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Recurrent moderate-risk mutations in Finnish breast and ovarian cancer patients

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Mutations in *BRCA1* and *BRCA2* genes predispose to breast and ovarian cancer (BC/OC) with a high lifetime risk, whereas mutations in *PALB2*, *CHEK2*, *ATM*, *FANCM*, *RAD51C* and *RAD51D* genes cause a moderately elevated risk. In the Finnish population, recurrent mutations have been identified in all of these genes, the latest being *CHEK2* c.319+2T>A and c.444+1G>A. By genotyping 3,156 cases and 2,089 controls, we estimated the frequencies of *CHEK2* c.319+2T>A and c.444+1G>A in Finnish BC patients. *CHEK2* c.319+2T>A was detected in 0.7% of the patients, and it was associated with a high risk of BC in the unselected patient group (OR = 5.40 [95% CI 1.58–18.45], $p = 0.007$) and similarly in the familial patient group. *CHEK2* c.444+1G>A was identified in 0.1% of all patients. Additionally, we evaluated the combined prevalence of recurrent moderate-risk gene mutations in 2,487 BC patients, 556 OC patients and 261 *BRCA1/2* carriers from 109 families. The overall frequency of the mutations was 13.3% in 1,141 *BRCA1/2*-negative familial BC patients, 7.5% in 1,727 unselected BC patients and 7.2% in 556 unselected OC patients. At least one moderate-risk gene mutation was found in 12.5% of *BRCA1* families and 7.1% of *BRCA1* index patients, as well as in 17.0% of *BRCA2* families and 11.3% of *BRCA2* index patients, and the mutations were associated with an additional risk in the *BRCA1/2* index patients (OR = 2.63 [1.15–5.48], $p = 0.011$). These results support gene panel testing of even multiple members of BC families where several mutations may segregate in different individuals.

Introduction

Breast cancer (BC) is the most common cancer in women worldwide.[1] One of the most significant risk factors for BC is a family history of the disease so that the risk increases along with the number of affected first-degree relatives.[2] The major high-risk genes predisposing to BC and ovarian cancer (OC) are *BRCA1* and *BRCA2*, with estimated cumulative risks of 72 and 69% for BC and 44 and 17% for OC, respectively, by the age of 80 years.[3] In Finland, *BRCA1/2* mutations have been detected in 21% of BC families.[4] Recurrent *BRCA1/2* founder mutations have been found in 1.8% of unselected BC patients,[5] in 26% of OC families,[6] and in 5.6% of unselected OC patients.[7]

Several genes with a moderate effect on BC or OC risk have been identified in recent years. Mutations in *PALB2* may cause a BC risk comparable to *BRCA2*,[8] whereas mutations in *CHEK2*, *ATM* and *FANCM* have been associated with a two- to threefold increased risk of BC compared to the general population, *FANCM* particularly with the risk of a triple-negative BC subtype.[9–15] *RAD51C* and *RAD51D* have been identified primarily as OC susceptibility genes, but have recently been linked also to triple-negative BC.[16–19] In addition, genome-wide association studies have reported numerous common risk-modifying alleles.[20]

In the Finnish population, the history of geographic isolation and genetic bottlenecks has led to reduced variation and enrichment of deleterious alleles.[21] Consequently, just a few recurrent mutations cover the majority of all pathogenic mutations found in BC and OC susceptibility genes in

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What's new?

In the Finnish population, a small number of recurrent mutations account for the majority of deleterious variations in breast and ovarian cancer susceptibility genes. The authors investigated 12 recurrent moderate-risk mutations in Finnish breast and ovarian cancer patients. These mutations were found with similar frequency in unselected patients and *BRCA1/2*-positive familial index patients, twice as often as in population controls. In addition, a novel association was identified between breast cancer risk and *CHEK2* variant c.319+2T>A. The findings highlight the relevance of gene panel testing for breast and ovarian cancer risk assessment and its potential use for assessing breast cancer families with members who may carry different mutations.

Finland. *PALB2* c.1592delT has been observed with a 0.7–0.9% frequency among unselected BC patients and a 2.0–2.7% frequency among familial patients in studies from different regions in Finland.[22,23] *CHEK2* c.1100delC has been found in 2.0% of unselected and in 5.5% of familial BC patients.[24] Three *FANCM* nonsense mutations, c.5101C>T, c.5791C>T and c.4025_4026delCT, were recently discovered in Finnish BC series.[14,15] Of these, c.5101C>T is the most common with a frequency of 2.8% in unselected patients, 3.1% in BC families and 5.6% in patients with triple-negative BC.[14] Two recurrent *ATM* mutations originally identified in Ataxia-telangiectasia families have been detected also in BC patients: c.6908dupA (previously marked as 6903insA) in 0.4% of unselected and in 0.6% of familial patients, and a pathogenic missense c.7570G>C in 0.2% of both unselected and familial patients.[25,26] Finally, two mutations in *RAD51C*, c.93delG and c.837+1G>A, and one in *RAD51D*, c.576+1G>A, were each observed in about 0.5% of unselected OC patients.[27,28] Besides these, a likely pathogenic *RAD51C* ex1–7 duplication was recently identified in 0.4% of unselected OC patients.[29]

Two *CHEK2* splicing mutations, an Eastern European founder mutation c.444+1G>A (alternatively IVS2+1G>A) and a novel c.319+2T>A, were recently discovered in Finnish BC patients.[29,30] Here, we estimate the frequencies of *CHEK2* c.444 +1G>A and c.319+2T>A among Finnish BC and OC patients and evaluate the BC risk associated with c.319+2T>A. We report the frequencies of *ATM* c.6908dupA and c.7570G>C in a large series of BC and OC patients from Southern Finland. Furthermore, we define the overall prevalence of known recurrent mutations in *PALB2*, *CHEK2*, *FANCM*, *ATM*, *RAD51C* and *RAD51D* genes in Finnish BC and OC patients and study the cooccurrence of these mutations in BC patients and families, including families carrying *BRCA1* or *BRCA2* mutations.

Materials and Methods

Patients

The patient series consisted of 3,156 female BC patients from the Helsinki and Tampere University Hospital regions in Southern Finland, as well as of 556 OC patients and 261 *BRCA1/2* carriers from Helsinki. The patient series are described in detail below. The study was carried out with informed consent obtained from the patients and with approval by the Ethics committees of the Helsinki University Hospital and the Tampere University Hospital.

Helsinki and Tampere BC series

The unselected Helsinki BC series, unselected for age and family history, was collected prospectively at the Helsinki University Hospital Department of Oncology in 1997–1998 and 2000[5,31] and Department of Surgery in 2001–2004.[32] The series consisted of 884 and 986 consecutive, newly diagnosed BC patients (79 and 87% of all new cases during the collection periods, respectively). Of these, 1,727 patients with invasive disease were included in the analysis. The familial Helsinki BC series consisted of 1,141 index patients, including 381 familial cases collected among the unselected series and 760 additional familial patients collected at the Departments of Oncology and Clinical Genetics until 2015 as previously described.[24,32,33] Six hundred and nine families had at least three first- or second-degree relatives with BC or OC and 532 families had two affected first-degree relatives. *BRCA1/2*-positive patients had been excluded from the familial series. In total, 2,487 female BC patients from the Helsinki region were included in the study (Supporting Information Fig. S1).

The unselected Tampere BC series was collected at the Tampere University Hospital as previously described.[5,32] The series consisted of 408 consecutive, newly diagnosed patients collected in 1997–1999 (75% of all new cases during the collection period), with additional 336 incident patients collected in 1996–2004. Of these, 669 female patients with invasive tumor were included in the study (Supporting Information Fig. S1). Two hundred and thirty-four of the patients had a positive family history with at least one first- or second-degree relative diagnosed with BC or OC. For the Helsinki and Tampere BC series, genomic DNA was isolated from peripheral blood samples.

OC series

The unselected Helsinki OC series was collected at the Helsinki University Hospital Department of Obstetrics and Gynecology. Two hundred and thirty-three patients with invasive disease were collected retrospectively during routine follow-up visits to the clinic in 1998 as previously described.[7] Additional patients were collected prospectively in 1998–2006. Altogether 556 cases were included in the series. For 433 patients genomic DNA was

isolated from blood and for 123 patients the DNA sample was extracted from tumor tissue.

BRCA1/2 family series

The *BRCA1/2*-positive series consisted of 126 *BRCA1* mutation carriers (including four males) from 56 families and 135 *BRCA2* mutation carriers (including 13 males) from 53 families. In 30 *BRCA1* and 31 *BRCA2* families, only the index patient was genotyped. All index patients had either BC or OC (including one male with BC) or both cancers. The index patients were initially identified from the familial or unselected Helsinki BC series as previously described,[4,5] or in diagnostic testing at the Helsinki University Hospital Department of Clinical Genetics. The *BRCA1/2* mutations of the families are listed in the Supporting Information Table S1. Of the *BRCA1* carriers, 21 individuals were affected with both BC and OC, 60 with BC, 13 with OC, 6 with other cancer and 26 individuals were healthy. Of the *BRCA2* carriers, 10 had BC and OC, 70 had BC, 8 had OC, 18 had other cancer and 29 were healthy. Twenty-four *BRCA1/2* carriers belonged also to the Helsinki unselected BC series and nine to the unselected OC series. For all patients, genomic DNA was extracted from peripheral blood.

Population controls

The geographically matched population controls consisted of 1,273 healthy female blood donors from Helsinki and 816 from Tampere regions. The Helsinki controls were collected in 2002–2003.

Genotyping

PALB2 c.1592delT, *CHEK2* c.1100delC, *FANCM* c.5101C>T and c.5791C>T, *RAD51C* c.93delG and c.837+1G>A and *RAD51D* c.576+1G>A had been previously genotyped in the majority of the samples from the Helsinki region.[14,15,23,27,28,34] *FANCM* c.4025_4026delCT had been genotyped in one-third of the Helsinki BC cases.[15] Additional cases and controls were genotyped with TaqMan real-time PCR or with Sanger sequencing to obtain comparable sample counts for all mutations (Supporting Information Table S2). All study subjects from Helsinki and Tampere were genotyped for *ATM* c.6908dupA and c.7570G>C with TaqMan real-time PCR or with Sanger sequencing, for *CHEK2* c.319+2T>A with Sequenom MassARRAY or with Sanger sequencing and for *CHEK2* c.444+1G>A with Sanger sequencing (see Supporting Information Methods and Supporting Information Table S3 for details).

Statistical analyses

The statistical analyses were performed using R (version 3.4.4) environment for statistical computing (https://www.r-project.org). Associations were tested with Fisher's exact test. Combined analysis of the Helsinki and Tampere BC series was performed with region-stratified logistic regression. All p values are two-sided. Further details are provided in Supporting Information Methods.

Results

Frequencies of *CHEK2* c.319+2T>A and c.444+1G>A

CHEK2 c.319+2T>A was identified in 13/1725 (0.8%) unselected BC patients and in 11/1138 (1.0%) familial cases from the Helsinki region in comparison to 1/1271 (0.1%) population controls (Table 1). In the study subjects from the Tampere region, c.319+2T>A was observed in 5/653 (0.8%) unselected BC patients and in 2/806 (0.2%) population controls. In the region-stratified analysis combining the Helsinki and Tampere BC series, c.319+2T>A was significantly associated with increased risk of BC in the unselected patient group (odds ratio (OR) = 5.40 [95% confidence interval (CI) 1.58–18.45], p = 0.007) and in the familial patient group (OR = 6.04 [1.65–22.10], p = 0.007; Table 2). Of the mutation carriers with available histological data, 19/22 (86.4%) had estrogen receptor (ER)-positive BC. The average diagnosis age was 54.3 years (range 33–81) for *CHEK2* c.319+2T>A carriers and 56.5 years (range 21–95) for all BC patients in the combined dataset. In the Helsinki series, 3/18 (16.7%) carriers had bilateral BC and two carriers had breast and other cancer (cervical cancer or basalioma).

Three BC patients (3/3147, 0.1%), but none of the controls, were found to carry germline *CHEK2* c.444+1G>A (Table 1). The carriers were diagnosed with BC at the ages of 40, 65 and 79 years. One of the patients had bilateral BC, while another patient had both BC and OC. No *CHEK2* c.319+2T>A or c.444+1G>A carriers were detected in the unselected OC series.

Frequencies of *ATM* c.6908dupA and c.7570G>C

ATM c.6908dupA was identified in two BC patients from the Helsinki region, both being familial index cases (2/1140, 0.2%), and in 2/1271 (0.2%) controls (Table 1). However, the two mutation-positive controls were young at the time of entering the study (ages 19 and 27). In the Tampere series, 5/666 (0.8%) unselected BC patients, but none of the 812 controls, carried the c.6908dupA mutation. The average age of BC diagnosis was 53.7 years (range 30–81) among the carriers. One of the carriers was later diagnosed with bladder cancer.

In the Helsinki BC series, *ATM* c.7570G>C was identified in 2/1727 (0.1%) unselected patients and in 3/1141 (0.3%) familial patients, but in none of the controls (Table 1). In the Tampere BC series, c.7570G>C was not identified among the patients, but it was detected in one out of 815 (0.1%) population controls. The average age of BC diagnosis among the c.7570G>C carriers was 50.3 years (range 28–61). One of the carriers had bilateral BC, one had breast and thyroid cancer and one had BC and basalioma.

In the per-gene analysis of the Helsinki and Tampere BC series, combining *ATM* c.6908dupA and c.7570G>C, the frequency of the mutations was two- to threefold higher in the unselected group (OR = 2.63 [0.69–10.01], p = 0.156) and in the familial group (OR = 2.97 [0.71–12.37], p = 0.136; Table 2) than in the population controls. Ten out of 12 (83.3%) carriers had ER-positive tumors. In the unselected OC series, the c.6908dupA mutation was detected in 2/549 (0.4%) patients (OR = 2.32

Table 1. Frequencies of the *CHEK2* and *ATM* mutations in the Helsinki and Tampere breast cancer series

		Helsinki[1]		Tampere[2]	
Mutation	**Study cohort**	**Carriers/total**	**%**	**Carriers/total**	**%**
CHEK2 c.319+2T>A, rs587782401	Controls	1/1271	0.1	2/806	0.2
	All BC	18/2484	0.7	5/653	0.8
	Familial BC	11/1138	1.0	1/227	0.4
	Unselected BC	13/1725	0.8	5/653	0.8
	ER+	16/1882	0.9	3/499	0.6
	ER–	2/451	0.4	1/122	0.8
CHEK2 c.444+1G>A, rs121908698	Controls	0/1272	0	0/786	0
	All BC	2/2485	0.1	1/662	0.2
	Familial BC	1/1140	0.1	0/230	0
	Unselected BC	1/1725	0.1	1/662	0.2
	ER+	1/1881	0.1	0/507	0
	ER–	0/452	0	1/123	0.8
ATM c.6908dupA, p.Glu2304GlyfsTer69, rs773570504	Controls	2/1271	0.2	0/812	0
	All BC	2/2486	0.1	5/666	0.8
	Familial BC	2/1140	0.2	1/232	0.4
	Unselected BC	1/1726	0.1	5/666	0.8
	ER+	2/1882	0.1	4/510	0.8
	ER–	0/452	0	1/124	0.8
ATM c.7570G>C, p.Ala2524Pro, rs769142993	Controls	0/1272	0	1/815	0.1
	All BC	5/2487	0.2	0/669	0
	Familial BC	3/1141	0.3	0/234	0
	Unselected BC	2/1727	0.1	0/669	0
	ER+	4/1883	0.2	0/513	0
	ER–	1/452	0.2	0/124	0
ATM c.6908dupA and *ATM* c.7570G>C combined	Controls	2/1271	0.2	1/811	0.1
	All BC	7/2486	0.3	5/666	0.8
	Familial BC	5/1140	0.4	1/232	0.4
	Unselected BC	3/1726	0.2	5/666	0.8
	ER+	6/1882	0.3	4/510	0.8
	ER–	1/452	0.2	1/124	0.8

[1]The cohorts are overlapping with 381 individuals included both in the familial and in the unselected patient group in the Helsinki breast cancer series.
[2]The Tampere unselected patient group includes the familial patient group.

[0.17–32.07], $p = 0.589$, compared to the Helsinki population controls). No c.7570G>C carriers were identified among the unselected OC cases.

Overall frequencies of the moderate-risk mutations in the patients

The combined mutation frequency in the moderate-penetrance genes was determined in the Helsinki BC and OC series. At least one of the studied mutations in *PALB2*, *CHEK2*, *ATM*, *FANCM*, *RAD51C* or *RAD51D* genes was detected in 243/2487 (9.8%) BC patients (Table 3). In the unselected BC series, at least one mutation was present in 129/1727 (7.5%) patients, whereas in the familial BC series in 152/1141 (13.3%) patients. The most common mutations among the familial index patients were *CHEK2* c.1100delC detected in 67/1141 (5.9%) cases, *FANCM* c.5101C>T in 36/1141 (3.2%) cases and *PALB2* c.1592delT in 27/1141 (2.4%) cases.

In the unselected OC series, at least one studied mutation was detected in 40/556 (7.2%) patients. When only germline DNA samples were included, at least one mutation was found in 29/433 (6.7%) patients. *FANCM* was the most commonly mutated gene among the OC patients with 13 patients carrying the c.5101C>T and four carrying the c.5791C>T mutation (total 17/556, 3.1%). Mutations in the established OC genes *RAD51C* and *RAD51D* were identified in total in 9/556 (1.6%) unselected OC patients.

Table 2. Combined analysis of the *CHEK2* and *ATM* mutations in the Helsinki and Tampere breast cancer series

Mutation	Study cohort	OR	95% CI	*p*-value
CHEK2 c.319+2T>A, rs587782401	All BC	5.32	1.58–17.97	0.007
	Familial BC	6.04	1.65–22.10	0.007
	Unselected BC	5.40	1.58–18.45	0.007
	ER+	5.52	1.61–18.93	0.007
	ER–	4.34	0.84–22.38	0.080
ATM c.6908dupA, p.Glu2304GlyfsTer69, rs773570504	All BC	3.06	0.62–15.11	0.169
	Familial BC	2.12	0.34–13.36	0.422
	Unselected BC	3.08	0.62–15.42	0.171
	ER+	3.27	0.64–16.65	0.154
	ER–	1.81	0.16–20.79	0.633
ATM c.7570G>C, p.Ala2524Pro, rs769142993	All BC	3.07	0.35–26.84	0.311
	Familial BC	4.71	0.46–48.39	0.192
	Unselected BC	1.76	0.16–19.71	0.647
	ER+	3.32	0.36–30.56	0.289
	ER–	4.31	0.25–73.49	0.313
ATM c.6908dupA and *ATM* c.7570G>C combined	All BC	3.07	0.85–11.09	0.088
	Familial BC	2.97	0.71–12.37	0.136
	Unselected BC	2.63	0.69–10.01	0.156
	ER+	3.29	0.88–12.23	0.076
	ER–	2.58	0.42–16.05	0.309

Of the population controls, 47/1273 (3.7%) carried at least one moderate-risk mutation.

Double heterozygosity for mutations in the moderate-penetrance genes

In the Helsinki BC series, 11 double heterozygotes were identified for the studied *PALB2*, *CHEK2*, *ATM* or *FANCM* mutations among all 2,487 patients, so that 7/1141 (0.6%) familial and 7/1727 (0.4%) unselected patients carried mutations in more than one gene. Three out of the 11 double heterozygotes were included both in the familial and in the unselected series. Of the mutation-positive patients in the series, 4.6% familial and 5.4% unselected cases carried more than one mutation. All but one identified double heterozygotes carried either *CHEK2* c.1100delC or *FANCM* c.5101C>T, or both of the two mutations (Supporting Information Table S4). Two of the double heterozygotes had bilateral BC, and one had breast and bladder cancer. None of the double heterozygotes in the BC series had *RAD51C* or *RAD51D* mutations or were affected with OC. However, one double heterozygote for *ATM* c.6908dupA and *FANCM* c.5101C>T was identified among the unselected OC patients (1/556, 0.2%). One healthy control, enrolled at the age of 37 years, was identified as a heterozygote for *PALB2* c.1592delT and *FANCM* c.5101C>T out of 1,273 (0.1%) population controls.

The expected number of double heterozygotes based on the mutation frequencies in the unselected series of BC cases was 4.4, less than the observed seven. In the population controls, the expected number of double heterozygotes was 0.7, when one was observed. We saw a statistically significant difference between the frequency of the double heterozygotes in the 7/1141 familial BC patients (OR = 7.85 [1.01–353.57], $p = 0.030$), but not in the 7/1727 unselected BC patients (OR = 5.17 [0.66–233.26], $p = 0.149$), when compared to the 1/1273 population controls.

Moderate-risk gene mutations in *BRCA1/2* families

We tested a total of 261 *BRCA1* and *BRCA2* carriers from 109 families for the recurrent mutations in the moderate-penetrance genes. Four of the 56 (7.1%) *BRCA1* index patients and 6/53 (11.3%) *BRCA2* index patients had at least one of the tested *PALB2*, *CHEK2*, *ATM*, *FANCM* or *RAD51C* mutations (Table 4). Two index patients carried three mutations: one OC patient had *BRCA1* c.3485delA, *ATM* c.6908dupA and *RAD51C* c.93delG, and one BC patient had *BRCA2* c.7480C>T, *CHEK2* c.1100delC and *FANCM* c.5101C>T. However, when considering all genotyped *BRCA1/2* carriers, including additional family members from 48 of the 109 families, at least one moderate-risk mutation was detected in 7/56 (12.5%) *BRCA1* families and in 9/53 (17.0%) *BRCA2* families. More than one carrier of a moderate-risk mutation was detected in four families: two *BRCA1* families carrying *ATM* c.6908dupA, one *BRCA2* family with *CHEK2* c.1100delC and one *BRCA2* family with *FANCM* c.5101C>T.

The frequency of the moderate-penetrance gene mutations in *BRCA1/2* index patients was comparable to the frequency in the unselected BC patients, and significantly different from the

Table 3. Frequencies of the moderate-risk mutations in the Helsinki breast cancer series and ovarian cancer series

Mutation	All BC[1]		Familial BC		Unselected BC		Unselected OC		Controls		Average BC Diagnosis age[2]
	Carriers/total	%	Carriers/total	%	Carriers/total	%	Carriers/total	%	Carriers/total	%	
ATM c.6908dupA	2/2486	0.08	2/1140	0.18	1/1726	0.06	2/549	0.36	2/1271	0.16	41.8 (30.3–53.2)
ATM c.7570G>C	5/2487	0.20	3/1141	0.26	2/1727	0.12	0/556	0	0/1272	0	50.3 (28.0–61.1)
CHEK2 c.319+2T>A	18/2484	0.72	11/1138	0.97	13/1725	0.75	0/546	0	1/1271	0.08	55.2 (34.2–76.2)
CHEK2 c.444+1G>A	2/2485	0.08	1/1140	0.09	1/1725	0.06	0/534	0	0/1272	0	59.5 (39.8–79.2)
CHEK2 c.1100delC	99/2487	3.98	67/1141	5.87	49/1727	2.84	10/554	1.81	18/1261	1.43	52.8 (23.3–87.5)
FANCM c.4025_4026delCT	1/2487	0.04	1/1141	0.09	0/1727	0	0/556	0	1/1272	0.08	54.7
FANCM c.5101C>T	73/2487	2.94	36/1141	3.16	48/1727	2.78	13/552	2.36	18/1270	1.42	54.2 (31.0–87.5)
FANCM c.5791C>T	12/2487	0.48	5/1141	0.44	8/1727	0.46	4/553	0.72	3/1257	0.24	54.6 (28.0–79.9)
PALB2 c.1592delT	36/2487	1.45	27/1141	2.37	13/1727	0.75	3/556	0.54	2/1273	0.16	51.6 (33.3–79.8)
RAD51C c.93delG	2/2487	0.08	2/1141	0.18	0/1727	0	4/556	0.72	2/1269	0.16	52.3 (49.9–54.6)
RAD51C c.837+1G>A	1/2487	0.04	1/1141	0.09	0/1727	0	2/555	0.36	0/1268	0	45.0
RAD51D c.576+1G>A	3/2487	0.12	3/1141	0.26	1/1727	0.06	3/556	0.54	1/1273	0.08	43.7 (30.8–53.8)
Total[3]	243/2487	9.77	152/1141	13.32	129/1727	7.47	40/556	7.19	47/1273	3.69	

[1]All BC patients from the familial BC and the unselected BC patient groups after removing the overlap of 381 individuals in these groups.
[2]In the Helsinki breast cancer series.
[3]Double heterozygotes are included only once in the total frequencies.

frequency in the population controls (OR = 2.63 [1.15–5.48], $p = 0.011$). Overall, our data comply with the multiplicative risk model, which suggests that the risk effects associated with single mutations combine multiplicatively and that the second mutation confers additional increase in risk, which is comparable in magnitude to its nominal risk effect (Table 5). The tumor characteristics of all double heterozygotes with BC or OC are presented in the Supporting Information Table S4.

Discussion

In our study, we evaluated the overall prevalence of 12 recurrent moderate-risk mutations in Finnish BC and OC patients. These mutations in *PALB2*, *CHEK2*, *ATM*, *FANCM*, *RAD51C* and *RAD51D* genes were observed in 13.3% of the *BRCA1/2*-negative familial BC patients, in 7.5% of the unselected BC patients and in 7.2% of the unselected OC patients. The studied mutations are likely to cover the vast majority of all pathogenic mutations in these genes in the Finnish population. We have not detected any other pathogenic mutations in *PALB2*, *CHEK2*, *ATM*, *FANCM*, *RAD51C* or *RAD51D* among 231 BC or OC patients from 174 families in whole-genome, whole-exome or gene-panel sequencing[14,29] (unpublished data). To the best of our knowledge, no other moderate-risk mutations in these genes have been reported in Finnish BC or OC patients. Furthermore, no other loss-of-function mutations have been identified among over 12,000 Finns included in the GnomAD database in *RAD51D* gene and only a few very rare or single variants in the other genes.[35] Even if some rare mutations may remain undetected, our results represent the minimum overall frequencies of the moderate-risk mutations in these genes in the BC and OC patients in Finland.

Recently, Couch *et al.*[36] reported the overall frequency of pathogenic variants in 21 BC or OC risk and candidate genes to be 10.2% in a Caucasian BC cohort enriched with familial cases. However, when excluding *BRCA1/2* carriers, the combined frequency was 6.2%.[36] We observed the 12 recurrent moderate-risk mutations over twice as often in the *BRCA1/2*-negative familial index cases and the frequency of the mutations was 7.5% even in the unselected series of BC patients. Thus, in a population with a history of isolation, the effect of just a dozen founder mutations could be more substantial than the effect of multiple rare variants in many genes in outbred populations. Hence, the national guidelines of genetic counseling and personal risk stratification could be designed considering the mutation spectrum present in the population.

The polygenic risk model for BC suggests that the effects associated with genetic variants combine multiplicatively so that the variants retain their nominal risk effects in the combinations.[37] This has been validated for common low-penetrance variants and for common variants in combination with rare mutations.[38–40] However, as indicated by Sokolenko *et al.*,[41] only few studies have been published on BC patients heterozygous for high- and moderate-risk mutations. In the Helsinki

Table 4. Frequencies of the moderate-risk mutations in the *BRCA1/2* index patients and families

Mutation[1]	*BRCA1* index patients (%), *n* = 56	*BRCA1* families (%), *n* = 56	*BRCA2* index patients (%), *n* = 53	*BRCA2* families (%), *n* = 53	*BRCA1/2* index patients (%), *n* = 109	*BRCA1/2* families (%), *n* = 109	All *BRCA1/2* patients[2] (%), *n* = 206	Healthy *BRCA1/2* carriers (%), *n* = 55
ATM c.6908dupA	2 (3.6)	2 (3.6)	0	0	2 (1.8)	2 (1.8)	3 (1.5)	3 (5.5)
ATM c.7570G>C	0	0	0	1 (1.9)	0	1 (0.9)	1 (0.5)	0
CHEK2 c.1100delC	0	1 (1.8)	1 (1.9)	3 (5.7)	1 (0.9)	4 (3.7)	4 (1.9)	2 (3.6)
FANCM c.5101C>T	1 (1.8)	3 (5.4)	3 (5.7)	3 (5.7)	4 (3.7)	6 (5.5)	7 (3.4)	2 (3.6)
FANCM c.5791C>T	1 (1.8)	1 (1.8)	1 (1.9)	1 (1.9)	2 (1.8)	2 (1.8)	2 (1.0)	0
PALB2 c.1592delT	0	0	2 (3.8)	2 (3.8)	2 (1.8)	2 (1.8)	2 (1.0)	0
RAD51C c.93delG	1 (1.8)	1 (1.8)	0	0	1 (0.9)	1 (0.9)	1 (0.5)	0
Total[3]	4 (7.1)	7 (12.5)	6 (11.3)	9 (17.0)	10 (9.2)	16 (14.7)	18 (8.7)	7 (12.7)

[1]Only the mutations detected in the *BRCA1/2* families are presented in the table.
[2]*BRCA1/2* carriers including the index cases and family members with any cancer type.
[3]Triple heterozygotes are included only once in the total carrier frequencies.

BC series, 0.4% of unselected and 0.6% of *BRCA1/2*-negative familial patients carried a moderate-risk mutation in more than one gene. The frequency of the double heterozygotes was five times higher in the unselected BC cases (p = 0.149) and over seven times higher in the familial BC cases (p = 0.030) than in the population controls. The studied moderate-risk mutations were detected also in 9.2% of the *BRCA1/2*-positive index patients, more than twice as often as in the population controls (p = 0.011; Table 5) and in 14.7% of the *BRCA1/2* families. These findings comply with the multiplicative risk model, where the second mutation confers additional risk. If it did not, the mutation frequencies would be approximately the same in the carrier patients as in the population controls. However, the exact risk associated with any specific mutation combination remains to be evaluated in larger patient groups. Earlier studies have indicated that *CHEK2* or *ATM* mutations do not cause additional risk of BC in *BRCA1/2*-positive patients.[42,43] Here, *FANCM* variants appeared as the most common second mutations with 5.5% frequency in the *BRCA1/2* index cases. However, even when excluding *FANCM* mutations from the double heterozygote analyses, the ORs were similar, even though statistically less significant (Supporting Information Table S5). It is also important to notice that in the *BRCA1/2* families, the moderate-risk gene mutation was not always observed in the proband, but in another *BRCA1/2* carrier in the family.

Even though we pooled the mutations for the double heterozygote analyses, we appreciate that the moderate-penetrance genes have different risk effects and that these effects can be subtype-specific. *CHEK2* mutations predispose especially to ER-positive disease[11] and are, like *ATM* mutations, associated with approximately two- to threefold increased risk of BC.[9–13] However, for patients with a family history of the disease, the risk estimates are often further increased, which is likely due to other risk-increasing variants in the families. The estimated lifetime risk of BC for *CHEK2* mutation carriers ranges from about 20% for women without affected relatives to up to approximately 40% for women with positive family history.[9–11] *PALB2* mutations are associated with about sixfold increased risk, and the estimated cumulative risk of BC caused by *PALB2* mutations is 35% by the age of 70 years, but even 58% for carriers with a strong family

Table 5. Frequencies of the second moderate-risk mutation in the carriers of *BRCA1/2* or moderate-penetrance mutation

	Second mutation				
Group	Carriers/total	%	OR[1]	95% CI	*p*-value
Moderate-risk mutation carrier[2]	8/129[3]	6.2	1.72	0.69–3.79	0.155
BRCA1 index patient	4/56	7.1	2.01	0.51–5.80	0.164
BRCA2 index patient	6/53	11.3	3.32	1.11–8.34	0.016
BRCA1/2 index patient	10/109	9.2	2.63	1.15–5.48	0.011
Moderate-risk mutation carrier[2] or *BRCA1/2* index patient	17/237[3,4]	7.2	2.01	1.06–3.65	0.021

[1]The frequency of a second mutation in the mutation carrier groups was compared to the overall frequency of moderate-risk mutations in the Helsinki population controls (47/1273, 3.7%).
[2]In the Helsinki unselected breast cancer series.
[3]Includes one *CHEK2* c.1100delC homozygote and one *BRCA2* c.7480C>T, *CHEK2* c.1100delC and *FANCM* c.5101C>T triple heterozygote.
[4]One *BRCA2* index patient included also in the Helsinki unselected breast cancer series is counted only once in the total frequencies.

history of the disease.[8] *RAD51C* and *RAD51D* have recently been suggested as risk genes for triple-negative BC,[19] while their contribution to OC with a five- to sixfold increased risk has been acknowledged longer.[16–18] We have previously identified *FANCM* as a BC predisposing gene in the Finnish population with approximately twofold increased risk of BC.[14,15] The highest risk was seen in the patients with the triple-negative subtype. Similar results have been published in other studies.[44,45] *FANCM* has also been suggested as an OC susceptibility gene.[46] Here, 3.1% of the unselected OC patients carried any of the three *FANCM* mutations.

We report a novel association between *CHEK2* c.319 +2T>A and BC risk. This mutation, located at a canonical splice site, was previously observed in a gene panel sequencing of BC patients from Finland[30] and in a Norwegian patient with Cowden-like syndrome, thyroid cancer and bilateral BC.[47] We identified c.319+2T>A in 0.7% of BC patients in the combined Helsinki and Tampere series. The mutation was associated with a 5.4-fold risk of BC in the unselected patient group, higher than the previous estimates for *CHEK2* mutations. The risk associated with c.319+2T>A here may be an overestimate and the true risk closer to the approximately threefold risk associated with the truncating *CHEK2* mutations.[9,10,13] However, our estimate of sixfold increased risk in the familial patient group is in line with the earlier studies on *CHEK2*.[9,10] We have previously detected the Eastern European founder mutation *CHEK2* c.444+1G>A in a gene panel sequencing of Finnish BC and OC patients.[29] As the c.444+1G>A mutation was rare (0.1%) in our BC series, we were not able to confirm the associated risk in this study.

The carrier frequencies of *ATM* c.6908dupA and c.7570G>C have previously been estimated in a Northern Finnish study.[26] Here, we genotyped these rare *ATM* mutations in a large series of 3,156 BC patients and 2,089 population controls to establish the carrier frequencies in Southern Finland. *ATM* c.6908dupA was detected in 0.8% of unselected BC patients from the Tampere region, but only in 0.2% of familial and 0.1% of unselected cases from the Helsinki region. *ATM* c.7570G>C was identified in 0.3% of familial and 0.1% of unselected BC patients in the Helsinki series, but absent in the Tampere patients. In a study by Pylkäs *et al.*,[26] a haplotype analysis suggested both c.6908dupA and c.7570G>C to be unique founder mutations, the former originating from the Tampere region. The frequency of these pathogenic *ATM* mutations was about two- to threefold higher in cases in comparison to the population controls, which is consistent with previous results on *ATM*.[13,48] As the c.6908dupA and c.7570G>C mutations are rare, a family-based penetrance study might be better suited to estimate the risk effect associated with these mutations.

Genetic testing allows personalized risk assessment and can lead to disease-preventive actions. An improved diagnostic yield can be achieved with gene panels compared to single gene-based tests. In our study, moderate-penetrance mutations were identified with a relatively high overall frequency in the patients and the results support the utilization of gene panels in clinical testing, possibly for more than one individual in the family. Several pathogenic variants may segregate also in a *BRCA*-positive family and thus, the family members testing negative for *BRCA1/2* mutations may remain at an increased risk of BC or OC due to other predisposing mutations. Furthermore, Manchanda *et al.*[49] estimated that, in UK and USA, population-based testing of *BRCA1/2* and moderate-risk genes would be more cost-effective than testing by clinical or family history-based criteria. In a founder population, like the Finns, screening the few recurrent risk mutations could give similar cost-benefits.

In conclusion, our study provides overall frequencies for recurrent moderate-risk gene mutations in BC and OC patients in Southern Finland. The frequency of the moderate-penetrance gene mutations was significantly different from the frequency in the population controls also in the *BRCA1/2* index patients and our data support the multiplicative risk model, with the second mutation conferring additional increase in risk. Our results underline the benefits of gene panel testing, possibly for multiple members of BC families where several pathogenic variants may segregate in different individuals.

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Author contributions

AN, TAM, LMP and HN designed the study and drafted the manuscript. AN, LMP, JIK, TAM, TH and SL carried out the mutation screening. AN and TAM performed the statistical analyses with LMP. KA, CB, RB, AK and JS contributed samples and patient information. All authors contributed to and approved the final manuscript.

Data availability

The data that support the findings of our study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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