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Genetic polymorphisms in the estrogen receptor beta (*ESR2*) gene and the risk of epithelial ovarian carcinoma

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Abstract

Ovarian cancer is influenced by exogenous and endogenous estrogens as suggested by experimental and epidemiological evidence. Estrogen receptor beta is a predominant estrogen receptor in the normal ovary. Polymorphisms in the estrogen receptor beta gene (*ESR2*) might influence epithelial ovarian risk through regulation of cell proliferation and apoptosis. This population-based case–control study included 313 women with epithelial ovarian carcinoma and 574 controls, frequency-matched on age and ethnicity. Unconditional logistic regression was used to test associations of rs1271572, rs1256030, rs1256031, and rs3020450 *ESR2* genotypes with ovarian cancer risk. Compared to homozygous common allele carriers, homozygous carriers of variant alleles for rs1271572 [odds ratio (OR) = 1.79, 95% confidence interval (CI):1.15–2.79, *p* global = 0.01] and rs1256030 [OR = 1.67, CI: 1.08–2.59, *p* global = 0.04], and women with haplotypes, including variant alleles of rs1271572, rs1256030, and rs1256031 SNPs [OR = 1.75, CI: 1.17–2.63, *p* global = 0.007], had significantly increased risk of ovarian carcinoma. The association of the rs1271572 genotype was strongest among women who had never used contraceptive steroids (*p* for interaction = 0.04). Our data suggest that *ESR2* might be a susceptibility marker for epithelial ovarian cancer.

Keywords

Epithelial ovarian cancer; *ESR2*; Single nucleotide polymorphisms; Case–control study

Introduction

Ovarian cancer is the leading cause of death from gynecological malignancy and is the fourth most frequent cause of death from cancer in women [1] mostly because of its late clinical manifestations and absence of screening methods for early detection. The majority of women who develop this cancer do not carry highly-penetrant *BRCA* gene mutations; therefore, discovering common low-penetrance gene mutations could be useful in detecting women at higher risk of ovarian cancer, allowing for an individualized approach to screening.

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There is evidence from experimental [2,3] and epidemiological studies [4,5] for a role of exogenous and endogenous estrogen in ovarian carcinogenesis. Estrogen effects are mediated by two estrogen receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), which belong to a family of nuclear receptors and act as ligand-dependent transcription factors [6]. Both ER sub-types are present in ovarian surface epithelial cells [7], but ERβ is the predominant type in the normal ovary [8-10]. It has been shown that estrogen induces cellular proliferation and has an antiapoptotic effect in the presence of ERα, whereas ERβ mediates estrogen-induced apoptosis [9]. Recent studies have suggested that ERβ might act as a tumor suppressor in ovarian cancer cells. A loss of ERβ expression or a decrease in the ERα/ERβ ratio in epithelial ovarian cancer as compared to normal tissue has been reported by several investigators [9,11,12]. Bardin et al. [13] demonstrated that an increased ERα/ERβ mRNA ratio observed in ovarian carcinoma was attributable to a selective decrease in ERβ mRNA expression without significant variation in ERα levels. A loss of ERβ expression could thus constitute a crucial step in ovarian carcinogenesis. Although the precise mechanism for a role of ERβ in ovarian carcinogenesis remains to be determined, recent in vivo and in vitro investigations provide evidence for an involvement of ERβ in the control of cellular proliferation, motility, and apoptosis in ovarian cancer. It is notable that ERβ expression is also lost in breast, colon, and prostate cancers [14-16], malignancies that share some etiologic features with ovarian cancer [17].

Based on these molecular, cellular, and animal studies, we hypothesized that genetic polymorphisms in the ERβ gene (*ESR2*) may affect susceptibility to epithelial ovarian cancer. We selected single nucleotide polymorphisms (SNPs) from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) database using the following criteria: (1) minor allele frequency of at least 20% among Caucasians and Japanese, who constitute the majority of our population; and (2) evidence from previous investigations for an association with the risk of cancer or other clinical outcomes. Two intronic SNPs (rs1256030 in intron 2 and rs1256031 in intron (3) and two SNPs in the 5′ area of *ESR2* gene (rs3020450 and rs1271572) were selected for this study. The rs1256030 polymorphism has been associated with the levels of urinary excretion of luteinizing hormone (LH) and estrone [18], and rs1256031 has been associated with left ventricular mass and left ventricular wall thickness in Framingham Offspring Study women [19]. The rs3020450 SNP is in strong linkage disequilibrium with rs2987983, a possible prostate cancer susceptibility gene [20]. The rs1271572 SNP has been reported to influence the risk of prostate cancer [20] and myocardial infarction [21].

Materials and methods

Study design and population

This population-based case–control study included 313 women 18 years of age or older who were diagnosed with primary histologically-confirmed epithelial ovarian carcinoma between 1993 and 2006. Incident cases were identified through the rapid-reporting system of the Hawaii Tumor Registry, which is part of the Surveillance, Epidemiology, and End-Results Program of the National Cancer Institute [22]. Control subjects (n = 574) were randomly selected from participants in an annual survey of representative households, conducted by the Hawaii Department of Health under statutory provision [23]. Controls were frequency-matched to cases based on ethnicity and 5-year age groups in an approximate 1:1.8 ratio. Eligibility criteria for controls included age 18 years or older, residency in Hawaii for a minimum of one year, no prior history of ovarian cancer, and having at least one intact ovary. The response rate was 64% for cases and 67% for controls. The study protocol was approved by the Institutional Review Board of the University of Hawaii. All study participants provided written informed consent.

Data collection

Study participants were interviewed using a structured pre-tested questionnaire that included socio-demographic and health-related information, menstrual, reproductive and gynecological histories, and exogenous hormone use [24]. Interviewers were uniformly trained and supervised to standardize interviewing and coding techniques.

Blood sample collection and genotyping

Thirty milliliters of blood was collected into vacutainers with ethylenediaminetetraacetic acid, using venipuncture performed by a trained certified phlebotomist at the participant's home. Vacutainers were immediately transported on ice to the laboratory and were processed within two - hours of collection by the laboratory technician. DNA was purified from whole blood using Qiagen Midi Kits (Qiagen, Valencia, CA). Genotyping was performed with the 5′ nuclease discrimination assay using TaqMan (Applied Biosystems, Foster City, California). Samples from cases and controls were intermixed on each plate. We included 66 pairs of blinded samples, randomly placed on each plate, to evaluate accuracy and reproducibility. In addition, each 384-well plate included eight non-DNA controls. Call rates were 98% for rs1271572 and rs1256030 and 99% for rs1256031 and rs3020450. The concordance rates among duplicates were 100% for all SNPs.

Statistical analysis

A goodness of fit chi-square test was used to assess whether allele frequency distributions among controls overall and in each ethnic group were consistent with Hardy–Weinberg equilibrium. Unconditional multiple logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of genotype with ovarian carcinoma risk. The genotype for each SNP was treated as a non-ordered categorical variable to test for heterogeneity and as an interval variable (with three levels: 0, 1, 2; one assigned to each genotype) to test for a gene-dose effect. Pair-wise linkage disequilibrium (D′) and correlation coefficients (r^2) were estimated using the HAPLOVIEW program [25]. The rs1271572, rs1256030, and rs1256031 SNPS were in relatively strong linkage disequilibrium (D′ range: 0.93–1.0 and r^2 range: 0.66–0.85) among all ethnic groups, and haplotypes were created for these three SNPs and assessed in relation to ovarian cancer risk; the haplotype including common alleles of all SNPs was used as a reference. The LD of rs3020450 with other SNPs was weak (r^2 range: 0.18–0.24). All multivariate models were adjusted for age, ethnicity, parity, history of a tubal ligation procedure, menopausal status, hysterectomy, and use of contraceptive and menopausal hormones. We also examined the interaction of the *ESR2* SNPs with age, ethnicity, menopausal status, and use of contraceptive and menopausal hormones by comparing models with and without interaction terms, using the likelihood ratio test. All *P*-values were based on two-tailed tests. Statistical significance was considered at a p value less than 0.05.

Results

The mean age of the study participants was 55.0 years (SD, 13.8; range 18–88). Women with a family history of ovarian cancer in first-degree relatives had a higher ovarian cancer risk. Parity, tubal ligation, use of contraceptive steroids, premenopausal status, and the use of menopausal estrogen in combination with progesterone (EPT) were inversely associated with ovarian cancer risk (Table 1).

The distribution of all *ESR2* genotypes among control subjects was consistent with Hardy–Weinberg equilibrium in each ethnic group and all groups combined (Table 2). Table 3 presents ORs and 95% CIs for the association of *ESR2* genotypes and haplotypes with epithelial ovarian carcinoma risk among Caucasian, Japanese, and all women combined (the sample sizes for

Hawaiian, Filipinas, and "others" were too small for independent analysis). Among all women combined, homozygous rs1271572 T and rs1256030 T variant allele carriers had significantly higher ovarian carcer risk when compared to homozygous common allele carriers (OR = 1.79; 95% CI:1.12–2.79 and OR = 1.67; 95% CI:1.08–2.59, respectively). The allele-dose effect was also statistically significant for rs1271572 and rs1256030 SNPs. Among Japanese women, homozygous rs1271572 T allele carriers also had statistically significant higher ovarian cancer risk (OR = 2.24; 95% CI:1.05–4.74; *p* for allele-dose effect = 0.049). Testing a recessive genetic model for three SNPs showed statistically significant results for rs1271572 and rs1256030 in both Caucasian and Japanese women, and in all women combined; for rs1256031, results were statistically significant only among Caucasian women. The genotype/haplotype associations with risk were generally consistent for each of the ethnic groups studied, and tests for heterogeneity among ethnic groups were not statistically significant for any of the SNPs. When carriers of the two most common rs1271572-rs1256030-rs1256031 haplotypes ("*GCT*" including all common alleles and "*ttc*" including all variant alleles) were compared, "*ttc*" haplotype carriers had significantly higher ovarian cancer risk among Caucasian, Japanese, and all women combined (Table 3). We did not find an association of the rs3020452 genotype with ovarian cancer risk in any of the ethnic groups or in all women combined, and therefore, did not include this SNP in the subgroup analyses.

The association between the rs1271572 genotype with the risk of ovarian cancer was stronger among never users than among ever users of contraceptive hormones (Table 4). Women who were homozygous carriers of the T allele had an OR of 3.09 (95% CI: 1.54–6.20; *p* for allele-dose effect = 0.003) compared to women who were carriers of the common G allele, among ever users. We did not find a significant interaction of age, menopausal status, or the use of menopausal hormones on the association of the *ESR2* SNPs with ovarian cancer risk. However, all of the *ESR2* genotype associations with risk were stronger among postmenopausal women using replacement hormones than among non-users (Table 5). The number of postmenopausal women who used progestin alone was too small to include in a subgroup analysis.

Discussion

In this investigation, we found that two common genetic polymorphisms (rs1271572 and rs1256030) in the *ESR2* gene, as well as the "*ttc*" haplotype including variant alleles of rs1271572, rs1256030, and rs1256031, which were in strong LD, were associated with the risk of epithelial ovarian cancer.

These *SNPs* have been previously studied in relation to breast, prostate, and ovarian cancers. Sun et al. [20] found an association of the rs1271572 polymorphism with the risk of prostate cancer among Chinese men. However, no evidence for a relation of the rs1256031 polymorphism with prostate cancer risk was reported by the Breast and Prostate Cancer Cohort Consortium [26]. The Breast and Prostate Cancer Cohort Consortium investigators reported no association of the rs1256030 SNPs with breast cancer risk [27]. One other recent study by Pearce et al. [26], using a haplotype approach, evaluated *ESR2* variation in relation to ovarian cancer risk. No statistically significant associations were reported, although one haplotype was associated with an increased risk of invasive clear cell carcinoma. Two of the five SNPs assessed by these investigators, rs1256031 and rs3020450, overlapped with our examination, and these were not found associated with ovarian cancer risk [28]. We also found no association of the rs3020450 with risk, and the association of the rs1256031 was weaker than for the rs1256030 and rs1271572 polymorphisms. The rs1256031 SNP is thought to capture variation in rs1256030 and rs1271572 among Caucasian subjects as these SNPs are in strong LD (r^2 = 0.9 for both SNPs, based on the HapMap and our data). However, the tagging by rs1256031 might be inadequate because of incomplete linkage or differences in allele frequency (0.42 for rs1271572, 0.44 for rs1256030, and 0.50 for rs1256031), and the rs125631 SNP was the least

strongly associated with risk in our study. In addition, we observed significant associations of rs1271572 and rs1256030 with risk only among homozygous variant allele carriers, and it is possible that a log-additive model does not provide the best fit for the association of the rs1256031 SNP with risk. Some other studies reported that association of rs1256031 [19], rs1271572 [21], and rs1256030 [29] with clinical outcome was limited only to homozygous variant allele carriers. Moreover, in our analysis, we accounted for the distribution of important potential confounding factors that might also contribute to differences in associations of *ESR2* SNPs with ovarian cancer risk. The likelihood that rs1271572 may be causally associated with the risk of ovarian cancer is supported by the finding that it maps to the promoter of the *ESR2* gene in a region of predicted intense transcription factor binding and might influence gene expression [27]. This possibility is further supported by the heightened association of rs1271572 with risk among Japanese women (OR = 2.24; 95% CI: 1.05–4.74) in whom the LD among all three SNPs was weaker than in Caucasians (r^2 range: 0.71–0.76).

Although the specific role of ERβ in carcinogenesis is not known, there is convincing evidence that ERβ inhibits proliferation and motility of ovarian cancer cells and plays an important role in apoptosis [30,31]. Since the identification of ERβ [32], several investigations have shown that a loss of ERβ expression is associated with the malignant phenotype in epithelial ovarian cancer [9,11,30]. A similar down-regulation of ERβ has also been noted in prostate, breast, and colon tumors [31,33,34]. Bardin et al. [13] reported that ERβ upregulates apoptosis in ovarian cancer cells. In normal ovary, ERβ has been shown to enhance FasL expression, a major apoptotic regulator [35]. The observed reduction of cyclin A2 mRNA levels in SK-OV-3 cells expressing ERβ is another possible mechanism underlying the growth inhibitory action of ERβ in ovarian cancer [36]. Cyclin A2 is a cell cycle regulator which is known to be estrogen responsive [37]. Treeck et al. [36] demonstrated that ERβ expresses a variety of antitumor effects in SK-OV-3 ovarian cells even in the absence of estradiol or functional ERα. Bardin et al. [48] showed that ERβ could block the estradiol-induced proliferation of ERα-expressing cells such as BG-1.

The rs1271572 SNP is located in the *ESR2* promoter close (−53 bp upstream) to the AP-4/MyoD binding site [38]. Transcription factor AP-4 contains multiple dimerization domains that function to promote homodimer formation and restrict heterocomplexes, providing a mechanism for the regulation of a dimer specificity [39]. Formation of homo- (ERβ/ERβ;ERα/ERα) or heterodimers (ERβ/ERα) upon ligand binding results in a unique ligand-ER complex that determines further interaction with specific receptor coactivator or corepressor proteins [40]. The MyoD transcription regulator plays a role in providing resistance of ER to proteolytic degradation [41]. Therefore, the rs1271572 sequence variation, if indeed functional, might interfere with some of the ERβ proposed antiproliferative effects by altering *ESR2* responsiveness to transcription regulators.

We observed that contraceptive hormone use modified the association of the rs1271572 and, to a lesser degree, rs1256030 polymorphisms with the risk of ovarian cancer. The association of these SNPs with ovarian cancer risk was stronger among women who never used contraceptive hormones than among contraceptive hormone users (p for interaction = 0.04). In contrast, an association of the rs1271572 genotype with ovarian cancer risk appeared to be stronger among users of menopausal hormones than among women who never used hormone replacement therapy, although the test for interaction was not statistically significant. Similar to endogenous estrogens, the biological effects of exogenous estrogens are thought to be mediated by ERs [42]. Moreover, experimental data indicates that the binding of different estrogens is stereo-specific and structure-dependent, and each estrogen imparts on ERβ a unique conformation, ultimately influencing its action [42]. Therefore, 17α-ethinyl estradiol, a synthetic component in contraceptive hormone regimens, and the main endogenous estrogen (17β-estradiol) might differentially alter the properties of ERβ and its further interaction with

a various transcription factors. In addition, 17α-ethinyl estradiol might act through ERα, to which it has high binding affinity [42-46]. Thus, among contraceptive hormone users, the observed effect of *ESR2* polymorphisms with risk might be attenuated. Among postmenopausal women who are not using menopausal hormones, the level of estrogen is low and ERβ and its functional variations might not play a prominent role. On the other hand, among women using menopausal hormones, the association of *ESR2* genotypes with risk may be of a greater importance as conjugated equine estrogens that were most often used as a hormone replacement therapy by our study participants appear to operate mostly through ERβ [42]. In final analysis, however, as there are currently no established models of ovarian carcinogenesis, we can only speculate on biological mechanisms consistent with the observed associations. Although the majority of epidemiological studies provide data for a protective effect of oral contraceptives against ovarian cancer, specific mechanisms of their action have yet to be clarified. In the recently published Million Women Study [47], menopausal hormone use was associated with an increased ovarian cancer risk. However, an association of EPT with ovarian cancer risk is uncertain as several studies reported no association [48,49] and a recent study by Rossing et al. [50] reported a decreased risk among past EPT users. In our study, the number of women who used unopposed estrogen or progestin was small, and EPT users had a decreased ovarian cancer risk when compared to women who never used menopausal hormones.

Strengths of this study include its population-basis, histologic confirmation of all case diagnoses, and stringent laboratory quality control procedures. The main limitation of this study is its small sample size. Given the sample size of 313 cases and 574 controls, we had >80% power with a critical value of 0.05 to detect an OR $\geq$ 1.33 using a log-additive model and an OR $\geq$ 1.66 using a recessive genetic model, for an allele frequency of 0.38, as found for rs1271572 and 1256031. Among Caucasian women, we had >80% power to detect an OR of 2.10 or higher for an allele frequency of 0.42, as found for these SNPs and a recessive mode of inheritance. These power limitations might have hampered our ability to identify weak associations with risk for rs125631 and rs3020450; however, we were able to detect the much stronger associations for the rs1271572 and rs1256030 SNPs.

In summary, our data suggest that *ESR2* gene polymorphisms may play a role in susceptibility to epithelial ovarian cancer. These findings will need to be replicated within a larger consortium of ovarian cancer studies.

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Table 1

Participant characteristics

Characteristics	Number (%) of participants		OR (95% CI)[a]
	Cases (*n* = 313)	Controls (*n* = 574)	
Age (years)			Matching factor
<45	70 (22)	117 (21)	
45–54	90 (29)	173 (30)	
55–64	75 (24)	146 (25)	
>64	78 (25)	138 (24)	
Ethnicity			Matching factor
Caucasian	72 (23)	148 (26)	
Japanese	94 (30)	173 (30)	
Filipino	36 (12)	79 (14)	
Hawaiian	69 (22)	106 (18)	
Other Asian	17 (5)	27 (5)	
Other	25 (8)	41 (7)	
Family history of ovarian cancer			
Yes	16 (5)	9 (2)	3.89 (1.67–9.76)
No	297 (95)	565 (98)	1.00 (reference)
Parity			
Nulliparous	91 (29)	82 (14)	1.00 (reference)
1	54 (17)	87 (15)	0.58 (0.35–0.94)
2	72 (23)	160 (28)	0.49 (0.31–0.77)
3	50 (16)	124 (22)	0.43 (0.26–0.71)
≥4	46 (15)	121 (21)	0.30 (0.17–0.52)
Used oral contraceptives			
Yes	144 (46)	394 (69)	0.32 (0.22–0.46)
No	169 (54)	180 (31)	1.00 (reference)
Had tubal ligation			
Yes	48 (15)	181 (32)	0.51 (0.34–0.77)
No	265 (85)	393 (68)	1.00 (reference)
Menopausal status			
Premenopausal	111 (35)	215 (37)	0.45 (0.21–0.98)
Postmenopausal	202 (65)	359 (63)	1.00 (reference)
Type of menopause			
Hysterectomy	32 (16)	47 (13)	0.91 (0.51–1.60)
Natural menopause	170 (84)	312 (87)	1.00 (reference)
Use of menopausal hormones			
Never used	106 (52)	149 (42)	1.00 (reference)
Estrogen only	32 (16)	50 (14)	0.81 (0.44–1.48)
Progestin only	12 (6)	19 (5)	0.69 (0.30–1.58)
Combined estrogen and progestin	52 (26)	141 (39)	0.62 (0.39–0.97)

[a] Odds ratio (OR) and 95% confidence intervals (CI) from unconditional logistic regression model including all listed variables

Table 2

Variant allele distribution in control subjects by ethnic group

***ESR2* SNPs**[a]	**Minor allele**[b]	**All (*n* = 574)**	**Caucasians (*n* = 148)**	**Japanese (*n* = 200)**	**Hawaiians (*n* = 106)**	**Filipinas (*n* = 79)**	**Other (*n* = 41)**	***p* for chi-square test**[c]
rs1271572	T	0.38	0.42	0.37	0.44	0.30	0.28	<0.01
rs1256030	T	0.46	0.44	0.38	0.43	0.26	0.30	<0.001
rs1256031	C	0.38	0.50	0.47	0.50	0.42	0.40	0.63
rs3020450	A	0.24	0.32	0.20	0.20	0.17	0.35	<0.0001

[a] Nomenclature was based on the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/, accessed 02/08/2008)

[b] Minor allele among Caucasians

[c] Chi-square test was based on a 3 × 5 table of genotype by ethnicity

Table 3

ESR2 SNP association with epithelial ovarian cancer risk

SNPs	All			Caucasian			Japanese		
	Cases	Controls	OR (95% CI)[a]	Cases	Controls	OR (95% CI)[b]	Cases	Controls	OR (95% CI)[b]
rs1271572 (SNP1)									
GG	99	212	1.00	21	42	1.00	28	61	1.00
GT	140	272	1.02 (0.73–1.43)	30	81	0.73 (0.36–1.49)	40	80	1.04 (0.55–1.96)
TT	66	77	1.79 (1.15–2.79)	19	20	1.92 (0.80–4.61)	25	27	2.24 (1.05–4.74)
p for trend			0.02			0.24			0.049
Any T vs. GG[c]	206	349	1.20 (0.88–1.66)	49	101	0.96 (0.49–1.87)	65	107	1.35 (0.75–2.42)
TT vs. any G[d]	239	494	1.78 (1.19–2.61)	51	123	2.42 (1.14–5.15)	68	141	2.26 (1.15–4.43)
rs1256030 (SNP2)									
CC	102	213	1.00	20	40	1.00	30	59	1.00
CT	142	266	1.04 (0.74–1.46)	30	82	0.69 (0.34–1.43)	28	79	0.95 (0.50–1.79)
TT	64	80	1.67 (1.08–2.59)	20	22	1.85 (0.77–4.43)	25	29	1.90 (0.89–4.02)
p for trend			0.04			0.23			0.13
Any T vs. CC[c]	206	346	1.20 (0.87–1.64)	50	104	0.93 (0.47–1.84)	53	108	1.18 (0.66–2.12)
TT vs. any C[d]	244	479	1.63 (1.10–2.41)	50	112	2.48 (1.18–5.18)	58	138	2.01 (1.04–3.90)
rs1256031 (SNP3)									
TT	84	164	1.00	20	24	1.00	27	41	1.00
TC	147	288	0.96 (0.67–1.36)	32	82	0.68 (0.33–1.41)	41	90	0.70 (0.36–1.38)
CC	80	116	1.30 (0.86–1.98)	20	39	1.57 (0.67–3.72)	25	42	1.15 (0.55–2.40)
p for trend			0.25			0.34			0.69
Any C vs. TT[c]	227	404	1.10 (0.77–1.50)	52	121	0.89 (0.45–1.76)	66	132	0.85 (0.46–1.59)
CC vs. any T[d]	231	452	1.32 (0.93–1.88)	52	106	2.12 (1.03–4.36)	68	131	1.50 (0.81–2.78)
rs3020450 (SNP4)									
GG	202	330	1.00	34	65	1.00	65	113	1.00
GA	91	209	0.72 (0.52–1.00)	29	68	0.81 (0.42–1.54)	21	54	0.75 (0.40–1.42)

SNPs	All			Caucasian			Japanese		
	Cases	Controls	OR (95% CI)[a]	Cases	Controls	OR (95% CI)[b]	Cases	Controls	OR (95% CI)[b]
AA	20	30	1.20 (0.64–2.27)	9	13	1.52 (0.55–4.19)	8	5	2.85 (0.83–9.85)
p for trend			0.40			0.78			0.57
Any A vs. GG[c]	111	239	0.78 (0.75–2.61)	38	81	0.87 (0.48–1.60)	29	59	0.97 (0.54–1.74)
AA vs. any G[d]	293	539	1.40 (0.75–2.61)	63	133	1.62 (0.62–4.24)	86	167	3.56 (1.00–12.64)
SNP1-SNP3 haplotypes									
GCT	220	441	1.00	50	118	1.00	66	128	1.00
tCT	4	4	1.72 (0.38–7.68)	0	1	—	0	0	—
GCc	16	30	0.89 (0.45–1.77)	0	0	—	2	10	0.39 (0.08–1.97)
Gtc	2	5	0.88 (0.16–4.90)	1	2	2.17 (0.17–28.3)	0	1	—
ttc	62	72	1.75 (1.17–2.63)	19	19	2.45 (1.14–5.27)	25	27	2.11 (1.07–4.15)

[a] Adjusted for age, ethnicity, parity, tubal ligation, hysterectomy, menopausal status, and use of contraceptive and menopausal hormones

[b] Adjusted for the same variables except ethnicity

[c] Testing dominant genetic model

[d] Testing recessive genetic model

Table 4

Interaction of *ESR2* genotypes with the use of contraceptive steroids

***ESR2* genotypes**	**Used contraceptive hormones**			**Never used contraceptive hormones**			***p* for the likelihood ratio test comparing models with and without interaction terms**
	Cases n	**Controls n**	**OR (95% CI)[a]**	**Cases n**	**Controls n**	**OR (95% CI)[a]**	
rs1271572 (SNP1)							
GG	52	143	1.00	47	69	1.00	
GT	59	184	0.85 (0.54–1.34)	81	88	1.29 (0.78–2.13)	
TT	28	58	1.21 (0.68–2.17)	38	19	3.09 (1.54–6.20)	0.04
p for trend			0.70			0.003	
rs1256030 (SNP2)							
CC	51	145	1.00	51	68	1.00	
CT	59	177	0.88 (0.56–1.39)	83	89	1.26 (0.77–2.06)	
TT	30	61	1.29 (0.73–2.28)	34	19	2.39 (1.20–4.76)	0.20
p for trend			0.49			0.02	
rs1256031 (SNP3)							
TT	34	83	1.00	33	46	1.00	
TC	66	195	0.77 (0.47–1.25)	93	81	1.17 (0.68–1.99)	
CC	43	112	0.86 (0.48–1.56)	52	41	1.81 (0.95–3.45)	
p for trend			0.95			0.07	0.15
SNP1-SNP3 haplotypes[b]							
GCT	105	300	1.00	115	141	1.00	
ttc	28	54	1.30 (0.76–2.24)	18	34	2.33 (1.18–4.57)	0.18

[a] Adjusted for age, ethnicity, parity, tubal ligation, hysterectomy, menopausal status, and use of menopausal hormones

[b] Only haplotypes carried by more than 5% of subjects in each subgroup were included in the table

Table 5

Joint association of ESR2 genotype and menopausal hormone[a] use among postmenopausal women

ESR2 genotypes	No menopausal hormones			Unopposed estrogen			Combined estrogen and progestin		
	Cases n	Controls n	OR (95% CI)[b]	Cases n	Controls n	OR (95% CI)[b]	Cases n	Controls n	OR (95% CI)[b]
rs1271572 (SNP1)									
GG	33	54	1.00	7	20	1.00	12	54	1.00
GT	50	75	1.09 (0.60–2.00)	15	22	1.87 (0.44–2.90)	23	65	1.67 (0.71–3.95)
TT	20	16	1.92 (0.83–4.43)	10	7	7.73 (1.36–43.9)	14	19	3.84 (1.34–11.01)
p for interaction[c]						0.75			0.54
rs1256030 (SNP2)									
CC	33	58	1.00	8	21	1.00	13	52	1.00
CT	52	71	0.89 (0.59–1.35)	14	20	1.73 (0.45–6.65)	25	65	1.58 (0.68–3.64)
TT	19	17	1.34 (0.77–1.33)	10	9	5.89 (1.16–29.81)	13	20	2.89 (1.02–8.15)
p for interaction[c]						0.82			0.78
rs1256031 (SNP3)									
TT	24	23	1.00	14	10	1.00	14	32	1.00
TC	55	82	0.84 (0.55–1.30)	12	20	1.96 (0.40- 9.55)	26	71	1.03 (0.44–2.41)
CC	27	43	1.12 (0.66–1.88)	5	19	16.02 (2.63–97.56)	12	37	1.24 (0.47–3.29)
p for interaction[c]						0.24			0.96
SNP1-SNP3 haplotypes									
GCT	77	123	1.00	17	38	1.00	34	106	1.00
ttc[d]	18	16	1.62 (0.75–3.52)	10	7	9.46 (6.79–50.05)	13	19	2.30 (0.94–5.68)
p for interaction[c]						0.52			0.63

[a] Risk estimates for women who used progestin only formulation are not available due to small numbers in subgroups

[b] Adjusted for age, ethnicity, parity, tubal ligation, hysterectomy, and history of contraceptive hormone use

[c] *p* for the likelihood ratio test comparing models with and without interaction terms

[d] Only haplotypes carried by more than 5% of subjects in each subgroup were included in the table