or cBioPortal; however, other variants involving this codon (p.N45T and p.N45S) have been reported as either likely pathogenic or variants of unknown significance.<sup>29</sup>

#### 3.3 Determination of LOH and Mutational Signature Analysis

Gene-level copy number analysis of somatic samples did not reveal LOH associated with any of the identified pathogenic alterations. Using both the tumor and matched normal sequencing data, we applied an allele-specific copy number analysis pipeline to identify potential copy-neutral LOH, which was not seen in any cases (example shown

in Supplementary Figure 1). To detect signature 3 (so called “BRCAness”) we used a computational method termed Signature Multivariate Analysis (SigMA). This validated technique uses a likelihood-based multivariate approach and machine learning that enables signature calling from low mutation counts, broadening its’ application to targeted gene panels which are much more commonly utilized in clinical practice than whole-exome or genome sequencing. The algorithm was unable to reliably call signatures in our samples, in large part due to the small number of exonic mutations in most samples (Supplementary Table 2). This finding was not unexpected given the known lower mutational burden of pediatric compared to adult cancers.<sup>30</sup>

#### 4. Discussion

Somatic pathogenic mutations in BRCA1/2 are identified rarely in pediatric cancers, with an estimated incidence of 0.66% (BRCA1) and 1.1% (BRCA2) from the St. Jude’s PeCAN data portal (data accumulated from PCGP, TARGET, and DKFZ sequencing studies with total n = 3047).<sup>31</sup> Several studies have investigated the specific incidence of germline mutations of BRCA1/2 in this population, and in some cases identified an incidence higher than the general population which is estimated at 0.16% and 0.25%, for BRCA1 and 2 respectively in the gnomAD database.<sup>32</sup> Specifically, sequencing studies of pediatric patients with medulloblastoma (MB) and rhabdomyosarcoma (RMS) have identified relatively high rates of germline BRCA2 pathogenic mutations giving an estimated relative risk of >4 for MB, and an odds ratio of 3.6 for RMS.<sup>33,34</sup> Recently, Ewing sarcoma was also found to have higher than expected rates of germline BRCA mutations.<sup>35</sup> A recent meta-analysis of 11 studies totaling 3795 children and adolescents with cancer and germline genomic data suggested that BRCA2 (odds ratio 3.81 (1.97 to 7.10)) but not BRCA1 (odds ratio 1.83 (0.77 to 3.91)) pathogenic variants were associated with statistically significant increased cancer risk when compared to two control populations.<sup>36</sup> These studies suggest a possible link between germline pathogenic variants and pediatric cancer development; however, proof of causality through the “second hit” mechanism observed in breast cancer has not been reported.

Although our study population was small, we identified a similar rate of germline (1.4%) and somatic (0.4%) BRCA1/2 mutations as previous studies. We did not observe LOH in patients with germline BRCA1/2 mutations. Lending further credence to our findings, a recent case-control study investigated the association of high- and moderate-penetrance germline pathogenic variants in associated vs. non-associated pediatric and adult-onset cancers.<sup>37</sup> This study identified 5 patients with BRCA1, BRCA2 or PALB2 pathogenic germline mutations and none had LOH or a second hit in the tumor.

It remains unclear why an increased frequency of BRCA1/2 germline mutations has been identified in pediatric cancer populations. Possible hypotheses include an alternative mechanism of germline BRCA1/2-induced oncogenesis in pediatric patients. Another possibility is the comparison of a pediatric cancer population to an unaffected adult population where some BRCA1/2 carriers are likely to have already presented with an early-onset cancer is not appropriate. Indeed, two published responses to the Kratz et al study disputed their findings by pointing out biases present within both control populations,

and propose that only a true age-matched control population could be used to properly determine risk.<sup>38,39</sup>

There are several limitations to our study. Because a targeted sequencing approach was used, there may be some localized LOH regions that could not be detected at the resolution of SNP sites on the panel. It's also possible that loss of the second allele could have occurred through mechanisms unable to be detected through targeted sequencing such as epigenetic silencing or enhancer hijacking. While we attempted to perform signature 3 analysis using the machine-learning predictor SigMA to address these issues, this did not result in any high-confidence results, likely due to the low mutational burden of most pediatric tumor samples and further limitations of panel sequencing coverage.

A recent paper from the SickKids Cancer Sequencing (KiCS) program suggests further work is needed to evaluate the performance of HR deficiency signature analysis in pediatric cancers.<sup>40</sup> Out of 293 patients with whole-genome somatic sequencing data, 76 (25.9%) were identified as having signature 3, which was enriched in the 25 patients with germline pathogenic/likely-pathogenic variants affecting the HR pathway (12/25, 48%). Similar to our study, LOH of the germline variant was found in a minority of pediatric tumors and did not always correlate with the presence of signature 3. Furthermore, the HRDetect algorithm was used as a parallel method and did not show meaningful correlation with samples the authors had determined to harbor signature 3, which further suggests biological differences between homologous recombination proficiency in pediatric vs. adult cancers.

In summary, BRCA1/2 pathogenic germline mutations, while identified more often in pediatric solid and CNS cancer patients than in unaffected adults, likely do not give rise to cancer through a two-hit mechanism and may not be responsible for tumorigenesis in the younger population. These findings should be validated in larger studies ideally using whole genome and whole transcriptome sequencing data.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviation Table

<b>HR</b>	Homologous Recombination
<b>FFPE</b>	Formalin fixed paraffin-embedded

<b>LOH</b>	Loss of heterozygosity
<b>TMB</b>	Tumor mutational burden
<b>MMR</b>	Mismatch repair
<b>VAF</b>	Variant Allele Frequency
<b>SNV</b>	Single Nucleotide Variant

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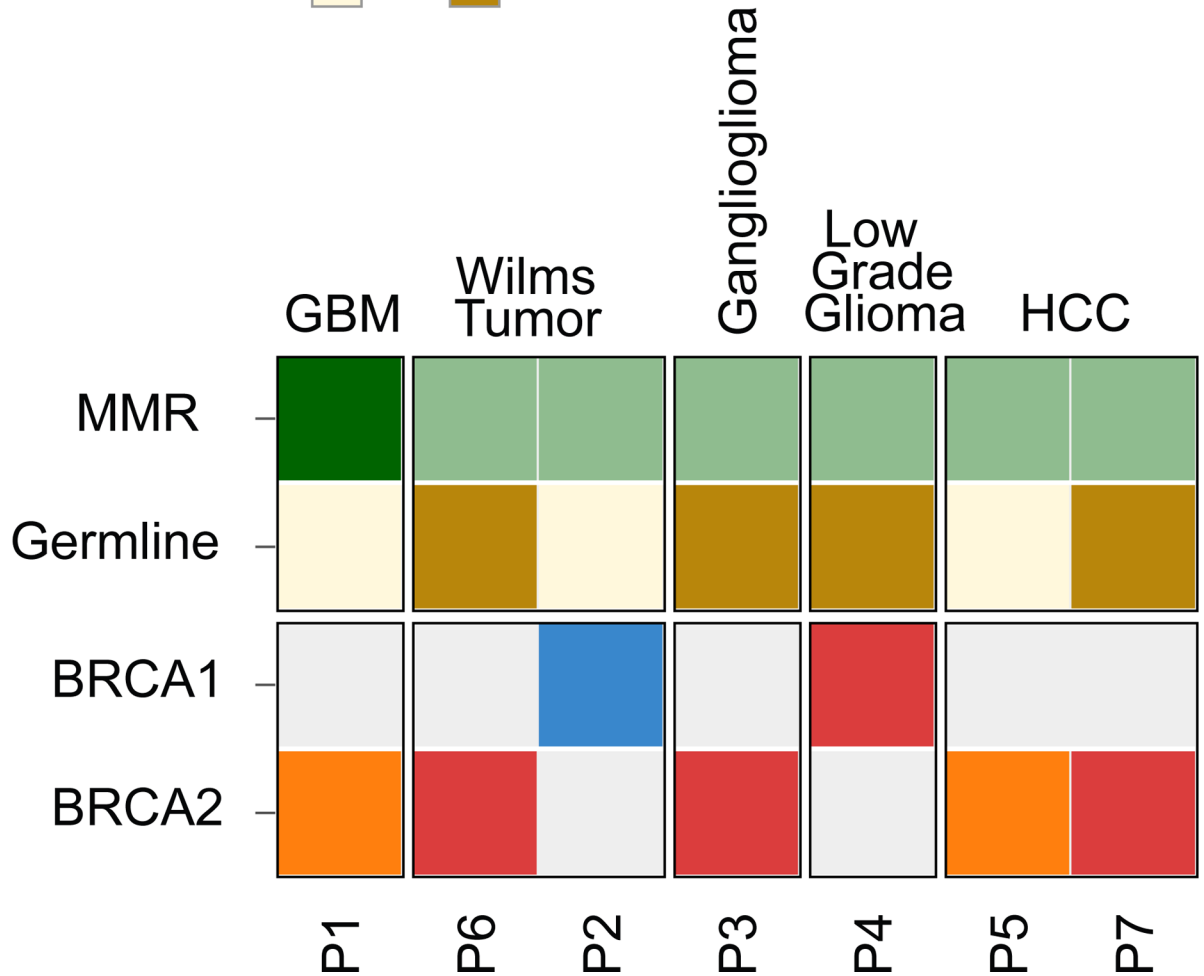
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**Mutation Types** ■ Missense ■ Frameshift ■ Nonsense

**MMR** ■ Deficient ■ Proficient

**Germline** ■ No ■ Yes



**Figure 1:** Heatmap of genomic features of patients with pathogenic BRCA1 and BRCA2 alterations

**TABLE 1:**

Clinical and molecular characteristics of patients with pathogenic BRCA1/2 mutations

Patient #	Age	Sex	Diagnosis	Gene	Variant c.	Variant p.	Germline	VAF (%)	LOH	TMB	MMR	SNV#
P1	13	M	Glioblastoma Multiforme	BRCA2	c.7480C>T	p.R2494*	No	46	No	302	deficient	421
P2	3	M	Wilms Tumor	BRCA1	c.5363G>T	p.G1788V	No	45	No	3	proficient	3
P3	15	M	Ganglioglioma	BRCA2	c.5128-5131delTATG	p.Y1710fs	Yes	39	No	11	proficient	1
P4	1	M	Low Grade Glioma	BRCA1	c.4136-4137delCT	p.S1379fs	Yes	41	No	10	proficient	2
P5	24	F	Hepatocellular Carcinoma	BRCA2	c.2581C>T	p.Q861*	No	23	No	9	proficient	6
P6	4	M	Wilms Tumor	BRCA2	c.5946delT	p.S1982fs	Yes	47	No	7	proficient	3
P7	14	F	Hepatocellular Carcinoma	BRCA2	c.3167-3171delAAAAG	p.K1057Tfs	Yes	36	No	8	proficient	9

\* Nonsense mutation

TMB= tumor mutational burden (per megabase); MMR = mismatch repair; VAF = Variant Allele Frequency; SNV = Single nucleotide variant

LOH = Loss of heterozygosity