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Germline pathogenic variant spectrum in 25 cancer susceptibility genes in Turkish breast and colorectal cancer patients and elderly controls

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Abstract

Inherited pathogenic variants account for 5% to 10% of all breast cancer (BC) and colorectal cancer (CRC) cases. Here, we sought to profile the pathogenic variants in 25 cancer susceptibility genes in Turkish population. Germline pathogenic variants were screened in 732 BC patients, 189 CRC patients and 490 cancer-free elderly controls, using next-generation sequencing-based multigene panel testing and multiplex ligation-dependent probe amplification testing. Pathogenic variants were detected in 17.2% of high-risk BC patients and 26.4% of high-risk CRC patients. More than 95% of these variants were clinically actionable. *BRCA1/2* and mismatch repair genes (*MLH1*, *MSH2* and *MSH6*) accounted for two-thirds of all pathogenic variants detected in high-risk BC and CRC patients, respectively. Pathogenic variants in *PALB2*, *CHEK2*, *ATM* and *TP53* were also prevalent in high-risk BC patients (4.5%). *BRCA1* exons 17-18 deletion and *CHEK2* c.592+3A>T were the most common variants predisposing to BC, and they are likely to be founder variants. Three frequent *MUTYH* pathogenic variants (c.884C>T, c.1437_1439delGGA and c.1187G>A) were responsible for all *MUTYH* biallelic cases (4.4% of high-risk CRC patients). The total pathogenic variant frequency was very low in controls (2.4%) and in low-risk BC

Abbreviations: BC, Breast cancer; CI, confidence interval; CNV, copy number variant; CRC, colorectal cancers; ExAC, Exome Aggregation Consortium; MLPA, multiplex ligation-dependent probe amplification; MMR, mismatch repair; NGS, next-generation sequencing; VUS, variant of uncertain significance; OC, ovarian cancer; OR, odds ratio.

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(3.9%) and CRC (6.1%) patients. Our study depicts the pathogenic variant spectrum and prevalence in Turkish BC and CRC patients, guiding clinicians and health authorities for genetic testing applications and variant classification in Turkish population.

KEYWORDS

breast cancer, colorectal cancers, hereditary cancer predisposition, multigene panel testing

1 | INTRODUCTION

Breast cancer (BC) and colorectal cancers (CRC) are among the most common malignancies.[1] A total of 5% to 10% of all BC and CRC cases result from inherited pathogenic variants in cancer susceptibility genes.[2,3] Germline genetic testing refines risk assessment and affects decisions on prophylactic interventions, surveillance and treatment options for the patients and their at-risk relatives.

The advances in next-generation sequencing (NGS) enabled simultaneous sequencing of multiple target genes, which has become the prevailing paradigm for clinical testing of hereditary cancer predisposition.[4] Multigene panel tests proved to be time-effective and cost-effective, especially for patients with a clinical presentation that warrants analysis of more than one gene.[5-7] However, lack of clear guidelines for moderate and low penetrance genes and increased frequency of variants of uncertain significance (VUS) in panel testing pose a challenge to counseling and risk management.[8,9] Clinical utility of panel tests depends on reliable estimations of gene- and variant-specific risks, which in turn necessitate more large-scale sequencing studies.

Prevalence of pathogenic variants and risk estimates might vary across populations.[10] The pathogenic variant spectrum in BC and CRC patients in Turkish population has not been fully explored, as previous studies were limited in sample size and/or confined to one or few genes.[11-13] Here, we aimed to determine the pathogenic variant frequency in 25 cancer susceptibility genes in a large cohort of Turkish BC and CRC patients using comprehensive cancer panels and multiplex ligation-dependent probe amplification (MLPA) tests. We examined correlations between pathogenic variants and clinical characteristics and compared variant frequencies between high-risk or all cancer patients and elderly controls.

What's New?

Inherited cancer-predisposing genetic variants vary in identity and frequency across populations, potentially impacting genetic testing for risk assessment and treatment. This study examined the spectrum and prevalence of pathogenic variants specifically among Turkish breast and colorectal cancer patients. A total of 25 cancer susceptibility genes were analyzed. Overall, pathogenic variants were mostly non-recurrent or recurrent at low frequencies in the study population. The most notable exception, occurring at greater frequency, was deletion of exons 17-18 in *BRCA1*, a potential founder variant that originated in northeastern Turkey. The findings could inform region-specific strategies for genetic screening in cancer prevention.

2 | MATERIALS AND METHODS

2.1 | The study population

The study population consisted of 1407 unrelated subjects, including 728 patients with BC, 185 patients with CRC, 4 patients with both BC and CRC, and 490 elderly controls. Subjects were registered at the Teaching and Research Hospitals of Umraniye (UEAH), Goztepe (GEAH) and Kartal (KEAH) in Istanbul, Turkey, between November 2016 and December 2019 and were referred to Genomic Laboratory (GLAB) of UEAH for genetic testing. Detailed demographic and clinical characteristics of all subjects, including gender, age at diagnosis, age at testing, histopathology reports, pedigree information and family cancer history, were provided by the referring clinicians at the first-registered hospital and requestioned by the medical geneticists at UEAH. Control subjects were above 65 years old with no personal cancer history and no first-degree relative with BC, CRC, ovarian cancer (OC) or endometrial cancer, selected among those who had other medical conditions such as hypertension, diabetes and flu. Genetic counseling was given to all participants after germline genetic testing. Genetic risk assessment for cancer predisposition was based on National Comprehensive Cancer Network (NCCN) guidelines[14,15] with minor modifications (Table S1). BC and CRC patients were stratified into high-risk and low-risk groups after reviewing their personal and family cancer histories.

2.2 | Multigene panel sequencing and bioinformatics analysis

Genomic DNAs were extracted from peripheral blood using QIAamp DNA Mini QIAcube kit (Qiagen). DNA libraries were prepared using three different commercial kits (Table S2). TruSight Cancer Panel kit (Illumina) was used for the first 96 subjects; BRCA Hereditary Cancer MASTR Plus and FAP MASTR kits (Multiplicom) were used for the next 259 subjects; and Sophia Hereditary Cancer Solution kit (Sophia Genetics) was used for the last 1052 subjects. Libraries prepared with TruSight and Multiplicom kits were sequenced on a MiSeq instrument (Illumina) with paired-end reads (2 × 300 bp), using MiSeq Reagent v3

kit. Libraries prepared with Sophia kit were sequenced on a NextSeq 500 instrument (Illumina) with paired-end reads (2 × 150 bp), using NextSeq 500/550 Mid Output v2 kit.

All sequencing kits covered the complete coding regions of *APC*, *ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *MUTYH*, *NBN*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11* and *TP53* genes. These 19 genes were regarded as the virtual common panel. *BARD1*, *MRE11*, *ABRAXAS1*, *XRCC2*, and *RAD50*, included in Multiplicom and Sophia kits, were sequenced in 93.2% of all subjects, and *PIK3CA*, included only in Sophia kit, was sequenced in 74.8% of all subjects. These 6 genes along with 19 common genes constituted the virtual comprehensive panel (25 genes in total). Test results were reported with regard to the comprehensive panel outcome unless otherwise specified. The penetrance and clinical actionability of cancer susceptibility genes are given in Table S3.

Sequence alignment, variant calling and annotation were performed using the Sophia DDM software (Sophia Genetics v4.2). Sequence reads were aligned to the reference human genome GRCH37/hg19. The threshold for variant calling was set at 50× coverage and 25% variant fraction. Low confidence regions on high and moderate penetrance genes were Sanger sequenced unless a pathogenic variant elsewhere was detected. All identified pathogenic variants were confirmed by Sanger sequencing. To unambiguously call variants at exons 9 and 11-15 of *PMS2* and exons 11-15 of *CHEK2*, which share high sequence homology with their respective pseudogenes, Sanger sequencing was performed after long-range PCR amplification with allele-specific primers and subsequent nested PCRs.

2.3 | Variant classification

Variants at the coding regions ±10 bp into introns were interpreted according to the five-tier variant classification system of the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP)[16] using the wInterVar web server.[17] Online databases (eg, ClinVar, dbSNP, HGMD, 1000 Genomes, GnomAD) and search engines (eg, PubMed, LitVar, OMIM, Human Splicing Finder, VarSome) were used to collect available information on variants, such as population frequencies, evolutionary conservation, *in silico* prediction scores, and published case-control, co-segregation and functional studies. Variants that were not reported in online databases were regarded as novel. Test results were classified as positive if a pathogenic/likely pathogenic variant (hereafter, referred to as pathogenic variant) was detected, inconclusive if only VUS values were detected, or negative if only benign/likely benign variants were detected. Due to the autosomal recessive inheritance of *MUTYH*-associated polyposis syndrome, only biallelic *MUTYH* pathogenic variants were regarded as a positive result.

2.4 | Detection of copy number variants

Around 60% of BC patients received MLPA testing for *BRCA1* and *BRCA2*, and around 64% of high-risk CRC patients received MLPA testing for *MLH1*, *MSH2*, *MSH6* and exon 9 of *EPCAM* (closely upstream of *MSH2*) as part of diagnostic testing at the request of the ordering clinician. Additional MLPA tests were ordered for *CDH1* and *STK11* in BC patients, and *STK11*, *APC*, *PMS2* and *BRCA1/2* in CRC patients if either a copy number variants (CNV) in any of these genes was predicted with high confidence in post-NGS CNV analysis or the patient had a family cancer history and/or clinical characteristic suggestive of a defect in the respective gene. Computational CNV predictions were performed successfully for more than 90% of all subjects using Sophia DDM software; however, predicted CNVs were not reported without confirmation by MLPA, which is the gold standard in identifying CNVs.[18] No gross deletion in any high-penetrance gene was predicted in control subjects; hence none of the controls received MLPA testing.

2.5 | Statistical analysis

Significant differences in age at diagnosis between groups were assessed by Wilcoxon rank-sum test. Fisher's exact test was used for all other analyses, including comparisons of pathogenic variant positive rates among patient groups and controls, comparisons of clinical characteristics between pathogenic variant carriers and noncarriers, and case-control analyses for gene-based risk assessment. For the latter analysis, odds ratios (ORs) were calculated using (a) controls of our study and (b) non-Finnish European controls of Exome Aggregation Consortium (ExAC) project as reported in previous studies.[19,20] Patients with more than one pathogenic variant were excluded to avoid risk overestimation. For all analyses involving comparison of controls of our study to BC patients, only female controls were used. For all analyses involving BC patients with the exception of positive test result rates in high-risk BC group, only female patients were used. All tests were two-sided, and the threshold of significance was set at $P < .05$. All analyses were performed using the R statistical software.

3 | RESULTS

3.1 | Germline genetic test outcomes

A total of 728 patients with BC, 185 patients with CRC, 4 patients with both BC and CRC and 490 healthy controls received germline genetic testing. The clinical characteristics of subjects are given in Table 1 and Table S4. According to genetic risk assessment criteria adapted from NCCN guidelines, 82.7% of BC cases and 48.1% of CRC cases were in the high-risk group for hereditary cancer, and the remaining cases were in the low-risk group.

Multigene panel testing for 25 cancer susceptibility genes supplemented with MLPA analysis for high penetrance genes identified 119 unique pathogenic/likely pathogenic variants in 149 subjects, excluding 4 *MUTYH* pathogenic variants found as a single copy (Table S5). Of which, 22.7% (27/119) of pathogenic variants were novel, and 16.0% (19/119) pathogenic variants were recurrent in multiple unrelated individuals. 25.5% (38/149) of all individuals with a positive result also carried at least one VUS. Four hundred four unique VUS (excluding those in *MUTYH*) were identified in 487 subjects. Of

TABLE 1 Clinical characteristics of study population

Clinical characteristics	n	%
Breast cancer patients	732[a]	100
Sex		
Female	720	98.4
Male	12	1.6
Age at diagnosis		
≤30	27	3.7
30 to 40	184	25.1
40 to 50	304	41.5
50 to 60	133	18.2
>60	84	11.5
Bilateral or multicentric BC	26	3.6[b]
Ovarian cancer in personal history	10	1.4[b]
TNBC ≤ 60 years	68	9.3
Receptor status		
ER+/ER−	518/141	70.8/19.3
PR+/PR−	467/191	63.8/26.1
HER2+/HER2−	191/417	26.1/57
Family history of cancer		
Yes	189	25.8
No	543	74.2
Colorectal cancer patients	189[a]	100
Sex		
Female	89	47.1
Male	100	52.9
Age at diagnosis		
≤40	22	11.6
40 to 50	46	24.3
50 to 60	51	27
>60	70	37
MMR status		
MMR proficient	88	46.6
MMR deficient	20	10.6
Family history of cancer		
Yes	37	19.6
No	152	80.4
Healthy controls	490	100
Sex		
Female	307	62.7
Male	183	37.3
Age at testing		
65 to 70	99	20.2
70 to 75	192	39.2
75 to 80	110	22.4
80 to 90	76	15.5
>90	13	2.7

Abbreviation: MMR, mismatch repair.
[a]Including four patients with both BC and CRC.
[b]Percentage in female BC cases.

which, 96 VUS were novel and 101 VUS were recurring in multiple individuals (Table S6).

The rate of pathogenic variant-positive individuals in low-risk BC patients (3.9%) was significantly lower than that in high-risk BC patients (17.2%; OR = 0.197, 95% confidence interval, CI = [0.079-0.495], $P = 3.0 \times 10^{-5}$), but not significantly higher than that in controls (2.6%; OR = 1.532, 95% CI = [0.491-4.775], $P = .54$) (Figure 1A). Likewise, the rate of positive individuals in low-risk CRC patients (6.1%) was significantly lower than that in high-risk CRC patients (26.4%; OR = 0.182, 95% CI = [0.071-0.470], $P = 1.3 \times 10^{-4}$), but not significantly higher than that in controls (2.4%; OR = 2.598, 95% CI = [0.951-7.098], $P = .10$). Around 30% or more of all subjects in each group received an inconclusive result due to presence of VUS. Inconclusive rates would be below 20% if smaller panels (eg, cancer-specific panels with only high- and moderate-penetrance genes) were used (Figure S1).

The proportion of clinically actionable pathogenic variants with regard to the cancer of the proband was significantly higher in high-risk patients than in low-risk patients (87.5% vs 40.0%, $P = .02$ for BC; 83.3% vs 33.3%, $P = .03$ for CRC) (Figure 1B). Most of the remaining pathogenic variants in high-risk patients were actionable for other cancers.

3.2 | Prevalence of pathogenic variants in BC patients

Among 605 high-risk BC patients, 17.2% (n = 104) were positive for germline pathogenic variants. Positives rates varied across patient subgroups, reaching as high as 50% for patients with both BC and OC (Table 2; Figure 2). Of which, 2 patients carried pathogenic variants in two different genes (*BRCA1/MUTYH*-biallelic and *NBN/MSH6*). Pathogenic variants in high- and moderate-penetrance BC genes were significantly enriched in high-risk BC patients compared to healthy female controls (13.1% vs 0.7%, $P = 8.7 \times 10^{-13}$; and 2.5% vs 0.3%, $P = 1.6 \times 10^{-2}$, respectively). *BRCA1* (n = 44) and *BRCA2* (n = 21) accounted for 61.3% of all pathogenic variants (or 10.7% of all cases) in high-risk BC patients. 43.2% (19/44) of *BRCA1* pathogenic variants were large genomic deletions. In contrast, no large deletion was detected in *BRCA2*. *BRCA1* exons 17-18 deletion (n = 13) was the most common pathogenic variant identified in our study after *MUTYH* pathogenic variants. However, this deleterious variant was not evenly distributed in Turkish population, but was rather confined to north-eastern Turkey. 2.3% (14/605) of high-risk BC patients carried pathogenic variants in other high-penetrance BC genes; *PALB2* (n = 9), *TP53* (n = 3), *STK11* (n = 1) and *CDH1* (n = 1). 2.5% (15/605) of high-risk BC patients carried pathogenic variants in moderate-penetrance genes *CHEK2* (n = 9) and *ATM* (n = 6). Notably, *CHEK2* c.592+3A>T (n = 5) was the most frequent pathogenic single nucleotide variant (SNV) among all BC patients.

The frequencies of pathogenic variants in low-penetrance or suspected BC genes (n = 6) and classical CRC-associated genes (n = 6) were not significantly higher in high-risk BC patients than in female controls (1.0% vs 1.3%, $P = .74$; 1.0% vs 0.3%, $P = .43$, respectively). Finally, among 127 low-risk BC patients, 3.9% (n = 5) of patients had a positive result; 2 pathogenic variants were in *CHEK2*, and 3 pathogenic

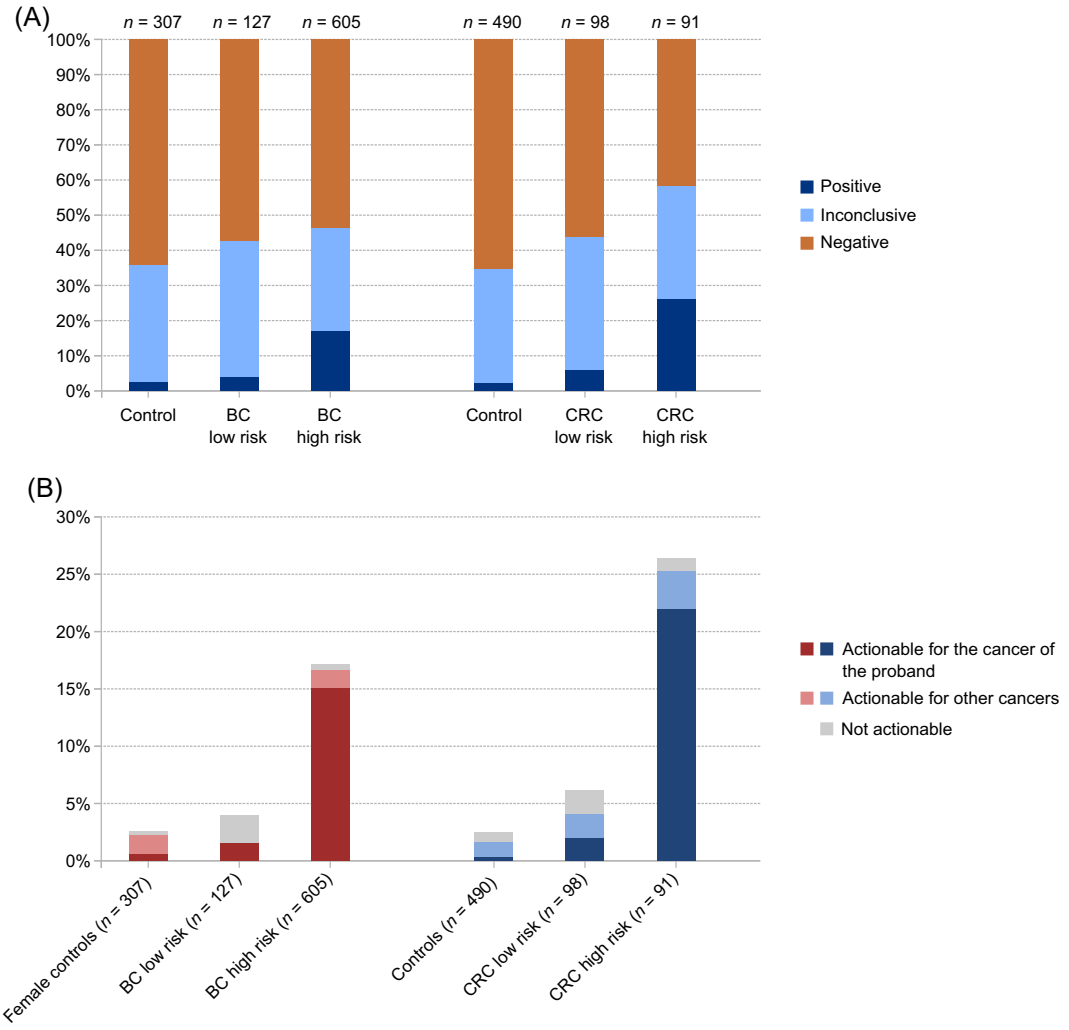

FIGURE 1 Germline genetic test outcomes by patient groups. A, Percentage of positive, inconclusive and negative test results. B, Percentage of patients with clinically actionable pathogenic variants. BC, breast cancer; CRC, colorectal cancer [Color figure can be viewed at wileyonlinelibrary.com]

variants were in low-penetrance genes. ORs of affected genes for BC risk are presented in Table S7.

3.3 | Clinical characteristics of BC patients with pathogenic variants

Women with pathogenic variants in *BRCA1* (41.3 years, $P = 4.8 \times 10^{-4}$), *BRCA2* (41.6 years, $P = 9.7 \times 10^{-3}$) and *TP53* (27.6 years, $P = 4.6 \times 10^{-3}$) were diagnosed with BC at a younger age compared to women with no pathogenic variants (46.7 years) (Table S8). The age at diagnosis was similar between carriers of *PALB2*, *ATM* and *CHEK2* pathogenic variants and noncarriers. Women with *BRCA1* pathogenic variants were more likely to report triple negative (ER– PR– HER2–) tumors (46.3% vs 12.4%, $P = 1.8 \times 10^{-8}$) compared to women with no pathogenic variants. In contrast, none of the patients with *ATM* or *CHEK2* pathogenic variants had ER/PR– HER2– tumor, consistent with previous reports.[19]

Compared to BC patients with no pathogenic variants, *BRCA1* and *BRCA2* pathogenic variant carriers were more likely to report family history of early-onset BC (≤45 years) (34.9% in *BRCA1*, and 35.0% in *BRCA2* vs 13.2%; $P = 4.8 \times 10^{-4}$ and $P = 1.3 \times 10^{-2}$, respectively) and family history of OC (9.3% in *BRCA1*, and 15.0% in *BRCA2* vs 2.9%; $P = 4.2 \times 10^{-2}$ and $P = 2.5 \times 10^{-2}$). In contrast, carriers of *PALB2*, *CHEK2* and *ATM* pathogenic variants were not associated with higher rates of family history. Carriers of *BRCA1* pathogenic variants were more likely to report personal history of multiple BC primaries (9.3% vs 2.9%, $P = 4.9 \times 10^{-2}$) and OC (11.6% vs 0.8%, $P = 1.9 \times 10^{-4}$) compared to noncarriers. *CHEK2* pathogenic variant carriers were also nominally associated with multiple BC primaries (18.2% vs 2.9%, $P = 4.5 \times 10^{-2}$), consistent with previous studies.[21] Of three patients with *TP53* pathogenic variants, two patients did not have any relative with cancer from Li-Fraumeni syndrome cancer spectrum. One BC patient with a pathogenic variant in *STK11* and another with a pathogenic variant in *CDH1* had family cancer histories suggestive of Peutz-Jeghers syndrome (three second-degree relatives with

TABLE 2 Pathogenic variants by genes and clinical characteristics

Subgroup characteristics (n)	Patients with pathogenic variants, n (%)	Affected genes (n)
Breast cancer (BC) patients		
High-risk (605)	104 (17.2)	*BRCA1* (44)[a], *BRCA2* (21), *PALB2* (9), *TP53* (3), *STK11* (1), *CDH1* (1), *CHEK2* (9), *ATM* (6), *BRIP1* (2), *RAD51C* (2), *NBN* (1)[b], *MRE11* (1), *MLH1* (2), *MSH6* (1)[b], *PMS2* (2), biallelic *MUTYH* (1)[a]
Low-risk (127)	5 (3.9)	*CHEK2* (2), *BARD1* (1), *XRCC2* (1), *RAD50* (1)
BC dx ≤50 y (515)	87 (16.9)	*BRCA1* (37)[a], *BRCA2* (17), *PALB2* (8), *TP53* (3), *CHEK2* (8), *ATM* (6), *BRIP1* (1), *RAD51C* (2), *NBN* (1)[b], *MRE11* (1), *MLH1* (1), *MSH6* (1)[b], *PMS2* (2), biallelic *MUTYH* (1)[a]
Multiple BC primaries (26)	8 (30.8)	*BRCA1* (4), *BRCA2* (1), *CHEK2* (2), *ATM* (1)
TNBC dx ≤60 y (68)	24 (35.3)	*BRCA1* (18), *BRCA2* (1), *PALB2* (2), *TP53* (1), *RAD51C* (2)
Personal OC history (10)	5 (50.0)	*BRCA1* (5)
Male BC (12)	1 (8.3)	*BRCA2* (1)
≥1 relative with BC dx ≤45 y (110)	28 (25.5)	*BRCA1* (15), *BRCA2* (7), *TP53* (1), *CHEK2* (1), *ATM* (2), *BRIP1* (1), *MLH1* (1)
≥2 relatives with BC (77)	15 (19.5)	*BRCA1* (5), *BRCA2* (4), *PALB2* (1), *TP53* (1), *CHEK2* (2), *ATM* (1), *BRIP1* (1)
≥1 relative with OC (25)	7 (28.0)	*BRCA1* (4), *BRCA2* (3)
Colorectal cancer (CRC) patients		
High-risk (91)	24 (26.4)	*BRCA1* (1), *CHEK2* (1), *ATM* (2), *BRIP1* (1), *MLH1* (8), *MSH2* (5), *MSH6* (2), biallelic *MUTYH* (4)
Low-risk (98)	6 (6.1)	*BRCA2* (2), *ATM* (1), *XRCC2* (1), *MLH1* (1), biallelic *MUTYH* (1)
CRC dx ≤50 y (68)	18 (26.5)	*CHEK2* (1), *BRIP1* (1), *MLH1* (8), *MSH2* (5), biallelic *MUTYH* (3)
MMR-deficient tumors (20)	10 (50.0)	*ATM* (1), *MLH1* (4), *MSH2* (4), *MSH6* (1)
≥1 relative with CRC/EC dx ≤50 y (23)	12 (52.2)	*CHEK2* (1), *MLH1* (6), *MSH2* (4), biallelic *MUTYH* (1)
≥2 relatives with CRC (30)	15 (50.0)	*BRCA1* (1), *CHEK2* (1), *ATM* (1), *MLH1* (6), *MSH2* (4), *MSH6* (1), biallelic *MUTYH* (1)
Controls		
Both sexes (490)	12 (2.5)	*BRCA2* (2), *CHEK2* (2), *ATM* (1), *BRIP1* (4), *NBN* (2), *PMS2* (1)
Females (307)	8 (2.6)	*BRCA2* (2), *CHEK2* (1), *BRIP1* (4), *PMS2* (1)

Note: a and b indicate individuals with two pathogenic variants (a, *BRCA1*/biallelic *MUTYH*; b, *NBN*/*MSH6*).
Abbreviations: BC, breast cancer; CRC, colorectal cancers; dx, age at diagnosis (y, years old); EC, endometrial cancer; MMR, mismatch repair; n, number of individuals; OC, ovarian cancer.

gastrointestinal cancers) and hereditary diffuse gastric cancer syndrome (two first-degree relatives with gastric cancer), respectively.

3.4 | Prevalence of pathogenic variants in CRC patients

26.4% (24/91) of all high-risk CRC patients and 50.0% (10/20) of CRC patients with mismatch repair (MMR)-deficient tumors were positive for pathogenic variants (Table 2; Figure 3). Pathogenic variants in Lynch syndrome genes (8 in *MLH1*, 5 in *MSH2* and 2 in *MSH6*) accounted for 62.5% of all pathogenic variants (or 16.5% of cases) in high-risk CRC patients. Biallelic *MUTYH* pathogenic variants accounted for 4.4% (4/91) of all patients in this group. 3.3% (3/91) of high-risk CRC patients had pathogenic variants in moderate-penetrance CRC genes *ATM* (n = 2) and *CHEK2* (n = 1), and 2.2% (2/91) had pathogenic variants in known or suspected BC susceptibility genes. Among 98 low-risk CRC patients, 6.1% (n = 6) had pathogenic variants; 3 of them were in high- or moderate-penetrance CRC genes, and 3 of them were in BC-related genes. ORs of affected genes for CRC are presented in Table S7.

Monoallelic *MUTYH* pathogenic variants were at similar frequencies in CRC patients and controls (2.1% vs 1.8%, $P = .762$). Three *MUTYH* pathogenic variants were frequently recurrent and were responsible for all biallelic *MUTYH* cases. Their allele frequencies in general Turkish population were estimated by calculating allele frequencies in non-CRC participants. Accordingly, *MUTYH* c.884C>T (0.0090 vs 0.00002643, $P < 2.2 \times 10^{-16}$) and c.1437-1439delGGA (0.0041 vs 0.0001319, $P = 1.8 \times 10^{-6}$) were significantly more

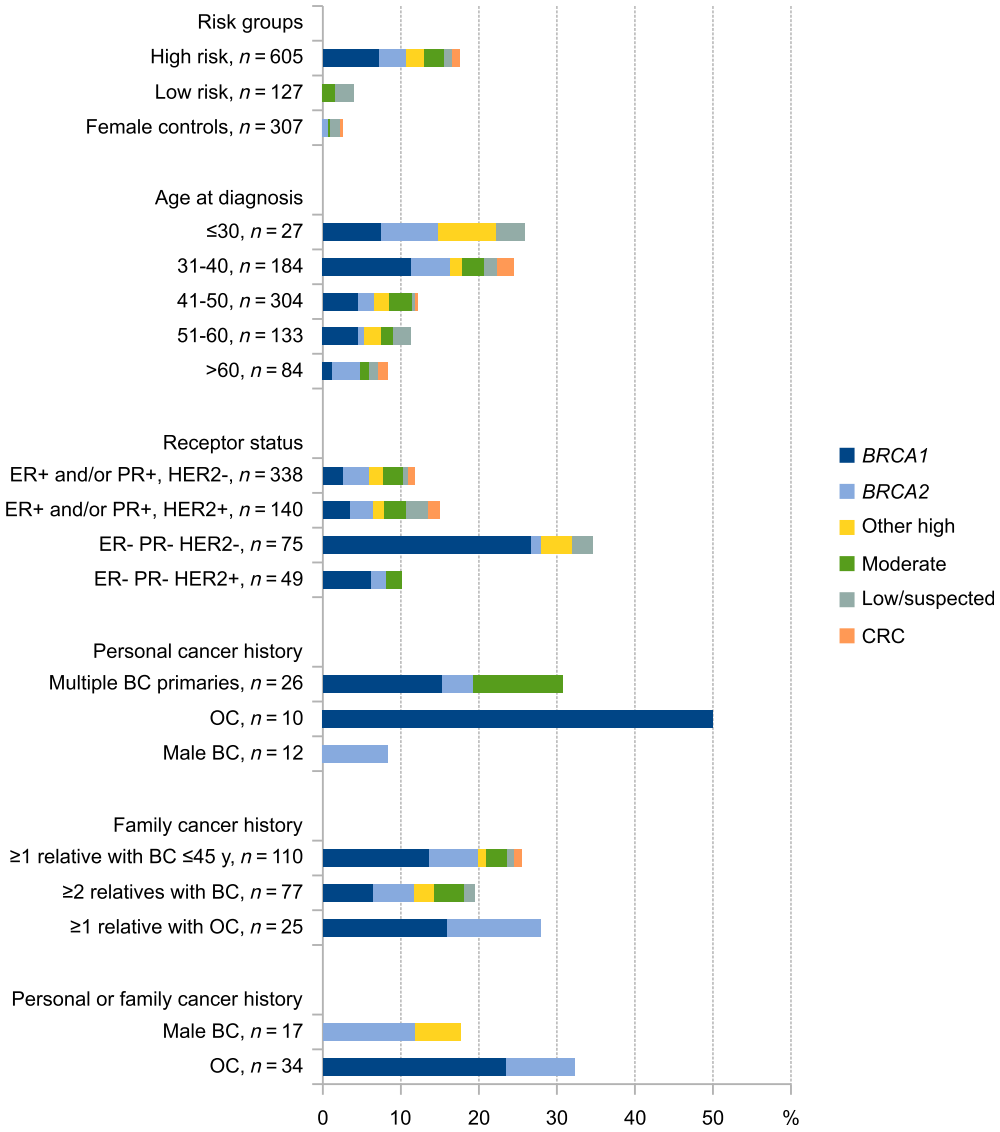

FIGURE 2 Frequency of affected genes by clinical characteristics in BC patients. Genes are grouped as follows: *BRCA1*, *BRCA2*, other high-penetrance BC genes (*PALB2*, *TP53*, *STK11*, *CDH1*); moderate-penetrance BC genes (*ATM*, *CHEK2*); low-penetrance/suspected BC or OC genes (*BRIP1*, *RAD51C*, *MRE11*, *NBN*, *BARD1*, *RAD50*, *XRCC2*); and CRC genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, biallelic *MUTYH*) [Color figure can be viewed at wileyonlinelibrary.com]

frequent in Turkish population compared to non-Finnish European population, whereas *MUTYH* c.1187G>A was found at similar frequencies (0.0057 vs 0.004894, $P = .67$).

3.5 | Clinical characteristics of CRC patients with pathogenic variants

The age at CRC diagnosis was significantly younger in *MLH1* (38.4 years, $P = 1.4 \times 10^{-4}$) and *MSH2* pathogenic variant carriers (42.3 years, $P = 7.5 \times 10^{-3}$), and older in *MSH6* pathogenic variant carriers (66.4 years, $P = .145$) than in CRC patients with no pathogenic variant (56.5 years), in agreement with previous reports[22] (Table S9). *MLH1* and *MSH2* pathogenic variant carriers were more likely to report having two or more relatives with CRC and/or endometrial cancer (71.4% vs 9.4% for noncarriers, $P = 4.5 \times 10^{-7}$), and having relatives with early-onset (≤50 years) CRC/endometrial cancer (71.4% vs 6.9%, $P = 5.4 \times 10^{-8}$). Among CRC patients with Lynch syndrome gene defects, for which tumor pathology results were available, 90.0% (9/10) showed loss of MMR gene expression in their tumors. The only exception to this was one *MSH6* pathogenic variant carrier with normal tumor staining. *MUTYH* biallelic pathogenic variant carriers were diagnosed with CRC at an average of 44.8 years old, and were not significantly enriched for a family history of CRC. Of 5 *MUTYH* biallelic pathogenic variant carriers, only one had a polyp history of 10 or more adenomas at the time of CRC diagnosis. Finally, one CRC patient with 5 or more serrated polyps was found to carry a pathogenic variant in *ATM*.

3.6 | Molecular characteristics of pathogenic variants and VUS in all participants

Pathogenic variants in all genes but *MUTYH* were mostly truncating variants (eg, nonsense, frame-shift and splice site alterations) (Figure S2). Missense and/or in-frame deletion pathogenic variants prevailed over truncating pathogenic variants only in *MUTYH* (97.4%), but were also

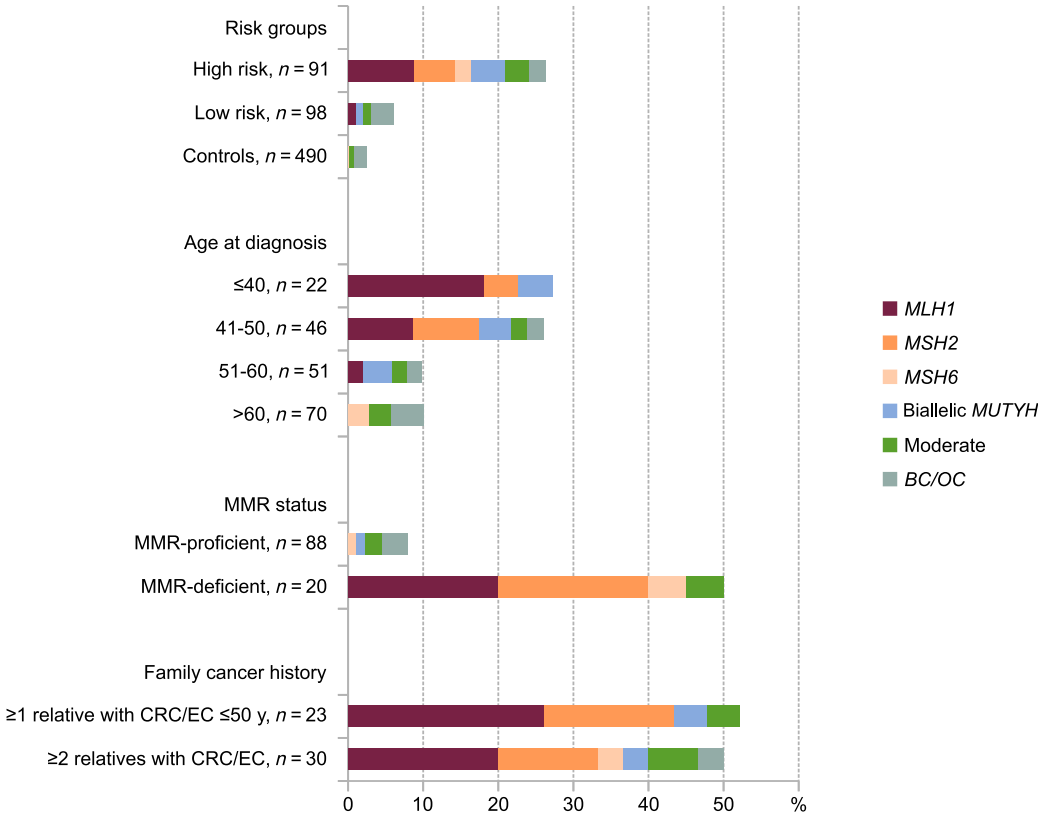

FIGURE 3 Frequency of affected genes by clinical characteristics in CRC patients. Genes are grouped as follows: *MLH1*, *MSH2*, *MSH6*, biallelic *MUTYH*, moderate-penetrance CRC genes (*ATM*, *CHEK2*), and BC or OC genes (*BRCA1*, *BRCA2*, *BRIP1*, *XRCC2*) [Color figure can be viewed at wileyonlinelibrary.com]

found at considerably high fraction (28.6%) in *CHEK2*. Even though most, or all, pathogenic variants were single nucleotide variants (SNVs) or small insertion-deletions (indels) in each gene, the number of deleterious CNVs (or CNVs) was also exceptionally high in *BRCA1* (42.2%). Pathogenic variants outnumbered VUS only in *BRCA1* and *MUTYH* genes. In contrast to pathogenic variants, the majority of VUS were missense variants (561/612, or 91.7%). Intronic/splice site variants were also common among VUS (7.7%). An exon duplication in *BRCA2* was the only CNV classified as VUS in our study. *ATM* (n = 74) had the highest number of VUS, followed by *CHEK2* (n = 59). However, *CHEK2* by far had the highest number of VUS per coding region length (36.2 per 1000 nucleotides), and the highest number of missense change per protein length (99.4 per 1000 amino acids) (Figure S3).

4 | DISCUSSION

Here, we describe the pathogenic variant spectrum and prevalence in 25 cancer susceptibility genes in a cohort of 732 BC patients, 189 CRC patients and 490 elderly controls of Turkish nationality. To the best of our knowledge, this is the largest assessment of pathogenic variants beyond *BRCA1/2* genes for BC patients and beyond Lynch syndrome genes for CRC patients in Turkish population. 27 of 119 pathogenic variants were newly identified in our study. Several recurrent pathogenic variants were identified; a fraction of them are likely to be founder in Turkish population. 17.2% of high-risk BC patients and 26.4% of high-risk CRC patients were found to have a germline pathogenic variant, as opposed to 2.4% of elderly controls. One major aspect of our study was its well-curated and detailed data on personal and family cancer histories. This enabled evaluation of pathogenic variant positivity rates for a range of risk criteria for hereditary cancer predisposition. These findings provide valuable data to healthcare providers and health authorities, which would be useful for their decision- or policy-making processes in genetic testing for different patient groups.

BRCA1/2 pathogenic variants accounted for 62.5% of all pathogenic variants in high-risk BC patients, explaining 10.7% of high-risk BC cases. *BRCA1* pathogenic variants were more frequent than *BRCA2* pathogenic variants as in other European and Middle Eastern, but not Asian, populations.[23-25] The most common pathogenic variant in BC patients was *BRCA1* exons 17-18 deletion. Interestingly, all carriers of this allele originated from the northeastern region of Turkey (Black Sea coastline from Giresun to Trabzon), consistent with previous reports.[26] Therefore, *BRCA1* exons 17-18 deletion is likely to be a founder pathogenic variant. Owing to this variant, the percentage of CNVs among all *BRCA1* pathogenic variants was very high in our study (43.2%), compared to most other populations (10-15%).[27,28] Inflated rates of *BRCA1* CNVs were also observed in Dutch population (27%) and northern Italian population (40%) because of founder CNVs.[29,30] 27.9% of all pathogenic variants in high-risk BC patients

were in non-*BRCA* high-penetrance and moderate-penetrance BC genes, explaining 4.8% of cases. These variants would have been missed if genetic testing was confined to *BRCA1/2*, and the clinical management of carriers would not have been tailored based on their genotype. Case-control analysis using controls from the ExAC project demonstrated significant associations of *BRCA1*, *BRCA2*, *PALB2*, *TP53*, *CHEK2* and *ATM* with BC. ORs for *PALB2* (OR = 14.02) and *TP53* (OR = 8.89) were consistent with their classification as high-penetrance genes, whereas *ATM* (OR = 2.82) and *CHEK2* (OR = 2.55) had ORs in agreement with their moderate-penetrance classification. However, these OR calculations might be confounded by the relatively small sample size of cases and potential overestimation bias (see later). *PTEN*, *CDH1* and *STK11* pathogenic variants were rare. The *CHEK2* c.592+3A>T splice donor site variant, which causes aberrant splicing and frameshift,[31] was very frequent, and a potential founder pathogenic variant in the Turkish population. Ashkenazi founder variant *BRCA1* c.5266dupC, which was previously reported as the most frequent pathogenic variant Turkish BC/OC patients,[12,13] was not identified among the BC cases in our study. The only pathogenic variant detected among 12 male BC patients was in *BRCA2*, which has been repeatedly found as the most frequently affected gene in male BC.[32] Low-penetrance BC genes and CRC-associated genes were not frequently affected in BC patients.

Lynch syndrome genes (62.5%) and biallelic *MUTYH* pathogenic variants (16.7%) accounted for 79.2% of all pathogenic variants detected in high-risk CRC patients, explaining 20.9% of the cases. An additional 12.5% of pathogenic variants were in moderate-penetrance genes *CHEK2* and *ATM*, for which management guidelines are evolving.[15] Case-control analysis demonstrated significant associations of *MLH1*, *MSH2*, *MUTYH* (biallelic) and *ATM* with CRC and supported evidence for *ATM* as a CRC susceptibility gene (OR = 4.66-5.30). MMR deficiency in tumor was a strong predictor of a pathogenic variant in Lynch syndrome genes, and vice versa, as reported previously.[33] In contrast, four of five biallelic *MUTYH* pathogenic variant carriers did not develop polyposis at the time of CRC diagnosis, and hence would have been missed if *APC/MUTYH* screening was confined to the clinical presentation of polyposis. Strikingly, parents of all *MUTYH* homozygous pathogenic variant carriers were reported to be relatives (ie, consanguinity) or have originated from the same town (ie, likely to be relatives). No pathogenic variant in *APC* was detected in this cohort, likely because patients with more than 30 polyps were referred for *APC/MUTYH* testing instead of comprehensive cancer panel testing.

CNVs comprised 17.2% of all pathogenic variants in high-risk cancer patients (7.6% without *BRCA1* exons 17-18 deletion). Computational CNV predictions and MLPA results for *BRCA1* were fully concordant. For most other genes, predictions were not reliable, as many predicted CNVs were recurrent on the same NGS run, suggestive of false positives, and many CNVs were not confirmed by MLPA tests. For instance, a total of 11 CNVs were predicted in *BRCA2* (6 of them being recurrent on the same run), and only one of them was confirmed by MLPA. Additionally, two MLPA-detected CNVs (in *STK11* and *MSH6*) were not computationally predicted. Therefore, current prediction algorithms are not always effective, and MLPA must be used not only as a confirmatory test, but also as a screening test at least for high-penetrance genes in the clinical setting.

National guidelines currently recommend germline genetic testing only for high-risk cancer patients.[34] We and others[8] found that the frequencies of pathogenic variants in low-risk BC/CRC patients were not significantly higher than that observed in cancer-free controls, supporting the risk-stratification approach in selecting patients for testing. However, sample sizes of the low-risk groups in our study were relatively small, and more extensive health economics and outcomes research is necessary for a comprehensive cost-benefit analysis of testing for low-risk patients.

Fourteen of 490 elderly healthy controls (2.9%) might be at high risk for hereditary predisposition to cancer, mostly due to their second-degree relatives with early-onset BC (≤45 years) or CRC (≤50 years). However, none of them carried pathogenic variants. Likewise, control subjects with pathogenic variants (n = 12) did not have a meaningful family history of BC or CRC.

High VUS rates decrease the clinical utility of panel testing.[35] Classification of VUS might change as more information accumulates.[36] We aim at establishing a publicly available reference dataset for 25 cancer susceptibility genes using a cohort of more than 1000 elderly controls to improve the interpretation of recurring VUS. For instance, among the three most frequent VUS in *CHEK2*, c.549G>C (p.Leu183Phe) was found in 6 BC patients, 2 CRC patients and 1 female control, and c.1053G>T (p.Glu351Asp) was found in 5 BC patients and 2 CRC patients, but not in controls. These two VUS are likely to be reclassified as likely pathogenic. On the other hand, c.1427C>T (p.Thr476Met) was found in 6 BC patients, 2 CRC patients, 3 female controls and 2 male controls, and it is likely to be reclassified as likely benign, if not, as a low-risk allele.

There are a few limitations in our study. Enrolled patients were not a series of consecutive cases, but rather a heterogeneous mixture of patients preselected by several referring clinicians. Risk assessment of genes and variants might have been overestimated as the case groups were enriched with high-risk patients (ie, higher frequency of pathogenic variants), and the controls were elderly cancer-free individuals instead of general population controls. Risk assessment was also performed using non-Finnish European controls from ExAC project; however, the sample size of cases was still not large enough to refine risk estimates. Another drawback of using ExAC controls was the potential differences in the genetic background between Turkish and Europeans. Estimations and comparisons of pathogenic variant positivity rates might have been confounded by the small sample sizes of low-risk BC, low-risk CRC and high-risk CRC groups. Participants were selected from three public hospitals in Istanbul, where diverse subpopulations across Turkey have intermixed. Despite this, the study population was likely not representative of the whole Turkish population because of neighborhood effect. Families of most participants originated from northern and eastern parts of Turkey, therefore, studies in other regions of Turkey might detect different recurring pathogenic variants (see References 12 and 13). Accordingly, the prevalence of *BRCA1* exons 17-18 deletion among all Turkish BC patients must be smaller than reported here. Certain genes that are known to contribute to hereditary BC or CRC risk

(eg, *NF1*, *RECQL*, *SMAD4*, *BMPR1A*, *POLE*, *POLD1*)[37] and CNVs in moderate- and low-penetrance genes were not investigated in our study. Nevertheless, their prevalence was previously reported to be low.[19,28]

In conclusion, our study represents one of the first steps in exploring the genetic underlying of BC and CRC predisposition in both well-established and moderate-to-low penetrance cancer susceptibility genes in Turkish population. Results of our study highlight the importance of multigene panel testing in diagnosing genetic predisposition to BC and CRC, and inform clinicians for risk assessment and variant classification processes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

All participants consented to the use of their specimens and data for research. Our study was approved by the ethical committee of Umraniye Teaching and Research Hospital (UEAH) (No: 49/24.03.2016).

DATA AVAILABILITY STATEMENT

Data are available from the corresponding authors upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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