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The Leu33Pro polymorphism in the *ITGB3* gene does not modify *BRCA1/2*-associated breast or ovarian cancer risks: results from a multicenter study among 15,542 *BRCA1* and *BRCA2* mutation carriers

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Abstract

Integrins containing the β_3 subunit are key players in tumor growth and metastasis. A functional Leu33Pro polymorphism (rs5918) in the β_3 subunit of the integrin gene (*ITGB3*) has previously been suggested to act as a modifier of ovarian cancer risk in Polish *BRCA1* mutation carriers. To investigate the association further, we genotyped 9,998 *BRCA1* and 5,544 *BRCA2* mutation carriers from 34 studies from the Consortium of Investigators of Modifiers of *BRCA1/2* for the *ITGB3* Leu33Pro polymorphism. Data were analysed within a Cox-proportional hazards framework using a retrospective likelihood approach. There was marginal evidence that the *ITGB3* polymorphism was associated with an increased risk of ovarian cancer for *BRCA1* mutation carriers (per-allele Hazard Ratio (HR) 1.11, 95% CI 1.00–1.23, p-trend 0.05). However, when the original Polish study was excluded from the analysis, the polymorphism was no longer significantly associated with ovarian cancer risk (HR 1.07, 95% CI 0.96–1.19, p-trend 0.25). There was no evidence of an association with ovarian cancer risk for *BRCA2* mutation carriers (HR 1.09, 95% CI 0.89–1.32). The polymorphism was not associated with breast cancer risk for

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either *BRCA1* or *BRCA2* mutation carriers. The *ITGB3* Leu33Pro polymorphism does not modify breast or ovarian cancer risk in *BRCA1* or *BRCA2* mutation carriers.

Keywords

ITGB3 Leu33Pro; *BRCA1*; *BRCA2*; Breast cancer; Ovarian cancer

Introduction

Women harbouring deleterious germline mutations in the *BRCA1* or *BRCA2* gene face high life-time risks of developing breast and ovarian cancers [1-4]. Recent estimates of breast cancer risk by the age of 70 years range from 47% [5] to 87% [6] for *BRCA1* mutation carriers and from 45% [7] to 84% [2] for *BRCA2* mutation carriers. The corresponding risk of ovarian cancer was estimated to range from 15% [8] to 68% [9] for *BRCA1* mutation carriers and 4.5% [8] to 31% [9] for *BRCA2* mutation carriers. Penetrance estimates vary by family ascertainment criteria and also between and within families, suggesting that other genetic or environmental factors modify the disease risks [10,11].

Integrins comprise a large family of cell surface receptors which control cell attachment to the extracellular matrix (ECM). They play a role in mammary gland biology with expression in all cell types within the gland [12] and activate intracellular signaling pathways that control proliferation, differentiation, apoptosis, cell motility, migration and survival [13,14]. Integrins consist of noncovalently linked α and β subunits. In mammals, combinations of 18α and 8β subunits form at least 25 different proteins that bind specific ECM components [14,15]. Two integrins containing the β_3 subunit, $\alpha v\beta_3$ and $\alpha IIb\beta_3$, are key players in tumor growth and metastasis. Increased expression of these integrins in melanomas, gliomas, ovarian and breast cancers correlates with invasive tumor properties [16-20], whereas their inhibition reduces tumor growth and metastasis through disruption of tumor angiogenesis [21,22].

The β_3 integrin gene (*ITGB3*) is a plausible candidate for breast and ovarian cancer susceptibility. A previously reported single nucleotide polymorphism (SNP) rs5918, a nucleotide substitution of T to C at codon 33 in the mature protein, causes a leucine to proline exchange [23]. This variant introduces a nick in the polypeptide chain just N-terminal of the hybrid domain of β_3, which is involved in dimerization with the αv and αIIb subunits during the formation of $\alpha v\beta_3$ and $\alpha IIb\beta_3$ integrins. It has been shown that *ITGB3* Leu33Pro polymorphism is of functional significance in that it increases the interactions with fibrinogen [24,25], results in an abnormal response to stimulation with thromboxane [26] and other agonists [27], enhances aggregation of platelets and generation of thrombins [28-30], decreases bleeding time [31], increases signaling through ERK2 of the MAPK [32] pathway and enhances cell migration [33].

A number of epidemiological studies have suggested associations of the *ITGB3* Leu33Pro with the risk of developing cancers including non-Hodgkin lymphoma [34], colon [34], kidney [35], breast [36-41] and ovarian cancer [36,42,43]. However, little data exist on the influence of this polymorphism on breast and ovarian cancer risk for women with *BRCA1* or *BRCA2* mutations. A recent study conducted of Polish *BRCA1* mutation carriers including 319 breast cancer cases, 146 ovarian cancer cases and 290 unaffected controls reported a significantly increased risk of ovarian cancer among carriers of the *ITGB3* 33Pro allele (OR_{adj} 2.51, 95% CI 1.30–4.84, $P = 0.006$) [44]. In order to verify this original finding and to estimate the possible association of *ITGB3* Leu33Pro with *BRCA2* related cancer risk, we

genotyped this polymorphism using a large series of *BRCA1* and *BRCA2* mutation carriers from the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA).

Materials and methods

Study sample

Eligible study subjects were women who carried a deleterious germline mutation in *BRCA1* or *BRCA2* and were 18 years old or older. Information on study subjects was submitted from 34 centers participating in CIMBA. Data collected included year of birth, mutation description, family membership, ethnicity, country of residence, age at last follow-up, ages at breast and ovarian cancer diagnosis and information on bilateral prophylactic mastectomy and oophorectomy. Only carriers of pathogenic *BRCA1* or *BRCA2* mutations were included in the study. These were mutations generating a premature termination codon (frameshifts, small deletions and insertions, nonsense mutations, splice site mutations, large genomic rearrangements), large in-frame deletions that spanned one or more exons, deletions of transcription regulatory regions (promoter and/or first exons) expected to cause lack of expression of mutant allele and missense variants classified as pathogenic by Breast Cancer Information Core (BIC) or using the algorithms of Goldgar et al. [45] and Chenevix-Trench et al. [46]. Truncating variants in exon 27 of *BRCA2* were excluded.

All carriers participated in clinical and research studies at the host institutions under ethically approved protocols. Further details of the CIMBA initiative can be found elsewhere [47].

Women who were self-reported as “non-white” and those who carried pathogenic mutations in both *BRCA1* and *BRCA2* were excluded from the analysis. Possible overlaps between studies were investigated by comparing the year of birth, mutation description, and the reported ages to identify duplicate individuals. Where possible, additional CIMBA genotyping data were used to ensure that duplicated individuals were only included once in the analysis.

Genotyping

Twenty-one centers genotyped the *ITGB3* Leu33Pro using a 5′ nuclease assay (TaqMan). PCR primers (5′-TCTCTTTGGGCTCCTGTCTTACA-3′ (forward) and 5′-GCAGATTCTCCTTCAGGTCACA-3′ (reverse) and probes (VIC-5′-TGAGCCCGGAGGCA-3′ and FAM-5′-TGAGCCCAGAGGCA-3′) were distributed centrally to each center. DNA samples from 11 centers were genotyped at the Queensland Institute of Medical Research, Brisbane, Australia, using iPLEX technology. PCR primers were 5′-ACGTTGGATGGCACAGTTATCCTTCAGCAG-3′ (forward) and 5′-ACGTTGGATGTCTTTGGGCTCCTGTCTTAC-3′ (reverse); the extension primer was 5′-AGCGAGGTGAGCCC-3′. One study performed genotyping by direct DNA sequencing on an ABI 3130XL Genetic Analyzer. PCR primers were 5′-GCTATTGGGAAGTGGTAGGGC-3′ (forward) and 5′-TGTCTCCAGAGCCCTTGTCG-3′ (reverse). IHCC geno-typed by PCR-based restriction fragment length polymorphism (RFLP) analysis as previously described [44].

In addition to the genotypes of patients, each study also provided genotypes for at least 2% of samples in duplicate, genotypes for a standard test plate containing 94 samples from the Coriell Cell Repository (New Jersey, USA), and cluster plots from Taqman analyses. Genotyping data were included in the analysis when they met the quality control criteria including an overall call rate of >95%, duplicate concordance and concordance of test plate genotypes of >98%. Samples that failed genotyping for two or more of genotyped SNPs at this CIMBA genotyping round (or ≥20% if typed using a multiplex platform) were also

excluded. All studies passed these quality criteria. As an additional genotyping quality criterion we also assessed Hardy–Weinberg Equilibrium (HWE) for unrelated mutation carriers for each study separately. There was no significant evidence of deviation from HWE for any of the studies and all the studies were therefore included in the analysis.

Statistical analysis

The aim of the analysis was to evaluate the association between the *ITGB3* Leu33Pro and risks of breast or ovarian cancer in *BRCA1* and *BRCA2* carriers. Women were classified according to their age of cancer diagnosis or their age at last observation. Three types of analysis were carried out. To evaluate the association with breast cancer risk, carriers were censored at the age of the first of the following events: breast cancer diagnosis (8,274 carriers), ovarian cancer diagnosis (1,507 carriers), bilateral prophylactic mastectomy (413 carriers) or age at last observation (5,348 carriers). Only those censored at a breast cancer diagnosis were assumed to be affected. To evaluate the association with ovarian cancer risk, carriers were censored at the age of ovarian cancer diagnosis (2,159 carriers), bilateral prophylactic oophorectomy (1,096) or age at last observation (12,287 carriers) whichever occurred first. Only those censored at ovarian cancer were assumed to be affected for the analysis. We did not censor at a breast cancer diagnosis in order to maximize the number of ovarian cancer cases used in the analysis. To evaluate whether our results were influenced by the fact we did not censor at breast cancer diagnosis, we also performed an analysis where individuals were censored at the first cancer diagnosis (breast or ovarian). In this we assumed that an individual was at risk of developing either breast or ovarian cancer.

Since *BRCA1* and *BRCA2* mutation carriers are not randomly sampled with respect to their disease status, standard methods of analysis can lead to biased estimates of the hazard ratios (HR) [46]. We therefore analyzed the data within a retrospective likelihood framework by modeling the likelihood of the observed genotypes conditional on the disease phenotypes. This approach is described in detail elsewhere [48]. Under this approach, the cancer incidence was assumed to depend on the underlying incidence through a Cox-proportional hazards model $\lambda_i(t) = \lambda_0(t) \exp(\beta_i)$, where $\exp(\beta_i)$ is the hazard ratio for genotype i and $\lambda_0(t)$ is the cancer incidence rate in the baseline category. The baseline age-specific incidence rates in the Cox proportional-hazards model were chosen such that the overall cancer incidence rates, averaged over all genotypic categories, agree with external estimates of incidence for *BRCA1* and *BRCA2* mutation carriers [11]. The effect of each SNP was modelled either as a per-allele HR (multiplicative model) or as separate HRs for heterozygotes and homozygotes, and these were estimated on the log scale (i.e. β_i). This analysis was performed separately for breast and ovarian cancer to evaluate the associations with each disease. In a further sensitivity analysis of the association with ovarian cancer risk, we extended the retrospective likelihood to allow for the fact that each mutation carrier is at risk of developing either breast or ovarian cancer. For this purpose we assumed that conditional on the genotype, the age-specific probability of developing ovarian cancer is independent of the probability of developing breast cancer. In this competing risk analysis, since the aim was to evaluate the association with ovarian cancer risk, we assumed that the SNP was not associated with the risk of developing breast cancer. Analyses were carried out with the pedigree-analysis software MENDEL [49]. We examined between-study heterogeneity by comparing the models that allowed for study-specific log-hazard ratios against models in which the same log-hazard ratio was assumed to apply to all studies. All analyses were stratified by study group and country of residence and used calendar-year- and cohort-specific breast cancer incidence rates for *BRCA1* and *BRCA2* mutation carriers [11].

To investigate whether SNP associations differed by mutation type, mutations were classified according to their predicted functional effect. Class 1 mutations include loss of

function mutations caused by reduced transcript and protein levels due to nonsense-mediated decay (NMD) and/or degradation or instability of truncated proteins [50-52], translation re-initiation but no production of stable protein [53], or the absence of expression caused by deletion of transcription regulatory regions. Class 2 mutations comprise mutations expected to generate stable mutant proteins: missense mutations, in-frame deletions/insertions and truncating mutations with premature stop codons occurring in the last exon and thus not triggering NMD. Mutations whose consequences at transcript or protein level could not be inferred were not considered for this classification.

Results

In this study, we investigated the effect of the *ITGB3* Leu33Pro on breast and ovarian cancer risk in female *BRCA1* and *BRCA2* mutation carriers from 34 centers participating in CIMBA. A total of 15,542 mutation carriers were eligible for inclusion in the analysis (9,998 *BRCA1* and 5,544 *BRCA2*, Table 1). The genotype frequencies and estimated HRs by mutation and disease status are shown in Table 2. There was no evidence of association between breast cancer risk and the *ITGB3* Leu33Pro for either *BRCA1* or *BRCA2* mutation carriers (per-allele HR 1.02, 95% CI 0.94–1.09 and 1.01, 95% CI 0.92–1.12 for *BRCA1* and *BRCA2*, respectively). There was no evidence of heterogeneity in the HRs across studies (p-het = 0.38 and 0.41 for *BRCA1* and *BRCA2*, respectively).

There was marginal evidence of association with ovarian cancer risk for *BRCA1* mutation carriers (per-allele HR 1.11, 95% CI 1.00–1.23, p-trend = 0.05) and there was no evidence of heterogeneity in the HRs across studies for *BRCA1* mutation carriers (p-het = 0.13). However, when the IHCC study, where the original association was found, was excluded there was no evidence of association (HR 1.07, 95% CI 0.96–1.19, p-trend = 0.25). *ITGB3* Leu33Pro was also not associated with ovarian cancer risk for *BRCA2* mutation carriers (perallele HR 1.09, 95% CI 0.89–1.32). In assessing between-study heterogeneity for *BRCA2* mutation carriers, study-specific log-hazard ratios converged to boundary conditions due to small numbers and it was not therefore possible to formally assess heterogeneity in the HRs between studies for *BRCA2*. To investigate whether ignoring breast cancer diagnoses influenced our results, we also analysed the data in a competing risks analysis framework and allowed individuals to be at risk of developing either breast or ovarian cancer. The HR estimates under this analysis were similar to the analysis which did not censor at a breast cancer diagnosis (Table 2).

Among *BRCA1* mutation carriers, 6,716 carried class 1 mutations (frameshifts—72%, nonsense mutations—21%, splice site mutations—4% and large deletions or duplications—3%) and 2,678 class 2 mutations (frameshifts—60%, missense mutations—26%, nonsense mutations—5%, splice site mutations—2% and large deletions or duplications—7%). To investigate whether our results differ by mutation type, we carried out separate analyses for *BRCA1* class 1 and class 2 mutations (Table 3). *ITGB3* Leu33Pro was not associated with either breast or ovarian cancer risk for class 1 mutation carriers. In addition, there was no evidence of an association with breast cancer risk for *BRCA1* class 2 mutations. However, there was evidence that the polymorphism is associated with ovarian cancer risk in *BRCA1* class 2 mutation carriers (per-allele HR 1.24 95% CI 1.00–1.53, p-trend = 0.048). Since the original study (IHCC), where an association was reported [44], consists predominantly of class 2 mutations (~94% of all *BRCA1* mutations in IHCC), we repeated the analysis by excluding the IHCC study and found no evidence of an association (HR 1.01, 95% CI 0.78–1.32, p-trend = 0.92).

Discussion

We genotyped of 9,998 *BRCA1* and 5,544 *BRCA2* mutation carriers to investigate the hypothesis that the *ITGB3* Leu33Pro polymorphism is associated with breast or ovarian cancer risk in *BRCA1* or *BRCA2* mutation carries. To our knowledge this is the largest study of its kind. Results from a smaller study had previously suggested that this polymorphism is associated with the risk of ovarian cancer in *BRCA1* mutation carriers [44]. However, our results suggest that *ITGB3* Leu33Pro is not associated with the risk of breast or ovarian cancer risk for *BRCA1* or *BRCA2* carriers. There was a marginal evidence of association with ovarian cancer risk for *BRCA1* mutation carriers, but when the original study, where the association was reported, was excluded, there was no evidence of an association.

Several studies have investigated the associations between *ITGB3* Leu33Pro with breast and ovarian cancer in the general population, but the results have been inconsistent [36-43]. One study reported an association between the homozygous 33Leu genotype and breast cancer risk [38], other studies suggested that the 33Pro allele is associated with breast cancer risk [36,37,39], and others reported no associations [40,41]. An association of *ITGB3* Leu33Pro was reported with ovarian cancer in the general population [43], but this was not confirmed in any other study [42]. In the latest and largest up to now study of 1,819 ovarian cancer patients, including 837 serous and 734 non-serous cases, and 2,353 controls performed by the Ovarian Cancer Association Consortium (OCAC) as part of a genome-wide association study [54], no significant association of *ITGB3* Leu33Pro with overall ovarian cancer risk was found (per-minor allele OR 0.99, 95% CI 0.88–1.12, p-trend = 0.95). Furthermore, no associations were found when patients were subdivided by histologic subtype: serous (OR 0.94, 95% CI 0.80–1.09) and non-serous (OR 1.08, 95% CI 0.92–1.27) tumors (personal communication, Honglin Song). Since the majority of the *BRCA1* mutation ovarian cancer tumors are serous [8], the absence of an association with ovarian cancer risk for *BRCA1* mutation carriers is consistent with the lack of association for serous tumors in the general population.

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Interdisciplinary Health Research International Team Breast Cancer susceptibility (INHERIT)

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Table 1

Number of eligible *BRCA1* and *BRCA2* carriers by study group

Study	Country[a]	BRCA1, N	BRCA2, N	Genotyping platform
Medical University of Vienna (MUV)	Austria	277	116	iPLEX[b]
Breast Cancer Family Registry (BCFR)	USA, Canada, Australia	490	356	Taqman
Copenhagen Breast Cancer Study (CBCS)	Denmark	91	51	Taqman
Spanish National Cancer Centre (CNIO)	Spain, Greece	167	197	Taqman
Deutsches Krebsforschungszentrum (DKFZ)	Germany	68	27	Taqman
Hereditary Breast and Ovarian study Netherlands (HEBON)	The Netherlands	768	291	iPLEX[b]
Epidemiological study of *BRCA1* & *BRCA2* mutation carriers (EMBRACE)	UK and Eire	807	632	iPLEX[b]
Fox Chase Cancer Centre (FCCC)	USA	80	54	iPLEX[b]
German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)	Germany	798	375	Taqman
Genetic Modifiers of cancer risk in *BRCA1/2* mutation carriers (GEMO)	France, USA	1116	561	Taqman
Georgetown University	USA	31	16	iPLEX[b]
Gynecologic Oncology Group (GOG)	USA	395	279	Taqman
Hospital Clinico San Carlos (HCSC)	Spain	109	95	Taqman
Helsinki Breast Cancer Study (HEBCS)	Finland	102	104	iPLEX[b]
International Hereditary Cancer Centre (IHCC)	Poland	695	0	RFLP
Iceland Landspitali—University Hospital (ILUH)	Iceland	0	87	Sequencing
Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERITBRCAs)	Quebec, Canada	73	82	Taqman
kConFab	Australia	488	388	iPLEX[b]
University of California Irvine (UCI)	USA	167	121	Taqman
Mayo Clinic (MAYO)	USA	213	117	iPLEX[b]
Milan Breast Cancer Study Group (MBCSG)	Italy	344	217	Taqman
Memorial Sloane Kettering Cancer Center (MSKCC)	USA	253	155	Taqman
National Cancer Institute (NCI)	USA	147	65	Taqman
National Israeli Cancer Control Center (NICCC)	Israel	312	199	Taqman
Ontario Cancer Genetics Network (OCGN)	Canada	214	169	Taqman

Study	Country[a]	BRCA1, N	BRCA2, N	Genotyping platform
Ohio State University Clinical Cancer Center (OSU CCG)	USA	59	31	Taqman
Odense University Hospital (OUH)	Denmark	215	131	Taqman
Pisa Breast Cancer Study (PBCS)	Italy	73	40	iPLEX[b]
Sheba Medical Centre (SMC)—Tel Hashomer	Israel	395	185	Taqman
Swedish Breast Cancer Study (SWE-BRCA)	Sweden	410	121	iPLEX[b]
N.N. Petrov Institute of Oncology (NNPIO)	Russia	67	0	Taqman
Modifier Study of Quantitative Effects on Disease (ModSQuaD)	Czech Republic, Belgium	269	130	Taqman
University of Turin Breast Cancer Study (UTBCS)	Italy	60	43	Taqman
University of Pennsylvania (UPENN)	USA	245	109	iPLEX[b]
Total		9998	5544	

[a] Country of the clinic at which carriers are recruited

[b] Indicates centralized genotyping (Queensland Institute of Medical Research)

Table 2

ITGB3 Leu33Pro (T>C, rs5918) genotype frequencies by disease status and hazard ratio estimates

Gene	Genotype	Unaffected (%)	Affected[a] (%)	HR	95% CI	*P*-value
Breast cancer analysis						
BRCA1	TT	3509 (73.2)	3769 (72.5)	1.00		
	TC	1183 (24.7)	1318 (25.3)	1.02	0.93–1.10	
	CC	104 (2.2)	115 (2.2)	1.03	0.80–1.33	
	2df test					0.92
	Per-allele			1.02	0.94–1.09	0.69
BRCA2	TT	1788 (72.3)	2231 (72.6)	1.00		
	TC	621 (25.1)	757 (24.6)	1.02	0.91–1.15	
	CC	63 (2.6)	84 (2.7)	0.99	0.71–1.37	
	2df test					0.93
	Per-allele			1.01	0.92–1.12	0.79
Ovarian cancer analysis						
No censoring at breast cancer						
BRCA1	TT	6065 (73.2)	1213 (70.9)	1.00		
	TC	2048 (24.7)	453 (26.5)	1.12	0.99–1.26	
	CC	174 (2.1)	45 (2.6)	1.21	0.88–1.65	
	2df test					0.14
	Per-allele			1.11	1.00–1.23	0.05
	Excluding IHCC					
	Per-allele			1.07	0.96–1.19	0.25
BRCA2	TT	3695 (72.5)	324 (72.3)	1.00		
	TC	1269 (24.9)	109 (24.3)	1.05	0.83–1.33	
	CC	132 (2.6)	15 (3.4)	1.34	0.74–2.40	
	2df test					0.60
	Per-allele			1.09	0.89–1.32	0.40
Censoring at breast cancer (competing risks analysis)[b]						
BRCA1	TT	6407 (73.9)	871 (71.6)	1.00		
	TC	2184 (24.9)	317 (26.1)	1.12	0.97–1.30	

Gene	Genotype	Unaffected (%)	Affected[a] (%)	HR	95% CI	*P*-value
	CC	191 (2.2)	28 (2.3)	1.06	0.71–1.60	
	2df test					0.32
	Per-allele			1.09	0.96–1.24	0.17
BRCA2	TT	3782 (72.5)	237 (72.0)	1.00		
	TC	1297 (24.9)	81 (24.6)	1.07	0.81–1.41	
	CC	136 (2.6)	11 (3.3)	1.35	0.68–2.70	
	2df test					0.64
	Per-allele			1.10	0.88–1.39	0.39

[a]Diagnosed with breast or ovarian cancer according to analysis

[b]*ITGB3* Leu33Pro assumed not to be associated with breast cancer risk

Table 3

ITGB3 Leu33Pro (T>C, rs5918) genotype frequencies by disease status and ovarian cancer hazard ratio estimates for *BRCA1* mutation carriers by mutation class

Gene	Genotype	Unaffected (%)	Affected[a] (%)	HR	95% CI	*P*-value
Breast cancer analysis						
BRCA1-Class1	TT	2444 (72.8)	2450 (72.9)	1.00		
	TC	840 (25.0)	835 (24.9)	1.01	0.91–1.12	
	CC	73 (2.2)	74 (2.9)	1.06	0.77–1.46	
	2df test					0.92
	Per-allele			1.02	0.93–1.11	0.72
BRCA1-Class2	TT	908 (75.4)	1086 (73.7)	1.00		
	TC	273 (22.7)	363 (24.6)	1.05	0.89–1.24	
	CC	23 (1.9)	25 (1.7)	0.90	0.52–1.55	
	2df test					0.76
	Per-allele			1.02	0.89–1.18	0.75
Ovarian cancer analysis						
BRCA1-Class1	TT	4029 (73.2)	865 (71.2)	1.00		
	TC	1357 (24.7)	318 (26.2)	1.08	0.93–1.25	
	CC	116 (2.1)	31 (2.6)	1.16	0.79–1.72	
	2df test					0.49
	Per-allele			1.08	0.95–1.22	0.23
BRCA1-Class2	TT	1701 (75.2)	293 (70.6)	1.00		
	TC	525 (23.2)	111 (26.8)	1.22	0.94–1.57	
	CC	37 (1.6)	11 (2.6)	1.62	0.89–2.95	
	2df test					0.12
	Per-allele			1.24	1.00–1.53	0.048
	Excluding IHCC:					
	Per-allele			1.01	0.78–1.32	0.92

[a]Diagnosed with breast or ovarian cancer according to analysis