ORIGINAL ARTICLE

Nordic collaborative study of the *BARD1* Cys557Ser allele in 3956 patients with cancer: enrichment in familial *BRCA1/BRCA2* mutation-negative breast cancer but not in other malignancies

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Background: *BARD1* was originally identified as a *BRCA1*-interacting protein but has also been described in tumour-suppressive functions independent of *BRCA1*. Several studies have indicated that the *BARD1* gene is a potential target for germline changes predisposing to breast and ovarian cancer. The C-terminal Cys557Ser change has previously been uncovered to associate with an increased risk of breast cancer and was recently shown to result in defective apoptotic activities.

Aim and methods: Conformation-sensitive gel electrophoresis, minisequencing, TaqMan assays, denaturing high-performance liquid chromatography analysis and DNA sequencing were used to investigate the prevalence of the Cys557Ser allele in a large Nordic case-control study cohort consisting of 2906 patients with breast or ovarian cancer, 734 with prostate cancer, 188 with colorectal cancer, 128 men with breast cancer, and 3591 controls from Finland, Iceland, Denmark, Sweden and Norway.

Results: The frequency of the *BARD1* Cys557Ser variant seemed to increase among patients from families with breast or ovarian cancer lacking *BRCA1* or *BRCA2* mutations: a significant difference was obtained compared with controls (6.8% v 2.7%; $p < 0.001$; odds ratio (OR) 2.6; 95% confidence interval (CI) 1.7 to 4.0) and with patients from *BRCA1/BRCA2* mutation-positive families (6.8% v 2.2%; $p = 0.01$; OR 3.2; 95% CI 1.2 to 8.3). In contrast, no major association with male breast, ovarian, colorectal or prostate cancer was observed. Additionally, a novel *BARD1* allele resulting in Ser558Pro was identified in familial breast cancer cases.

Conclusion: These results provide further evidence that *BARD1* Cys557Ser confers a slightly increased risk of breast cancer in women.

Five to ten per cent of all breast and ovarian cancers are proposed to be due to genetic predisposition,¹ and mutations in the two main susceptibility genes, *BRCA1* and *BRCA2*, are responsible for only about half of these cases.^{2–5} In the absence of evidence of additional commonly involved high-penetrance genes, a polygenic model, in which a large number of low-penetrance to moderate-penetrance genes together are responsible for the disease, has been proposed to explain the remaining familial cases.^{6–8} The *BARD1* gene has been reported to be targeted by germline and somatic mutations in a subset of breast and ovarian cancers,^{9–11} and has been considered to be a possible candidate to be associated with cancer susceptibility. In a recent search for germline mutations in *BARD1*, we observed a missense variant, Cys557Ser, present at considerably higher frequency in Finnish families with breast cancer than in healthy controls.¹¹

BARD1 has been implicated in multiple crucial cellular processes including DNA repair,^{12–14} RNA processing,^{15, 16} transcription,¹⁷ cell cycle regulation¹⁸ and apoptosis.¹⁹ *BARD1* is a major binding partner of *BRCA1*, and several breast cancer-associated mutations in the RING finger domain of *BRCA1* disrupt the ubiquitin ligase activity of the complex.^{20–22} To date, however, no mutations have been observed in the corresponding domain of *BARD1* and none of the possible disease-related changes elsewhere in the gene have been reported to affect *BRCA1*-dependent functions,

suggesting that *BARD1* may also have a *BRCA1*-independent role in tumour progression. The *BARD1* Cys557Ser mutation was originally classified as a harmless polymorphism,⁹ but has subsequently been considered to be a disease-associated variant.^{10, 11} Recently, the role of Cys557Ser as a pathogenic mutation was supported by a functional approach, showing that the variant protein is defective in growth-suppressive and apoptotic activities of *BARD1* and has an effect on p53 stabilisation.²³ Indeed, *BARD1* was previously implicated as a mediator between genotoxic stress and p53-dependent apoptosis independently of *BRCA1*.¹⁹ Moreover, the C-terminal part of *BARD1* comprising residue 557 was identified as the minimal region sufficient for apoptosis induction²⁴ and essential for binding to p53.²⁵ The region is also involved in binding to the Ewing's sarcoma protein, influencing its transrepression and transactivation functions,²⁶ to proto-oncoprotein Bcl3, thereby modulating transcription factor NFκB,¹⁷ and to CstF50, inhibiting the polyadenylation machinery.^{15, 16} Besides its function in apoptotic signalling, *BARD1* interacts with NFκB independently of its association with *BRCA1*.¹⁷

The effect of the *BARD1* Cys557Ser allele on risk of hereditary cancer needs to be evaluated more carefully. Therefore, we initiated a large collaborative Nordic study to determine its prevalence in DNA samples from 3034 patients with familial and unselected breast or ovarian cancer and 734

patients with prostate cancer, as well as in 188 patients with colorectal cancer and 3591 controls. Our results provide evidence of an association between heterozygosity for *BARD1* Cys557Ser and breast cancer risk, but do not indicate a major role in the development of ovarian, colorectal or prostate cancer.

PATIENTS AND METHODS

Patients and controls

Altogether, 3956 Nordic patients with cancer were included in screening for the presence of the Cys557Ser allele. They consisted of unselected patients ($n=1984$: Finland ($n=616$), Iceland ($n=1142$), Denmark ($n=226$)) and familial cases ($n=922$: Finland ($n=205$, of which 126 were also included in the previous study by Karppinen *et al.*¹¹), Iceland ($n=157$), Denmark ($n=219$), Sweden ($n=176$) and Norway ($n=165$)) of breast or ovarian cancer from the Nordic countries, as well as those with colorectal ($n=188$), male breast ($n=128$) and prostate ($n=734$) cancer from Finland. The DNA sample of the affected index case or the youngest patient available of each cancer family was studied.

Inclusion criteria for familial breast or ovarian cancer were ≥ 2 patients with breast or ovarian cancer in first-degree or second-degree relatives. Most of the families had ≥ 3 patients. Table 1 summarises additional data of the studied families. On the basis of mutation screening or haplotyping (Iceland), the division into *BRCA1/BRCA2*-associated ($n=250$) and *BRCA1/BRCA2*-non-associated ($n=593$) subgroups was possible for 843 of the studied 922 families with breast or ovarian cancer; for the remaining families the *BRCA1/BRCA2* mutation status was unknown. In all, 494 of the families were known to be associated with breast cancer, 202 with breast and ovarian cancer, and 10 with ovarian cancer. Five of the 119 Swedish and two of the 104 Icelandic familial cases were positive for *CHEK2* 1100delC. Owing to a lack of suitable controls, the Norwegian cases were excluded when counting the total figures in tables 2 and 3. Inclusion criteria for familial prostate cancer were ≥ 2 affected first-degree or second-degree relatives; and for colorectal cancer, malignancy in ≥ 2 first-degree relatives. The mean age at diagnosis of colorectal cancer was 66.1 (range 27–90) years and that at diagnosis of prostate cancer was 64.8 (range 44–86) years. All the patients with familial colorectal cancer had been previously studied for microsatellite instability and positive individuals were screened for mutations in the *MLH1* and *MSH2* genes. All the patients with familial prostate or colorectal cancer had been screened for the presence of *CHEK2* 1100delC.

The unselected cohorts of consecutive patients with various types of cancer (recruited without selection for or against family history) were collected as follows:

1. Blood samples from the Finnish patients with breast cancer ($n=616$) diagnosed between 2000 and 2004 at the Oulu University Hospital, Oulu, Finland (mean age at diagnosis was 58 years, range 29–95 years) and between 1986 and 1994 at the Tampere University Hospital, Tampere, Finland.
2. The Icelandic patients with breast cancer ($n=1142$) diagnosed at the University Hospital of Reykjavik, Reykjavik, Iceland, and through the cancer registry between 1987 and 2004 (mean age at diagnosis was 58 years, range 18–98 years).
3. The Danish patients with breast cancer ($n=226$) diagnosed between 1994 and 1995 at the Odense University Hospital, Odense, Denmark.
4. Population-based collection of Finnish patients with prostate cancer ($n=613$) and male breast cancer ($n=128$) at the Tampere University Hospital. In all,

613 unselected consecutive Finnish patients were diagnosed with prostate cancer between 2000 and 2001 (Mean age at diagnosis was 69.5 years, range 45–93 years) and *CHEK2* 1100delC had been analysed in 298 of these cases. Patients with male breast cancer were collected from all available patients diagnosed in Finland between 1967 and 1996 (mean age at diagnosis was 65.4 years, range 30–94 years). All patients with male breast cancer had been previously screened for Finnish *BRCA2* founder mutations and 109 for *CHEK2* 1100delC.

DNA samples from 3591 healthy people (1992 from Finland, of whom 1018 were also used in the study by Karppinen *et al.*¹¹ 931 from Iceland, 371 from Denmark and 297 from Sweden) served as population-based controls. Finnish controls were DNA samples from anonymous, voluntary and cancer-free blood donors obtained from the Finnish Red Cross Blood Centre in Tampere ($n=358$), Turku ($n=352$), Kuopio ($n=264$) and Oulu ($n=1018$), representing central, southwestern, eastern and northern areas in Finland, respectively. Samples from the Oulu region were collected in 2002 and the mean age was 41 (range 18–66) years. Finnish male controls included DNA samples from 440 male blood donors (age range 18–65 years) obtained from the Finnish Red Cross Blood Center in Tampere ($n=176$), Turku ($n=176$) and Kuopio ($n=88$). The Icelandic control group was a combination of three randomly selected sample sets: (1) 347 participants in an Icelandic National Diet Survey in 1990 (age range 15–74 years); (2) 479 people from the Icelandic genealogical database during 2003–4 (age range 18–75 years); and (3) 105 people from the Icelandic adult population with the help of the Icelandic Blood Bank and the Icelandic Heart Association between 1988 and 1990 (age range 20–77 years). Danish controls were cancer free and anonymous blood donors collected during 1994–5 and 2002 at the Odense University Hospital. The Swedish control group contained military conscripts aged about 18 years, representing a geographically matched unselected male population.

Informed consent to participate in the study was obtained from each patient, and the studies were approved by the appropriate ethics committees of each country or the participating university hospitals.

Mutation screening and sequence analyses

For identification of the *BARD1* Cys557Ser mutation, five different approaches were used. Mutations were detected either by conformation-sensitive gel electrophoresis,²⁷ mini-sequencing,²⁸ TaqMan assays (TaqMan MGB, Applied Biosystems, Foster City, California, USA), denaturing high-performance liquid chromatography (Transgenomic WAVE System, Transgenomic, San José, California, USA) or DNA sequencing (ABI 3730 Capillary Sequencer, Applied Biosystems). All *BARD1* Cys557Ser variants identified by different mutation screening methods were confirmed by direct sequencing of independently amplified templates. Primer sequences and reaction conditions are available on request.

Statistical analyses

The observed differences in carrier frequencies between the familial or unselected group of patients with breast cancer and controls were analysed by Pearson's χ^2 or Fisher's exact test. Mann-Whitney's U test was used to compare mean ages at disease onset between mutation carriers and non-carriers. The Mantel-Haenszel method was used for further estimation of the pooled odds ratios (ORs) for the combined figures from different countries. All p values were two sided. Data

Table 1 Patients with familial breast or ovarian cancer screened for *BARD1* Cys557Ser

	Number of cases	Mean (range) age at diagnosis (years)*	Family history of breast (no ovarian) cancer*	Family history of breast and ovarian cancer*	Family history of ovarian (no breast) cancer*
Index cases from families with breast or ovarian cancer	922	49.3 (22–99)	494	202	10
Finland	205	52.6 (28–80)	94	29	3
Iceland	157	52.9 (29–88)	75	22	0
Denmark	219	47.0 (22–81)	117	37	2
Sweden	176	47.0 (27–79)	110	61	5
Norway	165	46.9 (28–99)	98	53	0
<i>BRCA1/BRCA2</i> mutation-positive families	250	46.8 (27–79)	50	74	7
Finland	10	48.2 (28–64)	5	4	1
Iceland	53	48.7 (30–77)	ND	ND	0
Denmark	63	45.5 (25–70)	ND	ND	1
Sweden	97	45.8 (27–79)	41	51	5
Norway	27	45.8 (32–58)	4	19	0
<i>BRCA1/BRCA2</i> mutation-negative families	593	50.8 (22–99)	444	128	3
Finland	116	54.9 (28–80)	89	25	2
Iceland	104	57.2 (29–88)	75	22	0
Denmark	156	48.0 (22–81)	117	37	1
Sweden	79	46.9 (27–74)	69	10	0
Norway	138	47.1 (28–99)	94	34	0
<i>BARD1</i> Cys557Ser mutation-positive families	47	50.6 (29–82)	31	9	0
Finland	10	51.3 (42–76)	7	0	0
Iceland	9	60.3 (32–82)	5	2	0
Denmark	10	43.2 (31–54)	7	2	0
Sweden	10	46.7 (29–64)	8	2	0
Norway	8	51.4 (41–76)	4	3	0
<i>BARD1</i> Cys557Ser mutation-negative families	875	50.0 (22–99)	454	171	10
Finland	195	51.9 (28–80)	82	25	3
Iceland	148	56.9 (29–88)	70	20	0
Denmark	209	48.0 (22–81)	110	36	2
Sweden	166	47.0 (27–79)	102	59	0
Norway	157	46.3 (28–99)	90	31	5

ND, not determined.

*Includes only samples for which the particular classification is known.

were statistically analysed using SPSS V.12.0, STATA V.8.0 (StataCorp LP) or Confidence Interval Analysis V.2.0.0.41 for Windows.

RESULTS

Occurrence of the Cys557Ser variant was determined in 3956 patients with cancer and 3591 controls from the Nordic countries. The studied patients with cancer included 2906 with breast or ovarian cancer, 734 with prostate cancer, 188 with colorectal cancer and 128 with male breast cancer. The prevalence of heterozygotes seemed to be enriched among families with breast cancer and those with breast and ovarian cancer not displaying *BRCA1* or *BRCA2* mutations (tables 2–4). The pooled ORs for the combined figures were further estimated using the Mantel–Haenszel method. During the study, a novel *BARD1* variant, Ser558Pro, was observed in the Norwegian population.

Cys557Ser associates with familial breast or ovarian cancer

The *BARD1* Cys557Ser mutation was identified in 5.2% (39/757) of the index cases in families with breast or ovarian cancer from Finland, Iceland, Denmark and Sweden and in 2.7% (98/3591) of controls (table 2). This indicates about a twofold increased risk for familial cases (OR 1.9; 95% CI 1.3 to 2.8). The carrier frequencies varied considerably between different populations. In Finland, 4.9% of patients with familial breast or ovarian cancer were carriers, the frequency being significantly higher than in controls ($p = 0.004$; OR 3.1; 95% CI 1.5 to 6.5). Although the prevalence of heterozygotes

among families with breast or ovarian cancer from Sweden and Iceland was almost twofold higher than in controls, the differences were not statistically significant ($p = 0.3$ and $p = 0.1$, respectively). The frequency in Norwegian families was similar to that in other Nordic countries, but no control samples from Norway were available for comparison, and therefore the Norwegian patients were not included in the combined figures. The raised incidence among both unselected cases and controls from Denmark indicated a high carrier frequency of the variant in the Danish population. Additionally, unlike in the other Nordic populations, the prevalence in familial cases was slightly lower than in controls or in sporadic cases, and statistical significance was not observed between any of these groups. The age at diagnosis of breast cancer was found to be similar for mutation carriers and non-carriers among all patients with familial breast or ovarian cancer (50.6 v 50.0; $p = 1$; table 1), as well as among both Finnish (51.4 v 58.1 years; $p = 0.1$) and Icelandic (53.9 v 58.1 years; $p = 0.5$) unselected patients with breast cancer. During the screening process, two patients homozygotic for the variant allele were found, one from Denmark and the other from Iceland. The Danish woman was diagnosed with breast cancer at age 51 years. Her mother was heterozygotic for the allele and developed breast cancer at age 56 years, whereas her sister did not have the mutation and was cancer free at age 57 years. The Icelandic woman developed breast cancer at age 47 years and belonged to the group of unselected patients with breast cancer. Her family history of cancer was unknown. To analyse segregation of Cys557Ser with the disease in the other families studied, available family members with and

Table 2 Frequencies of *BARD1* Cys557Ser among patients with breast or ovarian cancer, and healthy controls

Subgroup and study population	Frequency	OR (95% CI)	p Value
Controls			
Finland*	1.6% (32/1992)		
Iceland	3.1% (29/931)		
Denmark	7.0% (26/371)		
Sweden	3.7% (11/297)†		
Total	2.7% (98/3591)		
Unselected cases with breast cancer			
Finland*	2.8% (17/616)	1.7 (0.96 to 3.15)	0.07
Iceland	4.7% (54/1142)	1.5 (0.98 to 2.45)	0.06
Denmark	7.5% (17/226)	1.1 (0.57 to 2.04)	0.81
Total	4.4% (88/1984)	1.7 (1.23 to 2.22)	0.001
Cases with familial breast or ovarian cancer			
Finland*	4.9% (10/205)	3.1 (1.52 to 6.49)	0.004
Iceland	5.7% (9/157)	1.9 (0.88 to 4.08)	0.1
Denmark	4.6% (10/219)	0.6 (0.30 to 1.34)	0.2
Sweden	5.7% (10/176)	1.6 (0.65 to 3.77)	0.3
Norway	4.8% (8/165)	ND	ND
Total‡	5.2% (39/757)	1.9 (1.32 to 2.83)	0.001

ND, not determined due to lack of controls.

*Partly included in Karppinen *et al.*¹¹

†Male controls.

‡Norway not included.

Table 3 Prevalence of *BARD1* Cys557Ser among patients with familial breast or ovarian cancer, according to family history

Subgroup and study population*	Frequency	OR (95% CI)	p Value
<i>BRCA1/BRCA2</i> mutation-positive families			
Finland†	0.0% (0/10)	0.0 (0.0 to 24.06)	0.68
Iceland	3.8% (2/53)	1.2 (0.28 to 5.25)	0.68
Denmark	1.6% (1/63)	0.2 (0.03 to 1.61)	0.15
Sweden	2.1% (2/97)	0.5 (0.12 to 2.51)	0.74
Norway	0.0% (0/27)	ND	ND
Total‡	2.2% (5/223)	0.8 (0.33 to 2.03)	0.66
<i>BRCA1/BRCA2</i> mutation-negative families			
Finland†	6.0% (7/116)	3.9 (1.70 to 9.11)	0.005
Iceland	6.7% (7/104)	2.2 (0.96 to 5.26)	0.08
Denmark	5.8% (9/156)	0.8 (0.37 to 1.78)	0.60
Sweden	10.1% (8/79)	2.9 (1.14 to 7.55)	0.04
Norway	5.8% (8/138)	ND	ND
Total‡	6.8% (31/455)	2.6 (1.72 to 3.95)	<0.001
Family history of breast cancer (without ovarian cancer)			
Finland†	7.4% (7/94)	4.9 (2.12 to 11.5)	0.001
Iceland	6.7% (5/75)	2.2 (0.83 to 5.92)	0.10
Denmark	6.0% (7/117)	0.8 (0.36 to 2.00)	0.70
Sweden	7.3% (8/110)	2.0 (0.80 to 5.21)	0.13
Norway	4.1% (4/98)	ND	ND
Total‡	6.8% (27/396)	2.6 (1.68 to 4.05)	<0.001
Family history of breast and ovarian cancer or only ovarian cancer§			
Finland†	0.0% (0/32)	0.0 (0.0 to 7.42)	0.47
Iceland	9.1% (2/22)	3.1 (0.70 to 13.9)	0.16
Denmark	5.1% (2/39)	0.7 (0.16 to 3.14)	1.00
Sweden	3.0% (2/66)	0.8 (0.18 to 3.76)	1.00
Norway	5.7% (3/53)	ND	ND
Total‡	3.8% (6/159)	1.4 (0.60 to 3.24)	0.45

ND, not determined due to lack of controls.

*Includes only samples for which the particular classification is known.

†Partly included in Karppinen *et al.*¹¹

‡Norway not included.

§Altogether 10 patients had a family history of only ovarian cancer (two from Denmark, five from Sweden and three from Finland), and none of them carried *BARD1* Cys557Ser mutation.

without cancer were tested for mutation status. Samples were obtained from Finland, Iceland, Norway and Sweden. The occurrence of the Cys557Ser allele in additional family members varied considerably between the studied families, and although many patients had the mutation, both healthy carriers and affected non-carriers were observed. On the basis of these data, the segregation of the allele with breast cancer seemed to be incomplete.

Prevalence of the Cys557Ser variant among different subgroups of patients from families with breast or ovarian cancer

When comparing subgroups from different countries, higher frequencies of the *BARD1* Cys557Ser allele were seen among patients from *BRCA1/BRCA2* mutation-negative families and also from families lacking cases of ovarian cancer (table 3). The overall prevalence of Cys557Ser carriers among patients without a family history of ovarian cancer was significantly higher than that in controls (6.8% v 2.7%; $p < 0.001$; OR 2.6; 95% CI 1.7 to 4.0). The combined OR calculated using the Mantel-Haenszel method was 1.8 ($p = 0.01$; 95% CI 1.2 to 2.9). By contrast, the frequency among patients with a family history of both breast and ovarian cancers or only ovarian cancer did not reach significance (3.8% v 2.7%; $p = 0.5$). Of these, 10 patients had a family history of only ovarian cancer and none of them carried the Cys557Ser allele. Moreover, the strongest association was found in patients without *BRCA1/BRCA2* mutations in the family. Within this subgroup, 6.8% carried the *BARD1* variant compared with 2.7% in controls ($p < 0.001$; OR 2.6; 95% CI 1.7 to 4.0). Also, the Mantel-Haenszel approximation showed a highly significant association ($p = 0.007$; OR 1.8; 95% CI 1.2 to 2.8). By contrast, only 2.2% of the cases from *BRCA1/BRCA2* mutation-positive families carried the allele, the frequency not differing from that of controls ($p = 0.7$), but being significantly lower than in the families without *BRCA1/BRCA2* mutations ($p = 0.01$; OR 3.2; 95% CI 1.2 to 8.3). The corresponding Mantel-Haenszel OR was 3.4 ($p = 0.01$; 95% CI 1.3 to 9.6).

BARD1 Cys557Ser seems not to associate with other types of cancer

To test whether the Cys557Ser variant also associates with male breast cancer or with other types of cancer, we determined its prevalence in 128 unselected patients with male breast cancer and with 188 patients with familial colorectal cancer, as well as in 121 patients with familial and 613 unselected patients with prostate cancer from Finland (table 4). Only 2.3% of both the men with breast cancer and male controls carried the mutation, indicating no association with malignancy. Nor did we detect a considerably increased frequency of Cys557Ser among patients with colorectal or prostate cancer. The prevalence among Finnish patients with familial prostate cancer (2.5%) and Finnish unselected (2.6%) patients with prostate cancer was similar to that in male controls. Equal frequencies were also observed between patients with familial colorectal cancer (1.6%) and population-based controls (1.6%). Our results indicate that besides female breast cancer, Cys557Ser does not associate with other studied cancer types.

Identification of a novel *BARD1* variant

During our study, a novel Ser558Pro missense mutation was observed in 3.1% (4/128) of affected index cases from Norwegian families with breast or ovarian cancer. In the absence of Norwegian controls and as the variant was not observed in any other of the studied populations, it was not possible to evaluate whether the mutation located next to Cys557Ser is disease related.

Table 4 Frequency of *BARD1* Cys557Ser among Finnish patients with male breast, prostate or colorectal cancer

Cancer type	Frequency		
	Familial	Unselected	Controls
Male breast cancer		2.3% (3/128)	2.3% (10/440)*†
Prostate cancer	2.5% (3/121)	2.6% (16/613)	2.3% (10/440)*†
Colorectal cancer	1.6% (3/188)		1.6% (32/1992)†

*Male controls.

†Although the frequency of *BARD1* Cys557Ser varied slightly between Finnish male (2.3%; 95% CI 1.2 to 4.1) and population (1.6%; 95% CI 1.1 to 2.3) controls, the observed values were within the calculated CI limits, and therefore the use of different sex-matched reference groups was appropriate.

DISCUSSION

The *BARD1* gene has been indicated as a possible target for mutations predisposing people to breast or ovarian cancer.⁹ Recently, to find additional genes conferring susceptibility to breast cancer, we analysed *BARD1* for germline mutations in Finnish patients with breast or ovarian cancer.¹¹ The markedly raised frequency of the Cys557Ser missense variant among the patients studied prompted us to investigate its role in predisposition to cancer more generally by using a large number of cases from the Nordic countries. This study provides evidence that Cys557Ser associates with incidence of breast cancer among *BRCA1/BRCA2* mutation-negative families. The prevalence among these patients was higher than in both controls and patients from families carrying *BRCA1* or *BRCA2* mutations. By contrast, we found no major association of the *BARD1* Cys557Ser variant with either male breast, ovarian, colorectal or prostate cancer, although these observations need to be confirmed with larger patient series. Additionally, the role of the novel Ser558Pro *BARD1* variant found in the Norwegian population remained unclear, although, interestingly, the variant locates between Cys557Ser and Gln564His, both of which have been associated with susceptibility to cancer.⁹⁻¹¹ Sequence comparison showed that Ser558 is conserved in *BARD1* homologues between several species, including human, mouse, dog and chicken (data not shown). Furthermore, serine to proline substitution represents a non-conservative amino acid change with the polar hydroxyl side chain of serine replaced by the non-polar hydrophobic side group of proline, and could therefore have a harmful effect on protein function.

The first evidence suggesting involvement of *BARD1* Cys557Ser in hereditary predisposition to breast or ovarian cancer was obtained from a study on patients from Italian *BRCA1/BRCA2* mutation-negative cancer families.¹⁰ Consistent with this indication, and also the notion from our earlier study,¹¹ current results showed a higher incidence of Cys557Ser among patients from families lacking *BRCA1/BRCA2* mutations. This trend was seen in different Nordic populations, particularly in Finland (6.0%, $p = 0.005$) and Sweden (10.1%, $p = 0.04$), but also in Iceland where the frequency was more than twofold that in controls, although the difference did not quite reach significance ($p = 0.08$). Moreover, patients from families without *BRCA1/BRCA2* mutations were more likely to carry the Cys557Ser mutation than those from *BRCA1/BRCA2* mutation-positive families ($p = 0.01$), among whom no association was observed when compared with controls ($p = 0.7$). To more critically analyse the combined data, Mantel-Haenszel pooled risk estimates were calculated for the comparisons. The Mantel-Haenszel combined OR values were similar to the initially obtained χ^2 values, showing significant association, thereby confirming the results. However, as could be expected as a result of allele frequency variation between different populations, the

Mantel–Haenszel test for homogeneity of the ORs showed heterogeneity in the combined data.

The presence of *BARD1* Cys557Ser with a carrier frequency of 2% in a Caucasian population with sporadic breast, ovarian or uterine tumours, a frequency of 2.5% among Italian *BRCA1/BRCA2* mutation-negative families with breast or ovarian cancer, and the absence in Japanese families implicated that *BARD1* Cys557Ser may be of European or Caucasian origin.^{9 10 29} Current results indicate that *BARD1* Cys557Ser is a common variant in different Nordic populations. The frequency of carriers among patients with familial breast or ovarian cancer varied from 4.6% to 4.9% in Denmark, Norway and Finland to 5.7% in Sweden and Iceland. Except from Denmark, where heterozygotes were present at an exceptionally high frequency in both unselected patients and controls (7.5% and 7.0%, respectively), the prevalence in general seemed to be highest among familial cases. In contrast with our observations, a recent study on patients with breast or ovarian cancer from southern Finland reported a low frequency (1.4%) of Cys557Ser, and found no association with cancer predisposition when compared with controls.³⁰ High variation in frequencies between the studies may be partly explained by sampling fluctuation. As our results show, *BARD1* Cys557Ser seems to be enriched in particular subgroups of patients, and differences within populations could be explained by an unequal distribution of patients studied between these groups. It is also possible that other breast cancer susceptibility alleles act together with Cys557Ser. The frequency variation could also be because the samples are of different geographical origin, as the prevalence of Cys557Ser seems to vary both between and within populations.

The high prevalence of *BARD1* Cys557Ser in general and its incomplete segregation with the disease suggest that the variant is a low-penetrance allele. The role is supported by the marked association observed among unselected patients with breast cancer compared with controls, even if this became apparent only when combining samples from different populations, thereby underlining the need for collaborative studies when dealing with low-penetrance genes. The enrichment of *BARD1* Cys557Ser particularly among patients from *BRCA1/BRCA2* mutation-negative cancer families further strengthens this implication. The observed frequencies among different study groups are consistent with a polygenic model of susceptibility to breast cancer, according to which susceptibility to cancer in the absence of mutations in the *BRCA1* or *BRCA2* genes may be attributable to the combined effect of defects in multiple genes with low to moderate penetrance.

Besides *BARD1* Cys557Ser, the breast cancer-associated *CHEK2* 1100delC variant is also enriched among patients from *BRCA1/BRCA2* mutation-negative families.^{31 32} Furthermore, the reported frequencies for the *CHEK2* allele in different subgroups of patients with familial or unselected breast cancer and healthy controls seem similar to those observed for *BARD1* Cys557Ser. To investigate the possible epistatic interaction between the *CHEK2* and *BARD1* alleles, their co-occurrence in the current patients with cancer was studied. *CHEK2* 1100delC mutation status was known for 119 Swedish and 104 Icelandic patients with familial breast or ovarian cancer. Five carriers were observed in the Swedish sample and two in the Icelandic population. Of these, only one Swedish patient diagnosed with breast cancer was also a carrier of *BARD1* Cys557Ser. Moreover, *BARD1* Cys557Ser was absent from 10 patients with *BRCA2*-positive male breast cancer and one with *CHEK2* 1100delC mutation-positive male breast cancer, as well as from two patients with colorectal cancer, of whom one was a carrier of a splice-site mutation in *MLH1* and the other of *CHEK2* 1100delC. In addition, none of

five unselected patients and four patients with familial prostate cancer positive for the *CHEK2* variant had *BARD1* Cys557Ser. These observations implicate that *BARD1* Cys557Ser and *CHEK2* 1100delC associate with increased risk of breast cancer, mainly independently of each other. Therefore, epistatic interaction between these two genes for cancer susceptibility seems unlikely.

In a recent study, several *BARD1* mutations including Cys557Ser were shown to abrogate growth-suppressive and apoptotic functions of *BARD1*, but seemed not to affect localisation or interaction with *BRCA1*.²³ The induction of apoptosis is an important mechanism for tumour suppression, and the decreased apoptotic activity of Cys557Ser matches its association with cancer. The suggestion of *BARD1* having a role in tumour suppression independently of *BRCA1* is in line with the observation of higher prevalence of Cys557Ser carriers among patients from *BRCA1/BRCA2* mutation-negative families. The independent role is further supported by a lower carrier frequency among patients with a family history of both breast and ovarian cancer or only ovarian cancer (although based on only six positive cases), as the presence of relatives with ovarian cancer in families with multiple breast cancer cases is a strong indicator for involvement of *BRCA1/BRCA2* mutations. It has been estimated that as many as 70% of the families with ≤ 4 patients with breast cancer and no patients with ovarian cancer may be explained by mutations in other predisposing genes. These results are consistent with the reports classifying *BARD1* Cys557Ser as a disease-associated variant conferring modest disease risk rather than a polymorphism. As low-penetrance alleles lead to incomplete cosegregation with cancer in many families and as the role of other factors is presently unknown, estimation of risk for cancer in these families is difficult. Therefore, inclusion of low-penetrance alleles for clinical screening is irrelevant at this point.

The association of *BARD1* Cys557Ser with cancer susceptibility has been controversial. Our current analysis of the variant in a large Nordic population with cancer gave further evidence of its association with breast cancer, but not importantly with any of the other studied malignancies. Cys557Ser increases risk for breast cancer about 2.6-fold among patients from families not associated with the *BRCA1/BRCA2* genes, and it is hypothesised that Cys557Ser may increase breast cancer risk by interacting together with other perhaps yet unknown susceptibility genes in a polygenic context.

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