Exploration of Endocrine and Metabolic Diseases

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Double heterozygous pathogenic variants in *BRCA2* and *CHEK2* in a girl with adrenocortical carcinoma

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Academic Editor: David Torpy, Royal Adelaide Hospital, The University of Adelaide, Australia
Received: January 17, 2025 **Accepted:** April 9, 2025 **Published:** April 17, 2025

Cite this article: Fincke VE, Sax I, Kunstreich M, Golas MM, Hofmann TG, Schlesner M, et al. Double heterozygous pathogenic variants in *BRCA2* and *CHEK2* in a girl with adrenocortical carcinoma. Explor Endocr Metab Dis. 2025;2:101429. https://doi.org/10.37349/eemd.2025.101429

Abstract

Pediatric adrenocortical tumors (pACTs) are rare endocrine neoplasms with variable prognosis, commonly associated with germline pathogenic variants (PVs) in the tumor suppressor gene *TP53*. Here, we report the case of a 3.1-year-old female presenting with virilization and Cushing syndrome due to a left-sided adrenal mass. The tumor was completely resected and confirmed as stage II adrenocortical carcinoma (ACC) based on the Wieneke index. Comprehensive molecular profiling revealed heterozygous germline PVs in *BRCA2* [c.9382C>T p.(Arg3128*)] and *CHEK2* [c.1232G>A p.(Trp411*)]. These findings suggest a potential role of impaired DNA damage repair in ACC pathogenesis, as both PVs are associated with hereditary breast and ovarian cancer (HBOC) syndromes and genomic instability. This case expands the genetic spectrum of pACT and underscores the importance of advanced molecular analyses in identifying rare germline alterations that may inform personalized treatment strategies and cancer prevention programs. Although no additional treatment was required in this case, *BRCA2* status highlights the potential for tailored therapeutic approaches, including poly(ADP-ribose) polymerase (PARP) inhibitors, in selected patients. Further research is warranted to explore the specific contributions of *BRCA2* and *CHEK2* PVs to ACC tumorigenesis and their implic ations for targeted therapies.

Keywords

Adrenocortical carcinoma, child, germline, double heterozygosity, *BRCA2*, *CHEK2*

Introduction

Pediatric adrenocortical tumors (pACTs) are very rare endocrine neoplasms that arise from the cortex of the adrenal gland and have a variable prognosis. The mainstay of therapy is radical surgical resection, while the benefit of aggressive systemic therapy in advanced disease, including mitotane and platinum-based chemotherapy, remains to be discerned [1, 2].

pACTs are recognized as childhood cancer with strong association to syndromes. Germline pathogenic variants (PVs) in the tumor suppressor gene *TP53* as cause of the Li-Fraumeni syndrome are reported in 50–65% of pediatric patients overall and up to 95% in patients from Southern Brazil due to the endemic *TP53* p.Arg337His founder variant [3]. Less frequently, pACT is associated with other genetic conditions such as Beckwith-Wiedemann syndrome or multiple endocrine neoplasia. Recently, pACT has been linked to Lynch syndrome [4].

Growing evidence suggests that PVs in genes related to DNA damage response and repair may play a role in childhood cancer; their exact contribution is not fully understood yet [5]. Comprehensive molecular tumor analysis is essential for determining their significance for cancer development and personalized cancer care [6].

Case report

We present the case of a 3.1-year-old female with virilization and Cushing syndrome along with a left-sided adrenal mass. Urinary steroid hormone profiling raised the suspicion of adrenocortical carcinoma (ACC). Complete tumor resection was performed, revealing a tumor volume of 420 mL and classified as a stage II (T2 N0 M0) according to the American Joint Committee on Cancer (AJCC) 7th staging system, with tumor-free resection margins. Malignancy was confirmed based on the Wieneke index. Germline testing of *TP53* was unremarkable. No additional treatment was administered, and eight years later the patient remained in first complete remission.

To further characterize the tumor, comprehensive molecular profiling was performed within the EpiGenPAT project, an in domo screening platform for pediatric ACC patients. Blood and fresh-frozen tumor tissue samples were collected with written informed consent from the patient's legal guardians. Tumor DNA was isolated using the Qiagen AllPrep DNA/RNA Mini Kit, while blood DNA was extracted using the Maxwell RSC Blood DNA Kit. DNA methylation analysis was performed on tumor DNA using Illumina's Infinium MethylationEPIC (850K) array, with raw data processed in R utilizing the 'IlluminaHumanMethylationEPICmanifest' and 'conumee' packages. Examination of copy number variations (CNVs) derived from DNA methylation revealed numerous alterations, such as a gain of chromosome 13 (Figure 1A), which encodes *BRCA2*, amongst others. The majority of the observed CNVs correspond to whole chromosome or chromosomal arm aneuploidies.

Whole-exome sequencing (WES) of tumor and blood DNA was conducted by Eurofins Genomics to diagnostic standards (ISO 17025) with a coverage of 50×. Raw sequencing data were adapter-trimmed using TrimGalore, mapped to the hg19 reference genome with Burrows-Wheeler Aligner Maximum Exact Matches (BWA-MEM) and duplicates were marked using Picard. Variants were called using six different algorithms (Freebayes, HaplotypeCaller, Bcftools mpileup, Strelka, Platypus, and VarScan2) and filtered for quality and coverage parameters. Variants were annotated with visual evoked potential (VEP) and filtered against gnomAD and in-house control datasets. Detailed parameters and protocols are available from the corresponding author upon request. Using this approach on tumor DNA, a heterozygous PV in *BRCA2* (NM_000059.4): c.9382C>T p.(Arg3128*) in exon 25 of 27 (Figure 1B) was found with a variant allele frequency (VAF) of 58%, alongside a heterozygous PV in *CHEK2* (NM_007194.4): c.1232G>A p.(Trp411*) in exon 11 of 15 (Figure 1C) with a VAF of 34%. Both variants were confirmed to be of heterozygous germline origin through genetic testing using the patient's blood sample. According to the American College of Medical Genetics and Genomics (ACMG) criteria 2015 both variants are classified as pathogenic (class 5). Degradation of the variant *BRCA2* and *CHEK2* mRNA through nonsense-mediated decay (NMD) likely leads to little or no expression of the variants.

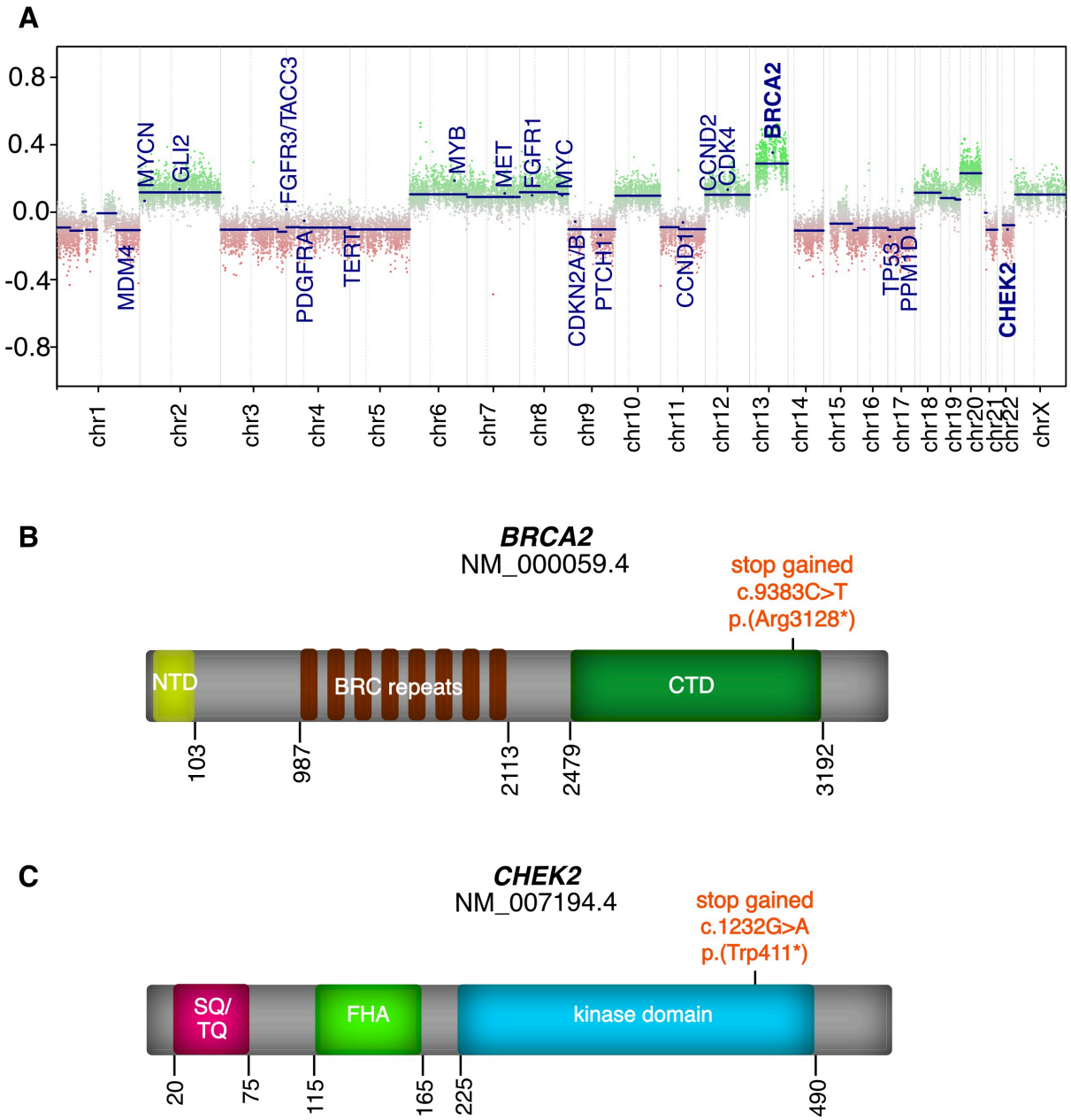

Figure 1. CNV profile and gene-specific mutations in the tumor sample. (**A**) Copy number variation (CNV) profile of the tumor, derived from the Illumina EPIC methylation array. Selected oncogenic and tumor suppressor genes, including *BRCA2* and *CHEK2*, are highlighted; (**B**) Observed mutation in the *BRCA2* gene; (**C**) Observed mutation in the *CHEK2* gene. NTD: N-terminal domain; BRC: BRCA2 repeat clusters; CTD: C-terminal domain; SQ/TQ: serine-glutamine/threonine-glutamine cluster domain; FHA: forkhead-associated

The patient's family history was unremarkable, with no reported cases of breast cancer, ovarian cancer, pancreatic ductal adenocarcinoma (PDAC), ACC, or other malignancies in first- or second-degree relatives. Information on cascade testing was not available.

WES of germline and tumor DNA identified an additional pathogenic (class 5) variant in IGSF3 (NM_001007237.3): c.1724G>A p.(Trp575Ter). IGSF3 encodes an immunoglobulin superfamily protein involved in cell adhesion and signaling; however, a role in ACC tumorigenesis has not been reported yet. No further high-confidence somatic single nucleotide variants (SNVs) in known cancer-related genes were detected.

Discussion

Here, we report the case of a child with ACC, harboring double heterozygous germline PVs in *BRCA2* and *CHEK2*. The c.9382C>T variant in *BRCA2*, resulting in a stop gain in exon 25, is listed in the population database gnomAD with a very low frequency. PVs in *BRCA2* are related to hereditary breast and ovarian cancer (HBOC). *CHEK2* is considered to be a moderate risk gene for HBOC. The c.1232G>A variant in *CHEK2* results in a stop gain in exon 11 and is not found in gnomAD.

Given that ACC is a prototypic tumor entity that displays substantial genomic instability, we assume that the stop codons introduced into *BRCA2* and *CHEK2* contribute to tumorigenesis by impairing DNA-damage repair. In the largest pan-genomic characterization of 91 adult [4] and 37 pediatric ACTs [3] to date, no PVs were reported in *BRCA2* and *CHEK2*.

El Ghorayeb et al. [7] found a c.8765delAG variant of germline origin in *BRCA2* in a 50-year-old male with ACC, supported by somatic tumor analyses showing loss-of-heterozygosity in the tumor, which suggested a causal link to ACC tumorigenesis. In another case involving a 48-year-old female with ACC, the c.1100delC variant in *CHEK2* was reported without molecular tumor analysis, leaving uncertainty regarding its association with tumorigenesis [8]. However, to our knowledge, no previous reports have described the co-occurrence of germline *BRCA2* and *CHEK2* variants in ACC. The co-occurrence of PVs in *CHEK2* and other predominantly high penetrance cancer predisposing genes has been previously documented in other tumor entities such as HBOC [9]. This includes double heterozygosity with PVs in *BRCA1* or *BRCA2* and less frequently in *ATM*, *CHEK2*, and other moderate risk genes. Double heterozygosity may potentially cause synergistic or additional effects leading to a more severe phenotype [10–12].

Given the involvement of *BRCA2* and *CHEK2* in repairing DNA damage, it is plausible that a compromised ability to effectively repair DNA results in an accumulation of genetic alterations and consequently increases the risk of tumor formation. Germline PVs in both genes may increase the cancer risk even further compared to having either PV alone. Thus, the PVs could contribute to genomic instability, which is a well-known characteristic associated with cancer development, particularly for ACCs. The observed CNVs are likely to disrupt normal gene function and regulatory elements further, thus serving as an additional mechanism in cancer initiation and/or progression.

In contrast to the typical biallelic inactivation observed in breast and ovarian cancers, *BRCA2* remained heterozygous in this case, with a VAF of 57% in blood and 53% in tumor, and no evidence of loss of heterozygosity (LOH) or a second-hit mutation. However, *CHEK2* exhibited LOH, with its VAF increasing from 34% in blood to 85% in tumor, suggesting biallelic inactivation. While *CHEK2* plays a key role in DNA damage response via checkpoint regulation, it is not classified as a core DNA repair deficiency gene, as it primarily functions in damage sensing rather than direct repair. Nevertheless, its inactivation in this case may have contributed to genomic instability through defective checkpoint control, which could indirectly impair DNA repair processes. WES analysis of *BRCA1*, *PALB2*, and *CHEK1* revealed no PVs, suggesting that genomic instability in this case is primarily linked to *CHEK2* inactivation, with a potential contributory role of *BRCA2*.

Trials assessing BRCA status have demonstrated an improved response to platinum agents; recently resulting in United States Food and Drug Administration (FDA)- and European Medicines Agency (EMA)-approval of poly(ADP-ribose) polymerase (PARP) inhibitors for adults with selected BRCA-positive cancers [13]. Extraordinary response to platinum-based chemotherapy was reported in a patient with metastatic pancreatic carcinoma associated with double heterozygous PVs in *BRCA2* and *CHEK2* [14]. In our patient's case of completely resected stage II ACC, no additional treatment was necessary as she remained disease-free after eight years. However, *BRCA2* status may open new avenues for personalized cancer care in certain patients with ACC, warranting further preclinical investigations of PARP-inhibition in ACC. A case report described an ACC patient with a germline *BRCA2* variant who received the PARP inhibitor rucaparib following chemotherapy, but the treatment showed only limited efficacy [15].

Patients with germline pathogenic *BRCA2* variants have a significantly increased lifetime risk of developing breast and ovarian cancer. *CHEK2* PVs are associated with a moderately increased breast cancer risk but have an unclear role in ovarian cancer predisposition. Given this patient's *BRCA2* variant, she may be at elevated risk for these malignancies later in life. Long-term surveillance, including breast magnetic resonance imaging screening and consideration of risk-reducing strategies such as mastectomy or salpingo-oophorectomy in adulthood, is recommended per established clinical guidelines, while no risk-reducing surgical strategies exist for ACC. Our findings connect pediatric ACC with hereditary genetic variations in *BRCA2* and *CHEK2*. Comprehensive analyses of ACCs are crucial for understanding the role of PVs in tumor development and tailoring personalized treatment, while additional research will be necessary to explore the specific roles of PVs in *BRCA2* and *CHEK2* in ACC development.

Abbreviations

ACC: adrenocortical carcinoma

CNVs: copy number variations

HBOC: hereditary breast and ovarian cancer

LOH: loss of heterozygosity

pACTs: pediatric adrenocortical tumors

PARP: poly(ADP-ribose) polymerase

PVs: pathogenic variants

VAF: variant allele frequency

WES: whole-exome sequencing

Declarations

Author contributions

VEF: Investigation, Data curation, Writing—original draft, Visualization. IS: Data curation. M Kunstreich: Investigation. MMG: Data curation, Writing—original draft. TGH and MS: Writing—review & editing. RC: Writing—original draft. AR: Investigation, Writing—review & editing. PDJ: Data curation, Writing—original draft, Supervision. M Kuhlen: Conceptualization, Investigation, Data curation, Writing—original draft, Supervision. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical approval

The MET studies were approved by the ethics committees of the University of Luebeck (IRB 97125) and Otto-von-Guericke-University Magdeburg (IRB 174/12 and 52/22), Germany. The EpiGenPAT project was approved by the Ethics Committee of the Ludwig Maximilians University Munich, Germany (IRB 21-0997).

Consent to participate

The informed consent to participate in the study was obtained from legal guardians.

Consent to publication

The informed consent to publication in the study was obtained from legal guardians.

Availability of data and materials

The data presented in this study are available upon reasonable request from the corresponding author. The data are not publicly available due to restrictions.

Funding

The research on pediatric cancer predisposition/double heterozygosity is supported by the Deutsche Forschungsgemeinschaft ([KU3764/3-1] and [HO2438/7-1]) and the EpiGenPAT project by intramurale Forschungsförderung, University of Augsburg (M Kuhlen). M Kuhlen is supported by Deutsche Krebshilfe [DKH 70115888]. PDJ is supported by the Max Eder Program of the Deutsche Krebshilfe. VEF receives a scholarship from the Konrad Adenauer Stiftung. The German MET studies were supported by Deutsche Kinderkrebsstiftung (grant numbers [DKS 2014.06], [DKS 2017.16], [DKS 2021.11], and [DKS 2024.16]) and Mitteldeutsche Kinderkrebsforschung. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Publisher's note

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