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The coexistence of a BRCA2 germline and a DICER1 somatic variant in two first-degree cousins suggests their potential synergic effect

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Cancer predisposition syndromes are recognized in about 10% of pediatric malignancies with several genes specifically involved in a subset of pediatric tumors such as *DICER1*, in pleuropulmonary blastoma, cystic nephroma, and brain sarcomas. By contrast, the role of *BRCA1/2* in pediatric cancer predisposition is still under investigation. We present two cases of young first-degree cousins, both carrying a germline *BRCA2* variant and developing tumors characterized by somatic *DICER1* mutations. Patient 1 presented with a cystic nephroma harboring a somatic *DICER1* variant (p.Asp1810Tyr), while patient 2 had a primary intracranial *DICER1*-mutated sarcoma showing a distinct somatic *DICER1* variant (p.Asp1709Glu) as well as biallelic inactivation of *TP53* (p.Val173Leu, VAF 91%) and *APC* (p.Ile1307Lys, VAF 95%) and a pathogenic variant in *KRAS* (p.Gln61His). Both patients carried the same germline *BRCA2* variant (p.Arg2842Cys) of unknown significance. The same variant was found in the mother of patient 2 and in the father of patient 1, who are siblings. A homologous recombination deficiency signature was not identified in any of the two tumors, possibly suggesting a reduction of BRCA2 activity. The association of *BRCA2* and *DICER1* variants in our cases hints at a potential cooperative role in cancer pathogenesis. Further studies are warranted to elucidate the interplay between *BRCA1/2* and *DICER1* variants and their implications for cancer predisposition and treatment in pediatric patients.

Keywords Cancer predisposition syndrome, Pediatric oncology, *BRCA* genes, *DICER1*, Cystic nephroma, Primary intracranial DICER1-mutated sarcoma

Cancer predisposition syndromes (CPSs) are described in about 10% of overall pediatric cancers[1]. The role of genetic factors influencing the onset and progression of cancer is gradually being identified and understood with increasing precision and translational efficacy. The best-known cancer-predisposition genes (CPGs) that play a role during pediatric age are *ALK, DICER1, ELP1, GATA2, NF1, PAX5, RB1, RET, RUNX1, SDHx, SMARCB1, SUFU, TP53,* and *WT1*[1].

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Among the genes associated with tumor predisposition in children, *DICER1* is one of the most explored. *DICER1* encodes an enzyme involved in the cytoplasmic microRNA (miRNA) processing. miRNAs play crucial roles in post-transcriptional gene regulation, and alterations in their processing can lead to dysregulation of gene expression, which in turn can contribute to tumorigenesis[2,3]. Notably, the Dicer protein is also linked to the DNA damage response and genome integrity maintenance, as it is involved in chromatin de-condensation during nucleotide excision repair[4], in small noncoding RNA accumulation at the level of DNA double-strand breaks (i.e., DNA damage response RNA)[5–8] and in resolution of three-stranded nucleic acid structures (i.e., R-loops) driving transcription-associated genomic instability[9]. DICER1 syndrome is a pediatric cancer predisposition syndrome typically caused by heterozygous germline *DICER1* loss-of-function variants accompanied by somatic second hit variants on the other allele within the sequence encoding the RNase IIIb domain. Individuals affected by DICER1 syndrome are predisposed to benign and malignant neoplasms in multiple organ systems, such as pleuropulmonary blastoma, cystic nephroma, and Sertoli–Leydig cell tumor of the ovary. Additionally, *DICER1* mutations have been reported in other pediatric cancers, including Wilms tumor, embryonal rhabdomyosarcoma[10], and certain rare types of brain tumors[11,12]. Nevertheless, sporadic tumors in which two somatic *DICER1* variants are identified have been described in the absence of an identified germline alteration[2].

By contrast, *BRCA1* and *BRCA2* are established autosomal dominant predisposition genes for breast, ovarian, pancreatic, and prostate cancers in adults[13,14], while their role in childhood cancer is poorly understood.

BRCA1 and *BRCA2* are involved in DNA damage response, specifically in the homologous recombination repair pathway. Cells with pathogenic variants in *BRCA* genes are error-prone at the DNA level and accumulate mutations leading to genome instability, showing a typical homologous recombination deficiency (HRD) signature. Tumors arising in this context show a “BRCAness phenotype”[15] and are likely to respond to Poly (ADP-ribose) polymerase inhibitors (PARPis)[16].

In the pediatric population, biallelic mutations in *BRCA2* can lead to clinical manifestations, whereas alterations in *BRCA1* are rare, typically presenting as the recessive disorder Fanconi anemia[17–19]. Children are not routinely tested for *BRCA1* and *BRCA2* variants unless Fanconi anemia is suspected. However, the increasing use of high-throughput technologies in clinical laboratories and the agnostic integration of blood and tumor sequencing has recently brought to light the role of germline mutations in *BRCA* genes and other DNA damage repair genes in several other types of pediatric cancers (osteosarcoma, Ewing sarcoma, neuroblastoma, medulloblastoma, acute lymphoblastic leukemia)[20].

Here, we report on two young first-degree cousins carrying a germline *BRCA2* variant and developing different solid tumor types, i.e., cystic nephroma and primary intracranial sarcoma, both characterized by somatic inactivation of *DICER1*. This report enables a better understanding of the biological bases of these pediatric tumors, allowing for more effective management of these oncological conditions and opening new opportunities for the development of targeted therapies and preventive strategies.

Results

A 14-month-old girl (patient 1) was admitted to our hospital with a palpable abdominal mass on the right side without other relevant symptoms. No anomalies were detected during pregnancy. An abdominal ultrasound revealed a large left renal mass, causing a compression of the excretory tract and distension of the pelvic cavities (Fig. 1). Tumor marker tests were performed and showed elevated renin values (185 µIU/mL with normal values of 2.8–39.9); other markers, such as alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), chromogranin A (CgA) and carcinoembryonic antigen (CEA) were negative. Chemotherapy was started according to the SIOP 2001 protocol; then, the patient underwent a left nephrectomy. On histological examination, the tumor had the characteristics of a cystic nephroma (Fig. 2). Sanger sequencing analysis showed the presence of a somatic pathogenic variant c.5428G > T (p.Asp1810Tyr) in the RNase IIIb domain of *DICER1* gene (NM_177438.3). Currently, the patient is in follow-up and is in complete remission of the disease at 24 months from diagnosis.

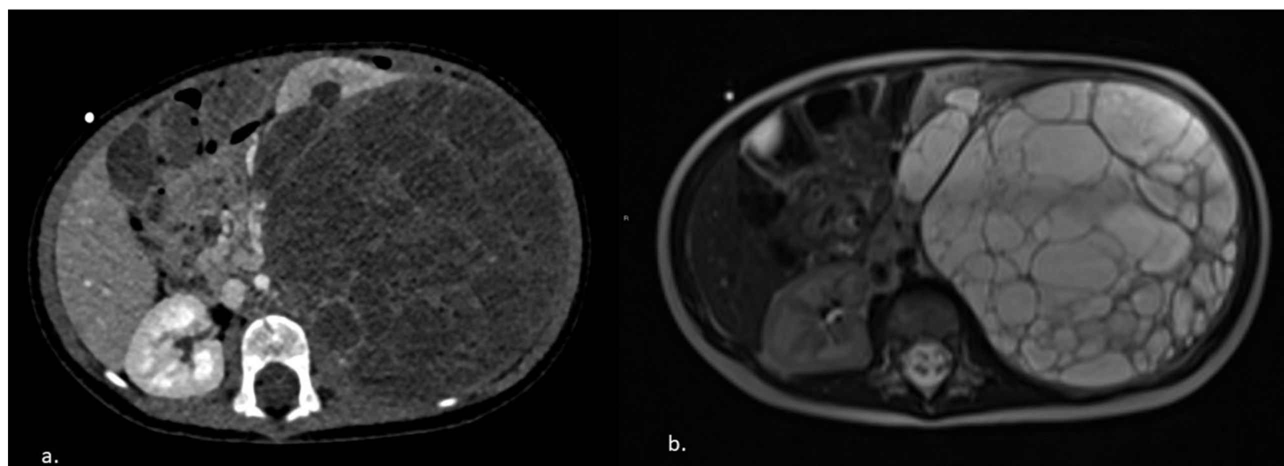

Fig. 1. Axial contrast-enhanced CT (**a**) and axial T2WI MRI (**b**) show a large cystic mass arising from the left kidney with multiple septations.

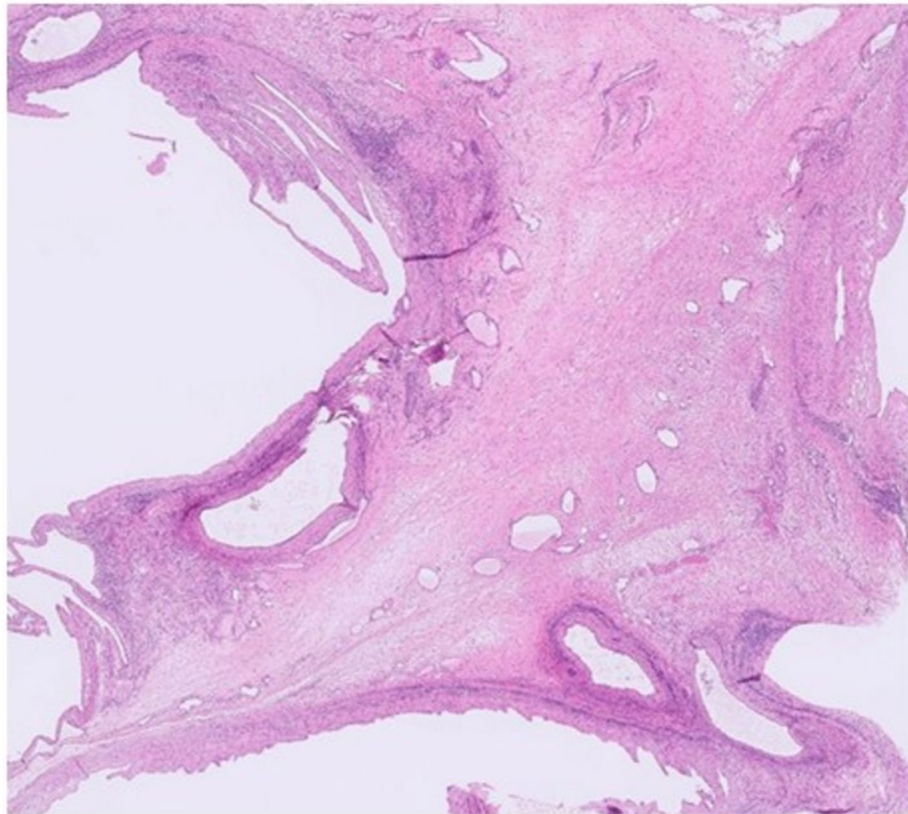

Fig. 2. At histological examination, the lesion of patient 1 consisted of multiple cysts lined by a cubic epithelium and separated by fibrous septa containing occasional abortive tubules.

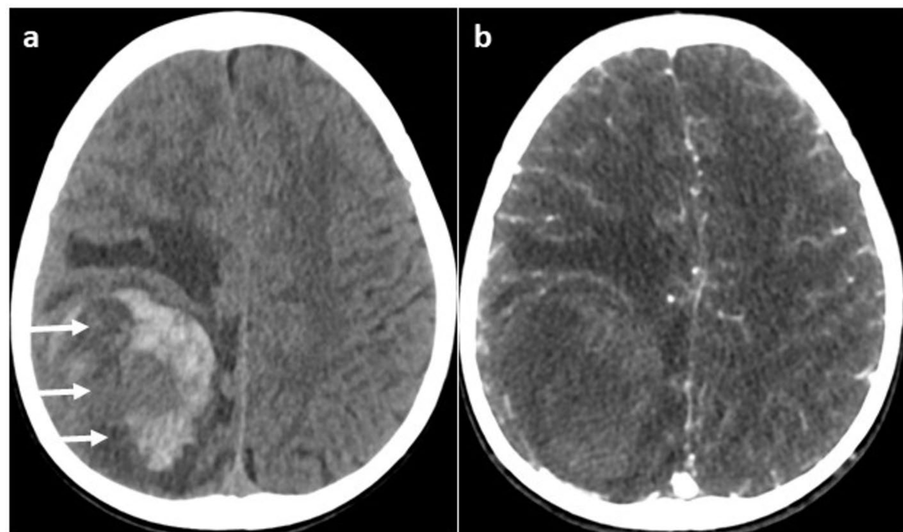

Fig. 3. Noncontrast (**a**) and contrast-enhanced (**b**) axial CT images show a large right fronto-parietal intracerebral hemorrhagic (ICH) lesion with cerebral herniation. No areas of contrast-enhancement or signs of vascular malformation are detected. The regions of hypo- and iso-attenuation (arrows) within the hyperattenuated ICH are suggestive of hyperacute/active hemorrhage, and the consensual presence of substantial surrounding edema raises suspicion of an underlying tumor.

Six months later, the first-degree cousin of patient 1, a 4-year-old girl (patient 2), was admitted to our hospital for a history of headache and vomiting associated with lethargy. Computed tomography (CT) showed a right frontoparietal lesion with signs of intra-tumoral hemorrhage (Fig. 3). For the progressive deterioration of clinical conditions and neurological status, the patient underwent urgent neurosurgical resection of the tumor. Histological examination revealed a malignant spindle cell sarcoma with focal nuclear pleomorphism and rare eosinophilic globules (Fig. 4A,B). Immunophenotypic features showed overexpression of p53 and loss of ATRX, mosaics from loss of H3K27me3 and focal expression of SALL4 and PRAME (Fig. 4C–G). The findings overall suggested a primary intracranial *DICER1*-mutated sarcoma, which was further confirmed by DNA methylation profiling and the identification of a somatic pathogenic variant c.5127T > A (p.Asp1709Glu) in the RNase IIIb domain of *DICER1* gene (NM_177438.3). The patient underwent a second neurosurgery, confirming gross total resection of the tumor. The cerebrospinal fluid (CSF) was negative for neoplastic cells, and postoperative imaging showed no metastatic lesions or extracranial localization. The patient was started on chemotherapy with a 4-cycle regimen of ifosfamide, carboplatin, etoposide (ICE)[21]. Subsequently, she underwent two cycles of high-dose chemotherapy with Thiotepa followed by autologous stem cell reinfusion and focal proton beam therapy (PBT). She is currently in complete remission at 18 months from the diagnosis.

Germline next-generation sequencing (NGS) analysis excluded the presence of variants in the *DICER1* gene and showed that both patients carried a heterozygous missense variant c.8524C > T (p.Arg2842Cys) in the *BRCA2* gene. The same variant was found in the mother of patient 2 and in the father of patient 1, who are siblings (Fig. 5). The variant is classified as a variant of uncertain significance (VUS) according to the American College of Medical Genetics and Genomics (ACMG) and functional assay demonstrates that this variant only partially complemented HR efficiency compared with wild-type *BRCA2*[22]. In patient 1, a heterozygous splicing variant c.250 + 1G > T (p.?) in the *FANCC* gene (NM_ 000136.3) with maternal segregation was also identified. This variant is considered as likely pathogenic and has never been reported in literature. In patient 2, an additional heterozygous missense variant c.3920T > A (p.Ile1307Lys) in the *APC* gene (NM_000038.6) with maternal segregation was recognized. This alteration is considered a risk allele for colorectal cancer and is not known to be

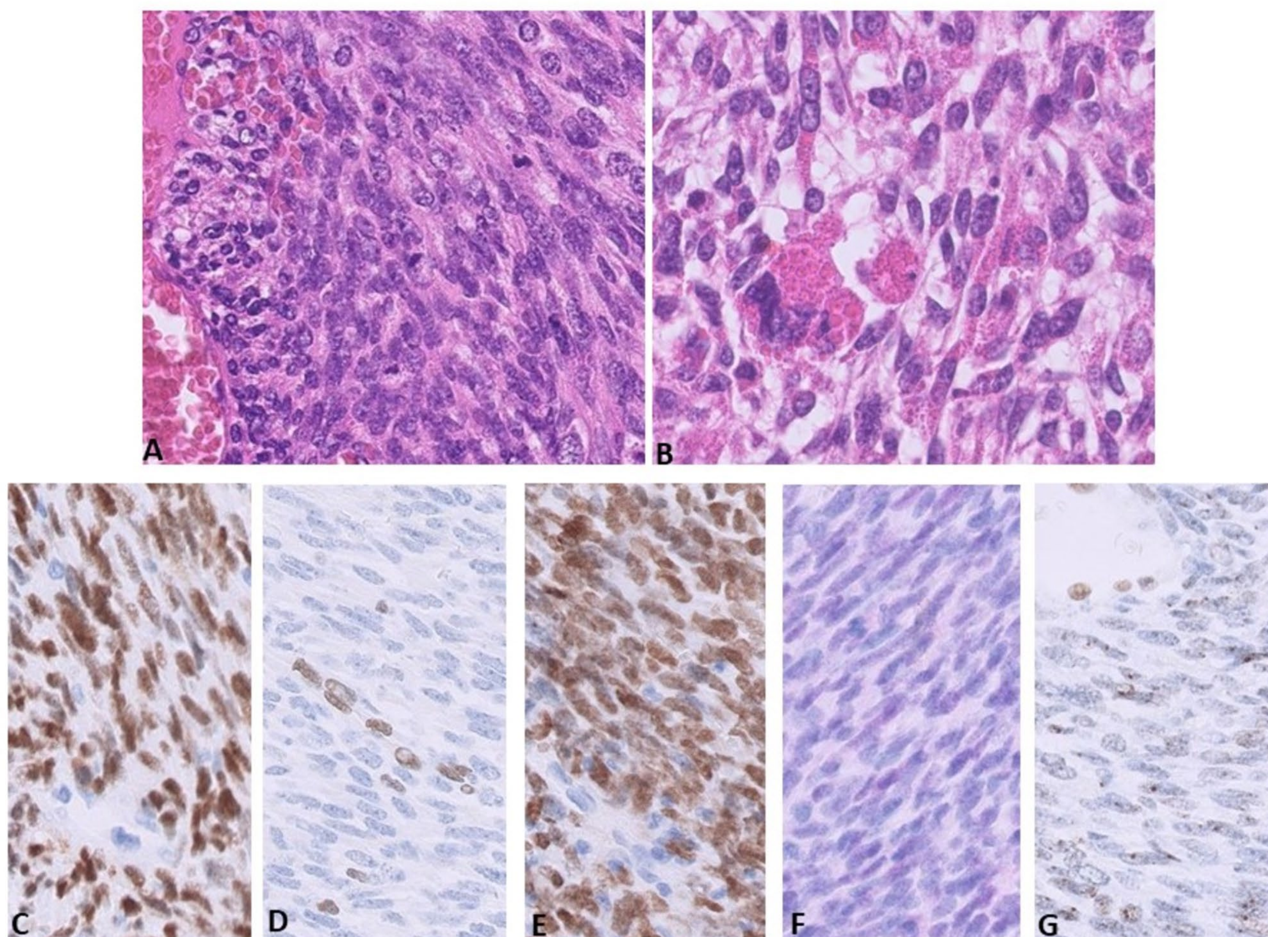

Fig. 4. At histological examination, the lesion of patient 2 consisted of fascicles of spindle cells with hyperchromatic nuclei, brisk mitoses (**A**), focal nuclear pleomorphism and rare eosinophilic globules (**B**). The tumor showed overexpression of p53 (**C**), loss of ATRX (**D**), overexpression of SALL4 (**E**) and PRAME (**F**) and mosaiciform loss of H3K27me3 (**G**).

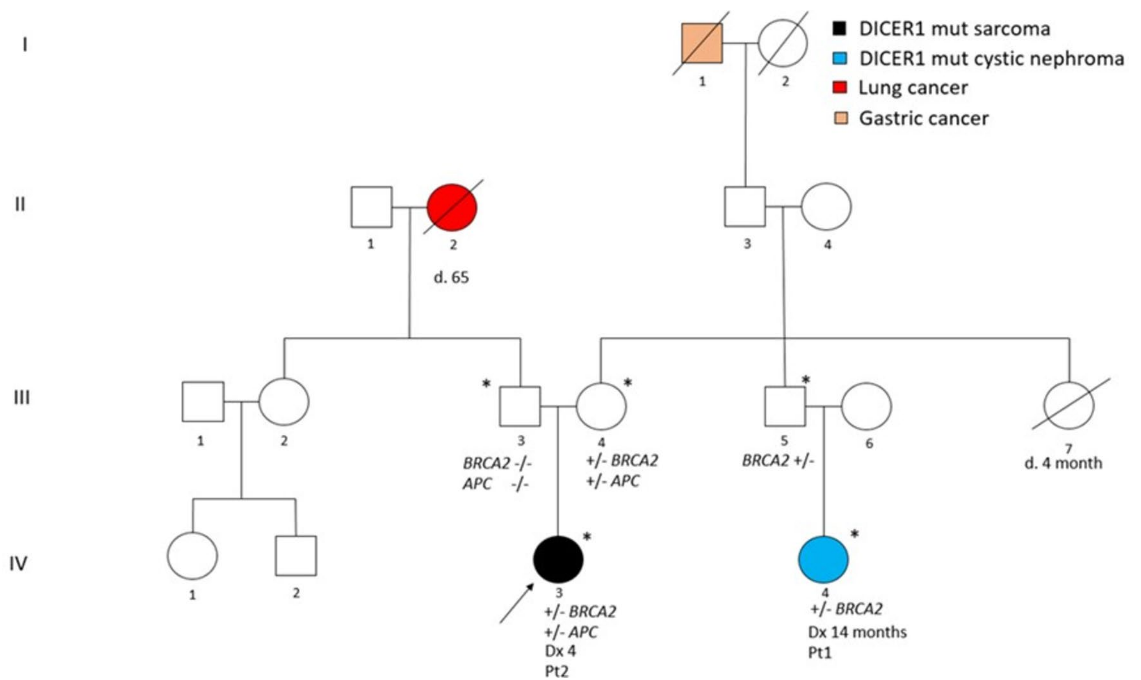

Fig. 5. Pedigree of the family. An asterisk indicates the individuals who were tested by DNA sequencing. Detection of the variants c.8524C > T in the *BRCA2* gene and c.3920T > A in the *APC* gene by genetic tests is indicated by the plus sign.

associated with polyposis[23]. Family history reported only one great-grandfather of both patients (not analyzed) with gastric cancer and a maternal grandmother of patient 2 with lung cancer (Fig. 5).

Given the insights from the germline data, somatic NGS analysis was performed through the OncoDeep kit on DNA extracted from formalin-fixed paraffin-embedded tumor tissues. Both samples showed low tumor mutational burden and stable MSI status. The analysis confirmed the PCR results on *DICER1* variants, unveiling a variant allele frequency (VAF) of 24% and 46% in patient 1 and 2 tumors, respectively. The germline heterozygous *BRCA2* variant c.8524C > T (p.Arg2842Cys) was also evident in both tumors. However, an HRD signature was not identified in any of the samples. Of note, the primary intracranial sarcoma (patient 2 tumor) harbored additional missense pathogenic/likely pathogenic variants: c.517G > C (p.Val173Leu) in *TP53* (NM_000546.6) with a VAF of 91%, c.3920T > A (p.Ile1307Lys) in *APC* (NM_000038.6) with a VAF of 95% and c.183A > C (p.Gln61His) in *KRAS* (NM_033360.4) with a VAF of 69% as well as multiple loss of heterozygosity involving other DNA repair genes (i.e. *APC, TP53, CHEK2, RAD21, FANCF*). Germline and somatic data are summarized in Table 1.

Based on the genetic testing results, both patients will undergo surveillance for breast and ovarian cancer as for clinical practice guidelines published[24]. Moreover, for patient 2 with a likely pathogenic variant in *APC*, the recommendations for *APC* conditions reported in the literature will be followed[25]. Such recommendations will also be applied to family members in whom these variants have been found.

Discussion

In the era of precision medicine, genetic profiling has become essential in pediatric oncology[26]. The identification of genetic alterations in childhood cancers allows both better prognostic definition and the development of targeted and personalized therapeutic strategies[27]. Mutations of *BRCA1* and *BRCA2* were identified in 1993 and 1994 and are associated with an increased risk of developing a wide spectrum of tumors during adulthood, typically breast and ovarian cancer, but also several other histotypes, such as pancreatic and prostate cancer and melanoma[28]. A role of *BRCA2* in childhood cancer predisposition has more recently emerged, and, remarkably, *BRCA2* has proved to be the second most mutated gene after *TP53* in the Pediatric Cancer Genome Project (PCGP)[29,30]. The spectrum of cancers observed in children carrying germline *BRCA2* mutations described to date includes leukemia, medulloblastoma, neuroblastoma, osteosarcoma, and Ewing sarcoma[20].

Here, we report on two young first-degree cousins, both developing a solid tumor with a distinct somatic mutation of *DICER1*. Patient 1 was affected by a cystic nephroma, a tumor typically considered pathognomonic of DICER1 syndrome[31]. Patient 2 presented with a primary intracranial *DICER1*-mutated sarcoma, which may be sporadic or occur in the context of DICER1 syndrome, with an isolated case described in an individual affected by neurofibromatosis type 1[32]. We were able to document a *DICER1* pathogenic mutation affecting the RNase IIIb domain in each tumor, namely p.Asp1810Tyr (patient 1 tumor) and p.Asp1709Glu (patient 2 tumor); the VAF of these variants (24% and 46% respectively) did not indicate a biallelic inactivation of *DICER1* in the tumors suggesting that Dicer1 activity was not completely abrogated. Intriguingly, the patients did not carry a *DICER1* germline mutation but shared the p.Arg2842Cys VUS in *BRCA2*. This variant is reported in a homozygous condition in primary ovarian insufficiency and was demonstrated to exhibit a reduced DSB-induced HR efficiency[22].

	Age at diagnosis	Tumor biomarkers			Gene	Cat	cDNA variant	Amino acid variant	Biological impact	Germline variants		Somatic variants	
		TMB score	MSI score	HRD score						VAF	Inheritance	VAF	CNA
Patient 1	14 months-old	Low (1.7)	Stable (4%)	Negative (23)	*BRCA2*	SNV	NM_000059.4:c.8524C > T	p.Arg2842Cys	LP	50%	Paternal	51%	–
					DICER1	SNV	NM_177438.3:c.5428G > T	p.Asp1810Tyr	LP	–		24%	–
					FANCC	SNV	NM_000136.3:c.250 + 1G > T	–	LP	50%	Maternal	47%	–
					BRCA2	SNV	NM_000059.4:c.8524C > T	p.Arg2842Cys	LP	50%	Maternal	46%	–
					DICER1	SNV	NM_177438.3:c.5127T > A	p.Asp1709Glu	LP	–		46%	–
					KRAS	SNV	NM_033360.4:c.183A > C	p.Gln61His	P	–		69%	–
					TP53	LOH	-	–	LP	–		–	1
Patient 2	4 years-old	Low (1.7)	Stable (3.7%)	Negative (18)	*TP53*	SNV	NM_000546.6:c.517G > C	p.Val173Leu	LP	–		91%	–
					APC	SNV	NM_000038.6:c.3920T > A	p.Ile1307Lys	LP	50%	Maternal	95%	–
					CHEK2	LOH	-	–	LP	–		–	1
					RAD21	LOH	-	–	LP	–		–	1
					FANCF	LOH	-	–	LP	–		–	1

Table 1. Molecular results from germline and somatic analysis. *TMB* Tumor mutational burden, *MSI* Microsatellite instability, *HRD* Homologous recombination deficiency. *SNV* Single nucleotide variant, *LOH* Loss of heterozygosity, *LP* Likely pathogenic variant, *VUS* Variant of uncertain significance, *P* Pathogenic variant, *VAF* Variant allele frequency, *CAN* Copy number alteration.

To shed light on the implication of the *BRCA2* p.Arg2842Cys variant in our cases, we sought to investigate the presence of the HRD signature in both tumors. However, we failed to demonstrate a BRCAness signature. It has to be highlighted that currently, no clinically validated diagnostic tests exist for determining BRCAness in pediatric cancer. The threshold we used in the analysis was specifically designated for this signature in adult ovarian and breast cancer and may not be accurate for the evaluation of other tumor entities occurring in children[33]. Hence, the possibility of a reduction of BRCA2 activity could not be completely excluded. On the other hand, both patients carried germline likely pathogenic variants on genes that are involved in cancer predisposition syndromes. Patient 1 harbored the splice variant c.250 + 1G > T (p.?) on the *FANCC* gene. The FANCC protein is present in the Fanconi anemia pathway and is involved in the DNA repair pathway, especially for DNA interstrand crosslink (ICL) repair[34,35], both in metabolic disorders and protection against oxidative stress-induced apoptosis[36,37]. Germline *FANCC* mutations are linked to familial breast cancer and rarely have been associated with renal tumor, and are not currently linked to any hereditary renal cancer predisposition syndromes[38]. Patient 2 also carried a likely pathogenic heterozygous *APC* missense variant, i.e., c.3920T > A (p.Ile1307Lys), associated with an increased risk of developing colon cancer. Germline pathogenic variants of *APC* cause familial adenomatous polyposis (FAP), an inherited colorectal cancer syndrome characterized by the development of hundreds to thousands of adenomas throughout the large bowel. Patients with FAP are also predisposed to developing other neoplasms, including desmoid tumors or aggressive fibromatosis, which are rare mesenchymal tumors of intermediate malignant potential[39]. Notably, the somatic VAF of the *APC* mutation was 96%, pointing to biallelic inactivation of the *APC* gene in the primary intracranial *DICER1*-mutated sarcoma. These data overall suggest a possible contribution of *APC* mutation to the pathobiology of this tumor.

To our knowledge, the coexistence of *BRCA2* and *DICER1* variants has not been reported up to now. The findings in the two patients of two different variants in the somatic hotspot region of *DICER1* may not be a simple coincidence, considering also the histotypes, which are typical of DICER1 syndrome and the absence of *DICER1* biallelic inactivation in the tumors. Some DNA repair factors have been described to have RNA-binding capacities, and screens investigating DNA damage repair mechanisms have identified RNA-binding proteins as a major group of novel factors involved in DNA repair[40]. Moreover, it is described that RNA is an important component of the DNA damage response and that it is required to maintain the fidelity of repair. Theoretically, mutations in components of RNA-dependent DNA repair could result in an increased mutation rate, thereby promoting carcinogenesis and cancer progression[40]. It is of note that the RNA endonuclease Dicer, besides its major role in the miRNA biogenesis, is a crucial player in the DNA damage response and genome integrity maintenance and is required to propagate Double-strand DNA repair[4–9] Depletion of RNA endonuclease results in deficient recruitment of repair factors to the damaged site, and reported assays show a significant reduction in both HR and non-homologous end-joining repair efficiency, comparable with that seen after *BRCA1* and *53BP1* depletion[8,41]. Based on our findings, it is tempting to speculate that *BRCA2* and *DICER1* variants may have a synergic effect resulting in the simultaneous impairment of relevant DNA repair pathways.

Conclusion

We reported for the first time the occurrence of two neoplasms typical of DICER-tumor predisposition syndrome in two patients carrying the same germline *BRCA2* variant and a somatic *DICER1* mutation. These findings suggest a potential cooperative tumorigenic role of DICER1 and BRCA2, both implicated in DNA repair pathways. Furthermore, this study demonstrates the importance of studying *BRCA* variants in the pediatric cancer patient population for the potential screening, risk-reductive interventions, and family planning options currently available for relatives of children carrying *BRCA* mutations. Extending the analysis of these genes to the pediatric population could provide relevant insights correlated to a possible predisposition to cancer.

Methods

Patients

Written informed consents for genetic analysis were obtained from/for all the participants. The study was approved by Institutional Review Board (IRB) of Bambino Gesù Children's Hospital (RAP-2024-002). All experimental work was carried out according to the declaration of Helsinki.

Germline NGS analysis

Genomic DNA was extracted using the DNA Blood Mini Kit (Qiagen, Hilden, NW, Germany) according to the manufacturer's instructions from circulating leukocytes of peripheral blood samples.

DNA quantification was performed using a Qubit fluorimeter (Life Technologies, Carlsbad, California, USA) with the dsDNA HS Assay kit following the manufacturer's instructions. The genetic analysis was performed through Next Generation Sequencing (NGS) by using a custom clinical exome panel (Twist Bioscience, South San Francisco, CA, USA) that contained more than 8500 genes, including the ones involved in cancer-predisposition syndromes, on NovaSeq 6000 platforms (Illumina, San Diego, CA, USA). The genes were analyzed according to clinical indication for patient 1: *BAP1, BRCA2, CDC73, CHEK2, DICER1, DIS3L2, EPCAM, FBXW7, FH, FLCN, MET, MITF, MLH1, MSH2, MSH6, PIK3CA, PMS2* (only exons 1–10), *PTEN, SDHA, SDHB, SDHC, SDHD, SMARCB1, TP53, TRIM28, TSC1, TSC2, VHL, WT1* and for patient 2: *APC, BLM, CDKN1C, DICER1, EPCAM, EXT1, EXT2, FH, HRAS, KIT, MLH1, MSH2, MSH6, NBN, NF1, PDGFRA, PMS2* (only exons 1–10), *PRKAR1A, PTCH1, RB1, RECQL4, SDHA, SDHB, SDHC, SDHD, SUFU, TP53, WRN, CDKN2A, POT1, PTCH2, TINF2, TSC1, TSC2, BRCA2*. The BaseSpace pipeline (Illumina, https://basespace.illumina.com/) and the GeneYX software (LifeMap Sciences) were used for the variant calling and annotating variants, respectively. Sequencing data were aligned to the hg19 human reference genome. Variants were examined for coverage and Qscore (minimum threshold of 30) and visualized by the Integrative Genome Viewer (IGV).

In addition, the reclassification of the variants of uncertain significance was based on the use of the database of variants of the general population—gnomAD (https://gnomad.broadinstitute.org/); the database of correlation between variants and phenotypes—ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/); the database of variants described in the literature—HGMD Professional (http://www.hgmd.org), the scientific literature reported on PubMed; the search engine of human genomic variants—Varsome (https://varsome.com/) and the pathogenicity prediction algorithms.

Somatic NGS analysis

DNA from cystic nephroma and intracranial sarcoma were extracted from formalin-fixed paraffin-embedded tumor tissue using Maxwell CSC instrument (Promega, Madison, USA) with the Maxwell RSC DNA FFPE kit (Promega, Madison, USA) according to the manufacturer's protocol; DNA concentrations were measured on a Qubit 2.0 Fluorometer (Thermofisher Scientific, Waltham, USA) using the Qubit dsDNA High Sensitivity.

Next-generation sequencing was performed through the OncoDeep kit (OncoDNA). The OncoDeep assay is a comprehensive genomic profiling performed in NGS and targeting 638 cancer-relevant genes. The assay detects indels, small nucleotide variants (SNVs), splice variants and copy-number/structural variations in several genes and also provides complex genomic signatures: tumor mutation burden (TMB), microsatellite status (MSI), and homologous recombination deficiency (HRD) status.

The OncoKDM v24.0.2 software (OncoDNA, Gosselies, Belgium) was used for the variant calling and annotating variants. Sequencing data were aligned to the hg19 human reference genome. Variants were examined for coverage and Qscore (minimum threshold of 150) and visualized by the Integrative Genome Viewer (IGV).

Variant classification was performed following the AMP/ASCO guidelines[42].

Data availability

The datasets generated during and/or analysed during the current study are available in clinVAR repository (submission ID somatic variant: SUB14648587, submission ID germline variant: SUB14646194, SCV005093812; SUB14646177, SCV005093810; SUB14646132, SCV005093809).

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