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A cross-sectional study of *CHEK2* pathogenic variants: cancer risk spectrum and clinical insights

CHEK2 patojenik varyantlarına dair kesitsel bir çalışma: Kanser risk spektrumu ve klinik değerlendirmeler

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ABSTRACT

Aims: *CHEK2* is a tumor suppressor gene involved in DNA damage response and a moderate-risk gene for breast cancer. However, its role in other malignancies remains unclear, and the clinical impact of biallelic *CHEK2* mutations is not well understood. This study aims to expand the cancer risk spectrum of *CHEK2*, including rare tumors, and to provide insights into the phenotypes associated with biallelic mutations and Multiple Inherited Neoplasia Alleles Syndrome (MINAS).

Materials and Methods: We analyzed 40 individuals from 34 families carrying *CHEK2* mutations, identified via multigene panel testing for hereditary cancer syndromes. Next-generation sequencing was performed for the probands, and segregation analysis in affected relatives was conducted using Sanger sequencing. Clinical data, including cancer type, age at diagnosis, and family history, were obtained from medical records and clinical evaluations.

Results: We identified 16 distinct *CHEK2* mutations, with c.1427C>T (p.Thr476Met) being the most frequent. Breast cancer was the most common diagnosis (75%), followed by thyroid cancer and rare tumors, including pancreatic neuroendocrine and cerebellopontine angle tumors. Multiple primary cancers occurred in 15% of patients, and 10% had MINAS, harboring additional variants in genes like PTEN and BRCA2. Biallelic *CHEK2* mutations were linked to severe phenotypes, including bilateral breast cancer and adolescent-onset polyposis.

Conclusions: Our findings broaden the *CHEK2*-associated cancer spectrum, extending beyond breast cancer to include rare malignancies and complex presentations. The identification of biallelic mutations and MINAS underscores the need for comprehensive genetic testing and tailored surveillance. These insights are crucial for refining risk assessment, enhancing prevention, and improving clinical management for individuals harboring *CHEK2* mutations.

Keywords: *CHEK2*, MINAS, polyposis, FATWO, hereditary cancer syndromes

ÖZ

Amaç: *CHEK2*, DNA hasar yanıtında rol oynayan bir tümör süpresör gendir ve meme kanseri için orta derecede risk faktörü olarak kabul edilir. Ancak, diğer malignitelerdeki rolü belirsizliğini korumaktadır ve biallelik *CHEK2* mutasyonlarının klinik etkileri tam olarak anlaşılamamıştır. Bu çalışma, *CHEK2* ile ilişkili kanser risk spektrumunu genişletmeyi, nadir tümörleri tanımlamayı ve biallelik mutasyonlar ile Multiple Inherited Neoplasia Alleles Syndrome (MINAS) fenotipleri hakkında yeni bilgiler sunmayı amaçlamaktadır.

Gereç ve Yöntemler: Kalıtsal kanser sendromları şüphesiyle multigen panel testi yapılan ve *CHEK2* mutasyonu saptanan 34 aileye ait 40 birey analiz edilmiştir. Probantlar için yeni nesil dizileme (NGS) yapılmış, etkilenen aile bireylerinde segregasyon analizi Sanger sekanslama ile gerçekleştirilmiştir. Kanser tipi, tanı yaşı ve aile öyküsü gibi klinik veriler tıbbi kayıtlar ve klinik değerlendirmeler yoluyla elde edilmiştir.

Bulgular: Toplam 16 farklı *CHEK2* mutasyonu tanımlanmış, bunlar arasında en sık c.1427C>T (p.Thr476Met) mutasyonu görülmüştür. Kohortta en yaygın tanı meme kanseri olup (%75), bunu tiroid kanseri takip etmiştir. Ayrıca, pankreatik nöroendokrin tümörler ve serebellopontin açı tümörleri gibi nadir maligniteler de gözlenmiştir. Hastaların %15'inde birden fazla birincil kanser bulunurken, %10'unda PTEN ve BRCA2 gibi ek varyantlar içeren MINAS saptanmıştır. Biallelik *CHEK2* mutasyonları, bilateral meme kanseri ve adolesan yaşta başlayan polipozis ile ilişkilendirilmiştir.

Sonuç: Bulgularımız, *CHEK2* ile ilişkili kanser spektrumunu genişleterek meme kanserinin ötesinde nadir maligniteleri ve kompleks klinik tabloları içermektedir. Biallelik mutasyonlar ve MINAS, kapsamlı genetik testlerin yanı sıra bireyselleştirilmiş izlem ve yönetim stratejilerinin önemini ortaya koymaktadır. Bu çalışmanın bulguları, risk değerlendirmesinin iyileştirilmesi, önleyici yaklaşımların geliştirilmesi ve *CHEK2* mutasyonu taşıyan bireylerin klinik yönetiminin optimize edilmesi açısından kritik öneme sahiptir.

Anahtar Kelimeler: *CHEK2*, MINAS, polipozis, FATWO, herediter kanser sendromları

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INTRODUCTION

CHEK2 encodes the checkpoint kinase 2 protein (CHK2), a tumor suppressor involved in the DNA damage response (DDR) pathway as part of the ATM-CHK2-p53 complex (1). The DDR is a signal amplification cascade that detects DNA damage, induces cell cycle arrest, and initiates DNA repair. Similar to mutations in many other genes involved in DDR and DNA repair pathways, mutations in the *CHEK2* play an active role in carcinogenesis (2).

Initial studies identified *CHEK2* germline mutations as predisposing to a moderate risk for breast cancer (3). Among these mutations, c.1100delC has been extensively studied and is associated with a significant increase in breast cancer risk (4). A meta-analysis of patients with this mutation estimated a cumulative risk of 37% for developing breast cancer by the age of 70 (5). While the loss-of-function (LOF) variants, such as c.1100delC, are typically classified as pathogenic variants (PVs), the clinical significance of other *CHEK2* variants, particularly missense mutations, remains less-defined (6). The effect of these missense variants is variable and highly dependent on whether critical protein domains within the CHK2 protein are affected (6).

Genetic testing for *CHEK2* is now a standard part of routine diagnostic Next Generation Sequencing (NGS) panels for various inherited cancers, with *CHEK2* being one of the most frequently identified genes harboring germline mutations (7). Despite the established role of *CHEK2* mutations in increasing the risk for hereditary cancers, the full spectrum of associated cancer risks is not yet fully understood. *CHEK2* germline mutations have been reported in large cohort studies and case-based publications across a variety of cancer types (8, 9). In the past, these mutations were even linked to Li-Fraumeni syndrome (10); however, this terminology is no longer in use (11). Recent studies have reinforced the increased risks for breast and prostate cancers (6, 12), leading to current cancer screening guidelines primarily focusing on these two cancer types for individuals with *CHEK2* mutations (13). However, these guidelines do not routinely address other potential cancer risks, highlighting the need for systematic data collection to more comprehensively define the full range of cancer risks in *CHEK2* mutation carriers. Furthermore, the American College of Medical Genetics and Genomics (ACMG) emphasizes the necessity of additional research to inform clinical management strategies for individuals with *CHEK2* mutations (14).

In this study, we present the clinical and genetic characteristics of 40 affected individuals from 34 families with *CHEK2* variants, identified through multigene panel testing for suspected hereditary cancer syndromes. Among these patients, we report atypical presentations, including rare cancers and the presence of concurrent pathogenic variants in other hereditary-cancer-related genes, underscoring the variability in *CHEK2*-related cancer phenotypes and the challenges in clinical interpretation.

MATERIALS AND METHODS

The study cohort includes 40 affected individuals from 34 families who were referred to medical genetics department with a suspicion of hereditary cancer syndrome and were found to have variants in the *CHEK2*, each with various cancer diagnoses. The study was conducted in accordance with the Declaration of Helsinki and the study protocol was approved by the institutional ethics committee of Hacettepe University (SBA 24/850, 17th September 2024). Written informed consent was obtained from the affected individuals for molecular testing and publication. Clinical data were gathered from both medical records and in-person evaluations between 2021 and 2024, including age at diagnosis, cancer type, family history of cancer, and other relevant clinical features and histopathological findings.

For genetic analysis, genomic DNA was extracted from peripheral blood samples of the index cases and their affected family members using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A targeted panel, consisting of the coding regions of at least 40 cancer predisposition genes *(APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, FANCC, FLCN, GALNT12, HOXB13, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS2, POLD1, POLE, PTCH1, PTEN, RAD51C, RAD51D, RB1, RET, SMAD4, STK11, TP53, VHL)* was performed on the DNA samples of the index cases using next-generation sequencing (NGS) technology.

Variant filtering steps were performed using the Seq Genomize Variant Analysis Platform, and variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines. Pathogenic and likely pathogenic variants were reported. Each identified variant was visually inspected using the Integrative Genomics Viewer (IGV). Segregation studies were conducted by Sanger sequencing for the affected family members of index cases carrying *CHEK2* variants.

Statistical Analysis

Descriptive statistics were used to summarize the data. Categorical variables were presented as frequencies and percentages, while continuous variables were reported as medians and ranges. No inferential statistical analyses were performed.

RESULTS

Clinical characteristics of affected individuals

This study included 40 patients with *CHEK2* variants from 34 unrelated families. Detailed demographic, clinical, and molecular data of the affected individuals are presented in Table 1. The cohort predominantly consisted of female patients (90%), with 4 male patients (10%). The ages at diagnosis ranged from 17 to 72 years, with a median age at diagnosis of 46 years. Breast cancer

Table 1. Clinical and genetic characteristics of the cohort.

ID	FAM ID	Gender	Current Age (y)	Diagnosis	Age at Cancer Diagnosis (y)	Family History	*CHEK2* (NM_007194.4) Variant	Zygosity	Concurrent Variant
P1	F1	F	47	Breast Cancer	43	Paternal grandfather stomach Ca, Paternal niece ALL	c.1427C>T (p.Thr476Met)	HET	No
P2	F2	F	40	Breast Cancer	37	Father colorectal Ca (AaD: 54y), Maternal aunt leukemia (AaD: 54y)	c.16del (p.Asp6Metfs*55)	HET	No
P3	F3	F	45	Breast Cancer	41	Father lymphoma AaD: 65y)	c.592+3A>T	HET	No
P4	F4	F	50	Breast Cancer	45	Maternal aunt colorectal Ca, Maternal cousin breast Ca, Father and 2 maternal uncle prostate Ca, Maternal cousin CNS tm	c.1427C>T (p.Thr476Met)	HET	No
P5	F5	F	55	FATWO	49	Sister breast cancer (AaD: 49y)	c.479T>C (p.Ile160Thr)	HET	No
P6		F	59	Breast Cancer	49	FATWO diagnosis in the sister (AaD: 49y)	c.479T>C (p.Ile160Thr)	HET	No
P7	F6	F	23	Ovarian Cancer	17	No	c.1427C>T (p.Thr476Met)	HET	No
P8	F7	F	29	Colorectal Cancer	26	Maternal grandmother breast cancer	c.100C>T (p.Gln34Ter)	HET	NM_001048174.2 *(MUTYH)*: c.775del (p.Ala259Profs*32) and c.800C>T (p.Pro267Leu) compound heterozygous
P9	F8	F	62	Breast Cancer	52	No	c.592+3A>T	HET	No
P10	F9	F	75	Bilateral Breast Cancer	69	No	c.444+1G>A	HET	No
P11	F10	F	74	Breast Cancer	72	Sister breast, colorectal and thoracic Ca, Son thyroid Ca, Mother leukemia, Maternal uncle stomach Ca	c.1427C>T (p.Thr476Met)	HET	No
P12		M	49	Thyroid Cancer	46	Mother breast Ca Maternal aunt breast, colorectal and thoracic Ca, Maternal grandmother leukemia, Brother of maternal grandmother stomach Ca		HET	No
P13	F11	M	22	Polyposis	17	Maternal uncle colorectal Ca	c.792+1G>T	HOM	No
P14	F12	F	66	Breast Cancer Endometrium Cancer	52 62	Sister breast Ca, Maternal aunt endometrium Ca, Maternal cousin lymphoma	c.190G>A (p.Glu64Lys)	HET	No
P15		F	68	Breast Cancer	42	Sister breast and endometrium Ca, Maternal aunt endometrium Ca, Maternal cousin lymphoma	c.190G>A (p.Glu64Lys)	HET	No
P16	F13	F	50	Breast Cancer	46	Maternal father colorectal Ca	c.1427C>T (p.Thr476Met)	HET	No
P17	F14	F	65	Breast Cancer	59	Three sisters breast Ca	c.427C>T (p.His143Tyr)	HET	No
P18	F15	F	53	Lobular Breast Cancer	46	Maternal grandfather stomach Ca, Maternal grandmother pancreatic Ca	c.433C>T (p.Arg145Trp)	HET	No
P19	F16	F	56	Bilateral Breast Cancer	47	Maternal uncle breast and prostate Ca	c.592+3A>T	HOM	No
P20	F17	F	39	Breast Cancer	36	No	c.1232G>A (p.Trp411Ter)	HET	No
P21	F18	F	51	Breast Cancer Thyroid Cancer	36 37	Brother colon Ca, Maternal uncle with stomach Ca; two of his sons colon Ca, his daughter breast Ca, Maternal aunt with colon Ca; her daughter breast cancer and her son colon Ca	c.1169A>C (p.Tyr390Ser)	HET	No
P22		F	65	Breast Cancer	61	Mother breast Ca, Father skin tm, Brother colon Ca, Paternal grandmother breast Ca, Maternal cousin breast and thyroid Ca, Another maternal cousin colon Ca, Maternal uncle with stomach Ca; two of his sons colon Ca, his daughter breast Ca	c.1169A>C (p.Tyr390Ser)	HET	No
P23		M	69	Colorectal Cancer	62	Mother breast Ca, Father skin tm, Sister breast Ca, Paternal grandmother breast Ca, Maternal cousin breast and thyroid Ca, Another maternal cousin colon Ca, Maternal uncle with stomach Ca; two of his sons colon Ca, his daughter breast Ca	c.1169A>C (p.Tyr390Ser)	HET	No

Continued

Table 1. Clinical and genetic characteristics of the cohort.

P24	F19	F	48	Breast Cancer	43	Two paternal cousins with early-onset breast Ca	c.499G>A (p.Gly167Arg)	HET	No
P25	F20	F	36	Breast Cancer	32	No	c.1169A>C (p.Tyr390Ser)	HET	No
P26	F21	M	51	Pancreatic Cancer	50	Mother gastric Ca, Sister leukemia (AaD: 66), Maternal cousin pancreatic Ca (AaD: 58), Maternal cousin breast Ca (AaD: 50)	c.1427C>T (p.Thr476Met)	HET	No
P27	F22	F	55	Breast Cancer	53	Mother bilateral breast Ca,		HET	No
P28		F	87	Bilateral Breast Cancer	70	Daughter breast Ca	c.1427C>T (p.Thr476Met) mutasyon	HET	No
P29	F23	F	54	Neurofibroma-tosis	40	Father subcutaneous nodules, Sister and maternal aunt axillary freckling, Sister and mother cafe au lait macules	c.1427C>T (p.Thr476Met)	HET	NM_001042492.3 *(NF1)*: c.76G>T (p.Gly26Ter)
P30	F24	F	68	Bilateral Breast Cancer	60	Sister breast Ca, Mother endometrium Ca, Father stomach Ca, Brother and paternal aunt bladder Ca, Maternal aunt and uncle with stomach Ca, Maternal aunts CNS tm	c.1427C>T (p.Thr476Met)	HET	NM_000059.4 *(BRCA2)*: c.3589A>T (p.Lys1197Ter)
P31	F25	F	66	Medullary Thyroid Cancer, Pancreatic NET	67 68	Father prostate Ca, Brother colorectal Ca, Sister cancer of unknown primary, Maternal uncle colorectal Ca, Paternal aunt colorectal Ca	c.1260C>A (p.Cys420Ter)	HET	No
P32	F26	F	37	Breast Cancer	35	Mother Thyroid Papillary Cancer		HET	No
P33	F27	F	62	Breast Cancer	51	Paternal cousin early-onset colorectal Ca	c.100C>T (p.Gln34Ter)	HET	No
P34	F28	F	45	Breast Cancer Thyroid Papillary Cancer	33 33	Nephew leukemia	c.592+3A>T	HET	No
P35	F29	F	46	Breast Cancer	34	No	c.1427C>T (p.Thr476Met)	HET	No
P36	F30	F	76	Breast Cancer Cerebellopontin tumor	54 69	Father prostate Ca, Sister endometrium Ca	c.1232G>A (p.Trp411Ter)	HET	No
P37	F31	F	57	Breast Cancer Thyroid Cancer	42 44	Father and paternal uncle prostate Ca, Paternal aunt and maternal uncle bladder Ca	c.1427C>T (p.Thr476Met)	HET	NM_000314.8 *(PTEN)*: c.407G>A (p.Cys136Tyr)
P38	F32	F	61	Primary serous peritonael carcinoma	57	No	c.592+3A>T	HET	No
P39	F33	F	46	Breast Cancer	42	Paternal uncle thyroid Ca, Paternal cousin leukemia	c.58C>T (p.Gln20Ter)	HET	No
P40	F34	F	52	Breast Cancer	49	Father CNS tm, Maternal aunt stomach Ca (Aad: 45y), Maternal niece stomach Ca (Aad: 35)	c.499G>T (p.Gly167Ter)	HET	No

Bilateral involvement is indicated. When known, the ages of cancer diagnoses in family members are shown in parentheses. ALL: acute lymphoblastic leukemia, AaD: age at diagnosis, Ca: cancer, CNS: central nervous system, F: female, FATWO: Female adnexal tumor of probable Wolffian Origin, HET: heterozygous, HOM: homozygous, M: male, NK: not known, tm: tumor, y: years.

was the most frequently observed diagnosis, affecting 30 patients (75%), 4 of whom had bilateral involvement. This was followed by thyroid cancer in 5 patients (12.5%), which included both medullary and papillary subtypes. Additionally, rare tumors were observed, including Wolffian Tumor (FATWO), a highly uncommon adnexal neoplasm, and Pancreatic Neuroendocrine Tumor. Furthermore, atypical presentations, such as cerebellopontine angle tumors, were identified, which have not been previously reported in association with *CHEK2* variants.

Notably, six patients (15%) presented with multiple primary cancers. These included one individual with medullary thyroid cancer and a pancreatic neuroendocrine tumor, another with a cerebellopontine angle tumor and breast cancer, three patients with breast and papillary thyroid cancers, and one with breast and endometrial cancers. Analysis of pedigrees revealed that 27 of the 34 probands had a significant positive family history of cancer among close relatives.

Genetic findings

Sixteen distinct pathogenic or likely pathogenic variants in *CHEK2* were identified across 34 unrelated probands, demonstrating a diverse spectrum of mutation types (Figure 1). These included missense mutations (n=7), nonsense mutations (n=5), splice-site mutations (n=3), and one frameshift mutation. The most recurrent variant was c.1427C>T (p.Thr476Met), detected in 11 probands.

Domain-specific analysis highlighted that missense mutations were predominantly localized within critical functional domains essential for CHK2's role in the DNA damage response. Of the seven missense mutations, the majority (n=6) were situated within the forkhead-

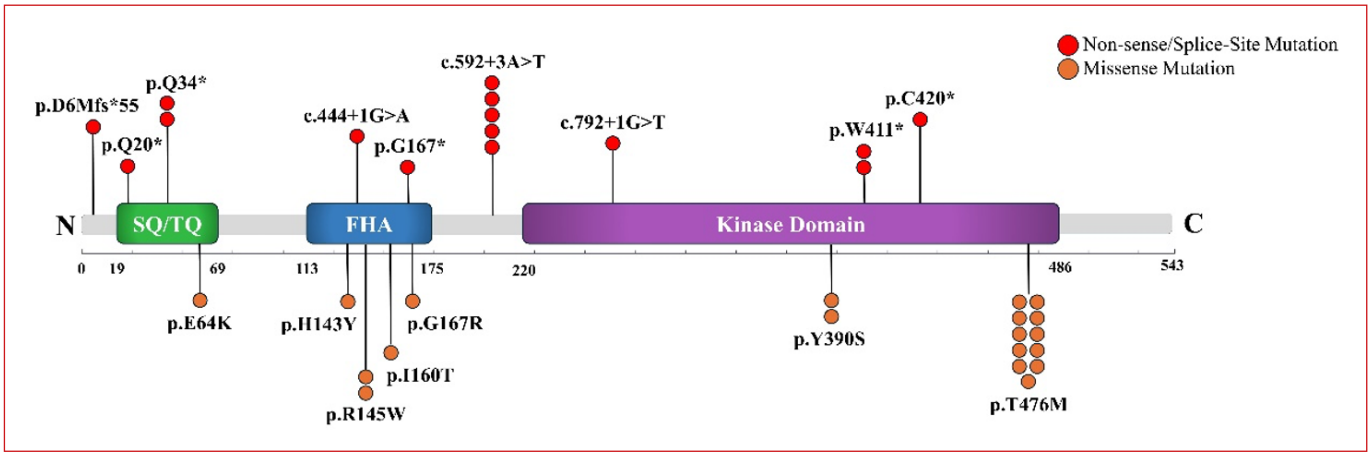

Figure 1. Schematic representation of *CHK2* protein domains and distribution of germline mutations.

The *CHK2* protein consists of three major functional domains: the SQ/TQ domain (amino acids 19–69), which mediates ATM binding; the Forkhead-Associated (FHA) domain (amino acids 113–175), involved in dimerization and activation through phosphorylation; and the Kinase domain (amino acids 220–486), responsible for phosphorylating downstream effector proteins. Each dot represents a family with the indicated mutation (Red: Non-sense/Splice-Site Mutation; Orange: Missense Mutation).

associated (FHA) domain (amino acids 113–175), responsible for dimerization and activation through phosphorylation, and the kinase domain (amino acids 220–486), which mediates phosphorylation of downstream effector proteins. Notably, the recurrent c.1427C>T (p.Thr476Met) variant lies within the kinase domain, suggesting that disrupted phosphorylation may be a key pathogenic mechanism. Additionally, one missense variant, c.190G>A (p.Glu64Lys), was identified within the SQ/TQ domain (amino acids 19–69), which may impair ATM-mediated phosphorylation.

Nonsense and splice-site mutations were distributed throughout the gene, typically resulting in truncated or non-functional proteins. Two patients were homozygous carriers of *CHEK2* mutations (c.792+1G>T and c.592+3A>T), with clinical manifestations including adolescent-onset colon polyposis and bilateral breast cancer, respectively.

Furthermore, 10% of patients (n=4) were found to have Multiple Inherited Neoplasia Alleles Syndrome (MINAS), harboring additional pathogenic variants in other cancer predisposition genes, such as *MUTYH, BRCA2, NF1,* and *PTEN*, adding complexity to their clinical presentations.

DISCUSSION

In this study, we report a cohort of 40 affected individuals from 34 families with *CHEK2* mutations, contributing novel clinical and genetic insights. Our findings further expand the phenotypic spectrum of *CHEK2*-related cancers and, with 75% of patients in our cohort diagnosed with breast cancer, reaffirm its well-established role in breast cancer predisposition. Notably, we found no patients diagnosed with prostate cancer in our cohort, despite its known association with increased risk in individuals with *CHEK2* mutations. This may reflect the tendency for genetic evaluations to overlook prostate cancer, which is frequently observed in men, potentially due to the underestimation of its genetic etiology.

Beyond the well-documented associations, our study highlights the presence of rare tumors in individuals with germline *CHEK2* mutations. We identified a case of Wolffian Tumor (FATWO), an extremely rare neoplasm that has previously been reported in only one patient with a *CHEK2* germline variant (15). Additionally, we describe a patient with cerebellopontine angle tumor, which, to our knowledge, has not been previously linked to *CHEK2* mutations. The presence of such rare tumor types in our cohort suggests that the oncogenic landscape of *CHEK2* may be broader than currently recognized. While larger studies are necessary to determine whether these associations reflect direct contributions of *CHEK2* dysfunction or occur by chance, our findings emphasize the importance of continued investigation into the full phenotypic spectrum of *CHEK2*-related cancers.

Among the 16 distinct mutations identified in our study, 7 were missense mutations (Figure 1). While truncating mutations—including nonsense, splice-site, and frameshift variants such as c.1100del—are well-established as pathogenic, missense mutations present greater challenges in classification. For instance, the p.Ile157Thr and p.Ser428Phe variants have been reported with conflicting interpretations, ranging from established risk alleles to variants of uncertain significance (VUS) or pathogenic mutations. Large-scale studies have further highlighted this uncertainty. These findings suggest that while the p.Ile157Thr and p.Ser428Phe

variants may exert some biological effect, their penetrance is likely too low to warrant clinical actionability (16). Given their limited clinical utility, individuals harboring these variants were excluded from our cohort. The most frequently observed missense mutation in our study was c.1427C>T (p.Thr476Met), located in the kinase domain of *CHEK2*, a region essential for its tumor suppressor function. Functional data strongly suggest that this variant disrupts CHK2's activity (17). However, the functional consequences of most *CHEK2* missense mutations remain poorly understood. Recently, multiplexed assays of variant effect (MAVEs) have emerged as a powerful tool to systematically assess the functional impact of VUS variants across hereditary cancer genes. Saturation genome editing approaches have been successfully applied to genes such as *BRCA2* and *RAD51D* (18, 19), providing large-scale functional insights that directly inform variant classification and patient management. Given the high prevalence of missense mutations in *CHEK2*, similar approaches are essential to refine risk stratification and guide clinical decision-making.

A particularly notable finding in our study is the presence of biallelic *CHEK2* mutations in two patients (Table 1), both of whom exhibited severe clinical phenotypes. One patient (P19), harboring the c.592+3A>T splice-site variant, developed bilateral breast cancer, a hallmark of high-risk hereditary cancer syndromes. Previous studies have suggested that biallelic *CHEK2* mutations, particularly involving truncating variants like c.1100del, may confer significantly higher cancer risks compared to monoallelic carriers (20). In cohorts analyzed for hereditary cancer, biallelic carriers have demonstrated a markedly elevated risk for invasive breast cancer (OR 8.69, 95% CI 3.69–20.47), with earlier onset and a higher frequency of bilateral tumors (21). These findings are consistent with our observation of bilateral breast cancer in a patient carrying the biallelic splice-site *CHEK2* variant. The second patient (P13), carrying the c.792+1G>T splice-site variant in a homozygous state, was the youngest patient in our cohort (17 years old) and was diagnosed with adolescent-onset polyposis. This patient presented with numerous adenomatous polyps and tubular adenomas in the duodenum and colon, many of which showed dysplasia. Recent studies have suggested that biallelic *CHEK2* mutations may represent a novel recessive hereditary cancer syndrome characterized by chromosomal instability and a predisposition to multiple cancers (22, 23). Although we were unable to evaluate chromosomal breakage in our patient, the presence of numerous dysplastic polyps at a remarkably young age, combined with the absence of mutations in known polyposis-associated genes, provides valuable support for this emerging entity.

In 10% of our cohort (n=4), Multiple Inherited Neoplasia Alleles Syndrome (MINAS) was identified, characterized by the co-occurrence of pathogenic variants in multiple hereditary cancer genes (Table 1). This emerging concept, increasingly recognized with the widespread use of multigene panels, presents significant challenges in risk assessment and clinical management of patients (24). Notably, a patient (P37) harboring both *CHEK2* and *PTEN* mutations was diagnosed with breast and thyroid cancer, with breast cancer diagnosed at age 42. While PTEN mutations are well-established in Cowden syndrome and associated with multiple malignancies (25), the potential contribution of *CHEK2* to breast cancer risk or age of onset in this patient remains uncertain. Given the tumor suppressor functions of both genes, their combined effect on tumorigenesis warrants further investigation. Another notable case involved a patient (P30) with both *CHEK2* and BRCA2 mutations, presenting with bilateral breast cancer at diagnosis and a strong family history of cancer on both maternal and paternal sides. Although segregation analysis was not available, the dual presence of *CHEK2* and BRCA2 mutations raises questions about their combined impact on disease penetrance and phenotype. While BRCA2 is strongly associated with high breast cancer risk, the contribution of *CHEK2* to the bilateral presentation and familial clustering in this case remains unclear. Similarly, a patient (P8) with *CHEK2* and biallelic *MUTYH* mutations developed colorectal cancer at age 26, suggesting that *CHEK2* may play a role in accelerating early-onset colorectal cancer in the context of MUTYH-associated polyposis. These cases highlight the complexity of interpreting multiple germline variants and their potential synergistic or additive effects.

Our findings contribute to the growing evidence surrounding biallelic *CHEK2* mutations and MINAS, emphasizing the need for further investigation. Future studies should focus not only on refining the clinical interpretation of *CHEK2* variants, particularly the missense mutations, but also on elucidating its role in a broader range of tumor types.

The predominance of breast cancer in our cohort underscores the clinical importance of *CHEK2* mutations for women's health and reaffirms their well-established role in hereditary breast cancer predisposition. Current NCCN guidelines estimate a lifetime breast cancer risk of 23–27% for women with germline *CHEK2* mutations, with a 10-year cumulative risk of 6–8% for contralateral breast cancer (13). Accordingly, annual mammography from age 40 and consideration of breast MRI beginning at 30–35 years are recommended, while decisions regarding risk-reducing mastectomy should be individualized according to family history. Gynecologic and breast cancers share several risk factors including inherited cancer-associated pathogenic gene variants, family cancer history, early menarche, late menopause, and obesity. The gynecology setting, therefore, may be an ideal environment for

breast cancer risk assessment and subsequent risk management, as many women present first to gynecologists for routine care. In this context, gynecologists play a pivotal role in recognizing hereditary cancer risk, initiating genetic evaluation, and guiding patients to appropriate preventive strategies. The identification of a rare gynecologic tumor (FATWO) in our series further highlights the broader oncologic relevance of *CHEK2* beyond breast cancer. In addition, the frequent familial clustering observed in our cohort emphasizes the importance of cascade testing and genetic counseling for at-risk relatives. Collectively, these findings highlight the need for multidisciplinary collaboration between medical genetics, gynecology, and oncology to optimize risk stratification, surveillance, prevention, and long-term outcomes in women carrying *CHEK2* mutations.

Conflict of interest: The authors declare no competing interests.

Author Contributions: CDD conceptualized and designed the study, performed data analysis, and drafted the manuscript. SA supervised the study and contributed to the manuscript revision. ÖÇA, NB, DDE, and NGL were responsible for genetic analyses, variant interpretation, and manuscript preparation. ÖD, ZA, and SA contributed to the collection and interpretation of clinical data. All authors critically reviewed and approved the final version of the manuscript.

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