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Selected Polymorphisms in Sex Hormone-Related Genes, Circulating Sex Hormones and Risk of Endometrial Cancer

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Abstract

Background—The role of estrogen and progesterone in the development of endometrial cancer is well documented. Few studies have examined the association of genetic variants in sex hormone-related genes with endometrial cancer risk.

Methods—We conducted a case-control study nested within three cohorts to examine the association of endometrial cancer risk with polymorphisms in hormone-related genes among 391 cases (92% postmenopausal at diagnosis) and 712 individually-matched controls. We also examined the association of these polymorphisms with circulating levels of sex hormones and

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SHBG in a cross-sectional analysis including 596 healthy postmenopausal women at blood donation (controls from this nested case-control study and from a nested case-control study of breast cancer in one of the three cohorts).

Results—Adjusting for endometrial cancer risk factors, the A allele of rs4775936 in *CYP19* was significantly associated ($OR_{per\ allele}$ = 1.22, 95% CI = 1.01–1.47, p_{trend} = 0.04), while the T allele of rs10046 was marginally associated with increased risk of endometrial cancer ($OR_{per\ allele}$ = 1.20, 95% CI = 0.99 – 1.45, p_{trend} = 0.06). *PGR* rs1042838 was also marginally associated with risk ($OR_{per\ allele}$ = 1.25, 95% CI = 0.96–1.61, p_{trend} = 0.09). No significant association was found for the other polymorphisms, i.e. *CYP1B1* rs1800440 and rs1056836, *UGT1A1* rs8175347, *SHBG* rs6259 and *ESR1* rs2234693. *Rs8175347* was significantly associated with postmenopausal levels of estradiol, free estradiol and estrone and rs6259 with SHBG and estradiol.

Conclusion—Our findings support an association between genetic variants in *CYP19, and* possibly *PGR*, and risk of endometrial cancer.

Keywords

endometrial cancer; estrogen; sex hormone-binding globulin; progesterone receptor; single nucleotide polymorphism

Introduction

The role of estrogens and progesterone in the development of endometrial cancer is well documented [1]. The few prospective studies that have reported on the association between endogenous sex hormones and endometrial cancer risk have shown that circulating levels of androgens and estrogens in postmenopausal women are positively related to risk [2–4]. A number of single-nucleotide polymorphisms (SNPs) and/or haplotypes in hormone-related genes have also been studied, with mixed results [5, 6]. We conducted a case-control study nested within three cohorts to examine the association of several SNPs and repeat polymorphisms in sex hormone-related genes with endometrial cancer risk in predominantly postmenopausal women.

Polymorphisms were studied from the following genes: CYP19, coding for aromatase which converts androgens to estrogens; CYP1B1, coding for the cytochrome P450 1B1 which hydroxylates estrogens to catecholestrogens; UGT1A1, coding for UDP-glucuronosyltransferase 1A1 which glucuronidates estrogens; SHBG, coding for sex hormone-binding globulin (SHBG) which binds estrogens and androgens, reducing their biological availability; and ESR1, coding for the estrogen receptor alpha. Specific polymorphisms (with minor allele frequencies >10%) were selected based on their potential functional impact [e.g. association with endogenous hormone or SHBG levels [7–13] or possible role in regulation of gene transcript expression involved in hormone signaling [14, 15]] and because at least one prior study had reported an association with endometrial cancer risk [7, 16–21] at the time of initiation of this study. The polymorphisms chosen were: CYP19, rs4775936 and rs10046 [7–9]; CYP1B1, rs1800440 and rs1056836 [20, 22]; UGT1A1, rs8175347 [17, 23]; SHBG, rs6259 [9, 18, 24]; PGR, rs1042838 [16, 25–27]; and ESR1, rs2234693 [21, 28, 29]. Because of budget limitations, we examined only a limited number of polymorphisms. We selected genetic variants for inclusion if an association with endometrial cancer had been reported but was not consistent across studies (or had not been assessed in more than one study) at the time of SNP selection for this study.

We also examined the association of these genetic variants with circulating levels of sex hormones and SHBG in healthy postmenopausal women, i.e. controls from this nested case-

control study and a parallel case-control study of breast cancer nested within one of the three cohorts [30].

Materials and Methods

Study Subjects

The case-control study was nested within three cohorts: the Northern Sweden Health and Disease Study (NSHDS) in Umeå, Sweden [31], the New York University Women's Health Study (NYUWHS) in New York City, USA [32], and the ORDET cohort in Milan, Italy [33]. The eligibility criteria, methods for data and biological sample collection and case ascertainment in each cohort were described previously in a manuscript reporting on the association of postmenopausal circulating levels of sex hormones and SHBG with risk of endometrial cancer in this same nested case-control study [3]. Incident invasive endometrial cancer cases (ICD-O codes 8010, 8140, 8210, 8260, 8310, 8323, 8380, 8382, 8441, 8460, 8461, 8480, 8481, 8560 and 8570) were included and two controls were individually-matched to each case. Controls were selected at random among participants from the same cohort who had not had a hysterectomy and were free of endometrial cancer at the time of diagnosis of the case, and who matched the case on menopausal status at enrollment and age (± 6 months) at, and date (± 3 months) of, blood donation.

Laboratory Methods

DNA extraction and genotyping—The NYUWHS started archiving blood clots and red blood cell precipitates (that had been prepared at time of blood collection by centrifugation of whole blood) about half-way through the recruitment period, resulting in such samples being available as a source of DNA for 42% of the study participants. For the remainder of the participants, DNA was extracted from serum. Samples were genotyped using TaqMan® [34, 35] with an ABI 7900 Real-Time PCR instrument (Applied Biosystems, Foster City, CA) and following the manufacturer's instructions. The percent of successful genotyping calls varied from 97 to 100%.

For the NSHDS and ORDET participants, DNA was isolated at the University of Umeå from buffy coat material. Genotyping was performed at the SNP Technology Platform at Uppsala University Hospital (www.genotyping.se) for seven SNPs. Five SNPs (rs4775936, rs10046, rs1800440, rs6259, rs2234693) were assayed using the GenomeLab SNPStream 12plex-system (Beckman Coulter) and two (rs1056836 and rs1042838) the FP-TDI system. *UGT1A1* rs8175347 was assayed at the German Cancer Research Center in Heidelberg using fluorescent fragment analysis on ABI PRISM 3100 Genetic analyzer (Applied Biosystems) with the GeneMapper software version 3.0 (Applied Biosystems). The percentage of samples with successful calls was 98% or greater for all SNPs except *CYP19* rs10046 which had 92% of samples called.

For each genetic variant, pilot studies were conducted at each of the genotyping centers prior to analyzing case-control samples. For the NYUWHS study, the pilot study included different biological type samples obtained from the same women: serum/clot/cell precipitate triplets from 50 women, serum/clot pairs from 34 women and serum/cell precipitate pairs from 34 women, for a total of 284 samples. For the NSHDS and ORDET, duplicate samples from 141 women (for a total of 282 samples) were included in the pilot study. Samples were re-labeled to prevent the laboratory personnel from identifying samples contributed by the same woman. The concordance between samples from the same participant was 99% or greater for all genetic variants analyzed.

Throughout all procedures, laboratory personnel were blinded as to the case/control status of the samples. Samples of a case and her controls were always analyzed on the same plate. Twelve quality control samples were included on each 96-well plate: 1 containing all reagents except template DNA, 4 samples selected from the pilot study with known genotype (1 homozygous wild-type, 1 homozygous variant and 2 heterozygotes) and 7 blinded duplicate samples. These quality control samples were interspersed at random on the plate.

Sex hormone and SHBG assays—Sex hormones and SHBG had been measured for women postmenopausal at blood donation who were included in a previous study of circulating sex hormones and endometrial cancer based on the same 3 cohorts, resulting in data available for 186 controls (84 from the NSHDS, 90 from the NYUWHS and 12 from ORDET). To increase the sample size, we also included the postmenopausal controls (n = 410) from a parallel case-control study of breast cancer nested within the NYUWHS for which the assays were done in the same laboratories during the same time period. Women were classified as postmenopausal if they reported not having a menstrual period in the 6 months before blood donation or having had a bilateral oophorectomy (n=4 controls). A total of 596 healthy women were included in these analyses, except for the two *CYP1B1* SNPs which were genotyped only for the endometrial cancer case-control study. Assay methods have been published previously [3, 30]. For the control women included in the initial NYUWHS endometrial case-control study (n = 80), estradiol, estrone, testosterone and androstenedione were measured using organic extraction and celite chromatography with the appropriate fractions analyzed by radioimmuno-assays (RIA) at the Clinical Studies Center of Quest Diagnostics Inc (Nichols Institute, San Juan Capistrano, CA). SHBG and DHEAS were measured using an immunometric chemiluminescent assay on an IMMULITE 2000 instrument at NYU. For all other women (n = 516), sex hormones and SHBG assays were carried out at the Hormone Laboratory at IARC, France. Estrone and androstenedione were measured by double antibody RIA with reagents from Diagnostic System Laboratories (Webster, TX), estradiol by ultrasensitive double antibody RIA with reagents from Diagnostic System Laboratories, testosterone and DHEAS by RIA with reagents from Immunotech (Marseille, France), and SHBG by immunoradiometric assay (IRMA) with reagents from Cis-Bio (Gif-sur-Yvette, France). Inter-batch coefficients of variation were ≤15% for all assays. Free estradiol and free testosterone were calculated using mass action equations and the concentrations of these two hormones and of SHBG, and assuming a constant serum albumin concentration [36].

Statistical Methods

The chi-square test was used to assess deviation from the Hardy-Weinberg equilibrium. As appropriate in studies with individual matching, the conditional logistic regression model was used to calculate odds ratios for endometrial cancer according to genotype, and the likelihood ratio test was used to assess statistical significance. Odds ratios are presented for each genotype (no genetic model assumed), as well as per allele (assuming an additive model), except for SNPs with less than 30 homozygous variant subjects (*CYP1B1* rs1800440 (n = 27) and *SHBG* rs6259 (n = 15)) for which homozygous and heterozygous subjects were grouped together. Simple models, adjusted for race only (and also controlling, through matching, for menopausal status, age at blood donation, and duration of sample storage), are presented as well as models adjusted for additional factors known to affect risk of endometrial cancer, i.e. age at menarche, nulliparity, oral contraceptive use, hormone replacement therapy use (containing estrogen and progestin or estrogen alone), and body mass index (BMI). Heterogeneity of the genetic associations by cohort was tested by comparing models with and without cross-product terms (cohort × genotype). Analyses

limited to endometrioid cases (n= 321) and their matched controls, and to Caucasians (n= 362 cases and 645 controls), were also conducted.

To compare hormone and SHBG levels in postmenopausal women according to genotype, geometric means and 95% confidence intervals were computed using general linear models. Geometric means were adjusted for cohort, assay laboratory (to control for technical variability between laboratories and assays), age (continuous), race (Caucasian, African-American, Hispanic, Other/Unknown), and BMI (continuous). A trend test was performed to assess whether the number of variant alleles (0, 1, or 2) was associated with hormone levels, except for SNPs with small numbers of variant homozygous (*CYP1B1* rs1800440and *SHBG* rs6259) for which homozygous and heterozygous carriers were grouped.

Results

A total of 391 cases and 712 controls were included in the nested case-control study (216 cases and 386 controls from the NSHDS, 129 cases and 238 controls from the NYUWHS and 46 cases and 88 controls from ORDET). Table 1 shows case and control subject characteristics. As expected, cases tended to have younger age at menarche and older age at menopause and to have higher weight and body mass index than controls. Also as expected, the proportions of nulliparous, ever users of hormone replacement therapy and diabetics were larger among cases than controls, while the proportion of ever oral contraceptive users was lower.

For all SNPs the allelic frequencies were within the ranges observed in previous studies and there was no evidence of deviation from the Hardy-Weinberg equilibrium in the control groups of each cohort except for *UGT1A1* rs8175347 ($p = 0.02$) in the NYUWHS, overall or in analysis limited to the Caucasian controls. *UGT1A1* rs8175347 frequencies were not consistent with Hardy-Weinberg equilibrium among controls in the Shanghai Endometrial Cancer Study [37] and the test for Hardy-Weinberg equilibrium was also marginally significant ($p=0.08$) in one group of Caucasian controls from the Nurses' Health Study [17]. Because of the high concordance between replicate samples observed in the pilot study and quality control samples included in the case-control study, and because there was no methodological evidence of genotyping errors, this polymorphism was retained in the statistical analysis. Minor allele frequencies did not differ appreciably in Caucasians or among women with endometrioid tumors.

Table 2 presents the odds ratios for endometrial cancer associated with the genetic polymorphisms. Adjusting for known risk factors of endometrial cancer, the A allele of rs4775936 in *CYP19* was significantly associated with increased risk of endometrial cancer ($OR_{per\ allele} = 1.22$, 95% CI = 1.01–1.47, $p_{trend} = 0.04$), while the T allele of rs10046 was marginally associated with risk ($OR_{per\ allele} = 1.20$, 95% CI = 0.99–1.45, $p_{trend} = 0.06$). *PGR* rs1042838 was also found to be marginally associated with risk ($OR_{per\ allele} = 1.25$, 95% CI = 0.96–1.61, $p_{trend} = 0.09$). No significant association with risk of endometrial cancer was found for the other SNPs examined. Results of the analysis limited to Caucasians were very similar to those of the overall analysis (data not shown). In analyses limited to the 321 sets with endometrioid tumors, adjusted odds ratios were slightly lower for the *CYP19* SNPs ($OR_{per\ allele} = 1.19$, 95% CI = 0.97–1.47, $p_{trend} = 0.10$ for rs4775936 and $OR_{per\ allele} = 1.16$, 95% CI = 0.93 – 1.43, $p_{trend} = 0.19$ for rs10046), and higher for *PGR* rs1042838 ($OR_{per\ allele} = 1.36$, 95% CI = 1.01–1.82, $p_{trend} = 0.04$). None of the tests of interaction by cohort were statistically significant.

Table 3 reports the geometric means of circulating estrogens and SHBG according to genotype among healthy postmenopausal women. The two *CYP1B1* polymorphisms (which

were genotyped only in the 186 endometrial cancer study controls) were associated with levels of estradiol and free estradiol: carrying the G allele of *CYP1B1* rs1800440 was associated with significantly lower levels of estradiol (p = 0.03) and free estradiol (p = 0.04), while carrying the G variant of the *CYP1B1* rs1056836 was associated with marginally higher levels of estradiol (p = 0.07) and free estradiol (p = 0.07). For polymorphisms genotyped in both the endometrial and breast cancer study controls (n=596), having 7 TA repeats in *UGT1A1* rs8175347 was associated with higher levels of estradiol (p = 0.05), estrone (p = 0.05), and free estradiol (p = 0.03) as compared to carrying 6 repeats and the A allele of *SHBG* rs6259 was associated with a higher level of SHBG (p = 0.05), as well as estradiol (p = 0.03). Associations were in the same direction, although no longer statistically significant, in analyses restricted to the controls for which hormones were measured in the same laboratory and with the same assay method. None of the other polymorphisms were associated with levels of estrogens or SHBG, and none of the polymorphisms were associated with levels of testosterone, androstenedione, free testosterone or DHEAS (data not shown).

Discussion

With the exception of one case-control study [6], our results for the two SNPs we examined in *CYP19* are consistent with the results of other studies. *CYP19* rs4775936 (A allele) and rs10046 (T allele), which are in linkage disequilibrium (r^2 = 0.79 in our study), were found to be associated with increased risk of endometrial cancer in two earlier studies [7, 38], including a pooled analysis including 4998 cases and 8285 controls from 10 studies [38] which found an association with rs749292, which is in in high linkage disequilibrium with rs10046 (r^2 > 0.83 [8]). Further, two pathway-based analyses support a role for *CYP19* variants in endometrial cancer development [6, 39]. *CYP19* codes for aromatase, the enzyme responsible for the formation of estrogens from androgens. The A allele of rs4775936 and the T allele of rs10046 have been shown to be associated with increased levels of estrogens and/or of estrogen to androgen ratios in several studies [7–9, 40]. Although we did not observe statistically significant differences in estrogen levels according to genotype in our study, levels were in the expected directions, i.e. higher levels with the A allele of rs4775936 and the T allele of rs10046.

It is well established that progesterone inhibits endometrial cell proliferation induced by estrogens through binding to the progesterone receptor [41]. *PGR* rs1042838, the polymorphism that we examined, is a non-synonymous SNP (V660L) in exon 4 which is in complete linkage disequilibrium with the PROGINS allele, an Alu insertion in intron 7, and with rs1042839, a silent SNP (H770H) in exon 5 [26]. The Alu insertion has been shown to reduce transcript stability, and the amino acid substitution (V660L) to result in lower efficiency in opposing proliferation of cells expressing the A isoform of the receptor [14], the main isoform mediating the anti-proliferative effects of progesterone in the endometrium [42]. The suggestive increase in risk we observed with *PGR* rs1042838 is consistent with these observations, as well as with results of some [6, 16, 25], but not all [26, 43, 44], other epidemiologic studies. The large study conducted by Lee et al., which examined 17 haplotype-tagging SNPs in the *PGR* gene, reported that the PROGINS allele, and haplotypes containing the PROGINS allele, were associated with increased endometrial cancer risk [25]. The PROGINS allele was also found to be associated with ovarian cancer of the endometrioid type, which shares histological features with endometrial cancer [45]. *PGR* rs1042838 was not associated with hormone levels in our study, which is in agreement with the largest study to date (3852 women), which did not observe an association between tagging polymorphisms in the *PGR* gene and estrogens, androgens, or SHBG [40].

We observed that *SHBG* rs6259 carriers of allele A had higher circulating levels of SHBG than non-carriers. Similar results were observed in several [9–11, 24, 46], although not all [47, 48], prospective and cross-sectional studies of healthy women that examined this association. This result is consistent with the observation that rs6259, a non-synonymous SNP leading to the introduction of an N-glycosylation site, reduces the rate of clearance of SHBG [49]. Higher levels of SHBG are associated with a reduced risk of endometrial cancer which is thought to result primarily from its binding to estrogens, reducing their bioavailability [3]. The observation of a 28% reduction in endometrial cancer risk in postmenopausal women carrying the rs6259 variant in a large Chinese case-control study was therefore in the expected direction [18]. However, a smaller Polish case-control study reported an increased risk of endometrial cancer associated with the variant allele [6]. In our study, *SHBG* rs6259 was not associated with risk, however, we had to group the homozygous variant and recessive alleles since the allelic frequency of rs6259 is quite low in Caucasians (10% in our study), as compared to Asians (~18%) [50], and thus may have not been able to observe an association with the variant genotype. We also observed an association of rs6259 allele A with higher levels of total estradiol. The only other study that reported on the association of rs6259 with circulating estrogen, which was larger than ours (1975 healthy postmenopausal women), found no difference in total estradiol according to genotype [9], so it is possible that our finding is due to chance.

We did not find an association between risk of endometrial cancer and the *UGT1A1* rs8175347, which is associated with a variable number of repeat TA in the $A(TA)_nTAA$ sequence of the promoter. Although an early study [17] found that carriers of the *28 allele (7 repeats) had a reduction in risk of endometrial cancer as compared to the wild type (*1, 6 repeats), several subsequent studies did not confirm this result [23, 37, 44, 51]. We found, though, that controls carrying the *28 allele had significantly higher circulating levels of estrogens than those carrying the *1 variant. A similar result was observed in another study, although it did not reach statistical significance [12], while a third study reported higher levels of estradiol in *28 homozygous carriers only; however this study had a small sample size (n = 87) [52]. The findings that estrogens may be higher among carriers of the *28 allele, though, are consistent with the observation that the *28 allele may be associated with reduced glucuronidation of various estrogens, including estradiol [12].

We did not observe an association of endometrial cancer risk with the two non-synonymous *CYP1B1* SNPs that we examined. Although some early studies found an increased risk with the G (Ser) allele of rs1800440 [19] or the G (Val) allele of rs1056836 [20], subsequent studies, including several larger studies, did not confirm these associations [6, 44, 51, 53–56]. Although a meta-analysis of 12 case-control and nested case-control studies (n = 2059 cases and 3381 controls) reported a positive association for the G vs. C allele of rs1056836 and endometrial cancer risk (OR = 1.23, 95% CI = 1.06, 1.43, p=0.007), there was significant heterogeneity across studies [57]. These two *CYP1B1* SNPs were associated with levels of circulating estradiol and free estradiol in our study: we observed lower levels of estradiol with the G allele of rs1800440 and higher levels with the G allele of rs1056836. De Vivo et al. also observed significant associations of these two SNPs with estradiol levels [13]. However, whereas the association was in the same direction as in our study for rs1056836, it was in the opposite direction for rs1800440. The largest two studies conducted to date [1975 [9] and 2721 [40] women], as well as another smaller study [58], did not observe any association between hormones and *CYP1B1* SNPs . Although differences in assay methodology or subject characteristics could contribute to these discrepancies, overall these inconsistent results suggest that, if these SNPs do affect estrogen levels, the effect is likely to be small.

In conclusion, we observed associations of SNPs in the *CYP19* and *PGR* genes with risk of endometrial cancer. These associations are consistent with the well documented roles of estrogens and progesterone in the development of endometrial cancer. Although the associations between *CYP19* and *PGR* SNPs and estrogen levels were not significant, they were in the expected direction based on previous reports in the literature [7–9] and the direction of their association with endometrial cancer risk (positive and inverse, respectively). The lack of a significant association between these SNPs and estrogen levels in our study may be due to several factors: 1) differences in the hormone assays used across studies; 2) we only had a single hormone measurement and only one or two SNPs genotyped in each gene, thus we may not have been able to capture more complex multi-SNP associations with hormones or account for intra-individual variability in hormone levels over time, and 3) associations between these genetic variants and endometrial cancer risk may not be exclusively mediated through changes in circulating hormone levels. The risk of endometrial cancer was not associated with the genetic variants we examined in the *CYP1B1, UGT1A1, SHBG* and *ESR1* genes. These results do not preclude a role of the genes carrying these variants since we examined only one or two SNPs for each gene. Although not associated with risk of endometrial cancer, some of the polymorphisms we examined were associated with circulating levels of sex hormones and SHBG: *SHBG* rs6259 was associated with levels of SHBG and estradiol, *UGT1A1* rs8175347 with levels of estradiol, free estradiol and estrone and *CYP1B1* rs1800440 with levels of estradiol and free estradiol. Similar observations were made in breast cancer studies where no association with disease risk was found for genetic variants significantly related to estrogen levels, despite the facts that estrogen levels are almost as strongly associated with breast cancer risk than with endometrial cancer risk, and that these studies were much larger than the current study [40, 59]. The impact of any single polymorphism on circulating hormone levels may be too small to result in a significant association with endometrial cancer risk, in particular in studies of moderate size.

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Table 1

Description of case and control subjects

Characteristic	Cases (n = 391)	Controls (n = 712)	p-value
Age at blood donation, years, median (10^{th}, 90^{th})	54.4 (44.0, 62.7)	54.5 (44.0, 63.0)	Matched
Age at diagnosis, years, median (10^{th}, 90^{th})	61.9 (51.6, 71.6)		
Lag time between blood donation and diagnosis, years, median (10^{th}, 90^{th})	7.6 (1.8, 13.2)		
Race, n (%)			0.26
Caucasian	362 (96%)	645 (94%)	
Other	16 (4%)	44 (6%)	
Missing	13	23	
Height, cm, median (10^{th}, 90^{th})	162.8 (154.0, 170.0)	162.0 (154.0, 169.0)	0.41
Weight, kg, median (10^{th}, 90^{th})	69.0 (54.0, 90.0)	64.0 (53.0, 82.0)	<0.0001
Body mass index, kg/m^2, n (%)			<0.0001
<25	143 (42%)	360 (57%)	
25-<30	105 (31%)	183 (29%)	
≥30	92 (27%)	91 (14%)	
Age at menarche, years, n (%)			0.01
<12	73 (20%)	96 (14%)	
12	78 (21%)	125 (19%)	
13	97 (26%)	201 (30%)	
>13	121 (33%)	250 (37%)	
Missing	22	40	
Postmenopausal at diagnosis, n (%)	**192 (92%)**	**542 (95%)**	**0.19**
Missing	**75**	**146**	
Age at menopause, years, n (%)			0.0002
<50	55 (22%)	162 (34%)	
50–52	95 (37%)	158 (33%)	
>52	103 (41%)	154 (33%)	
Missing	138	238	
Nulliparous, n (%)	83 (22%)	109 (16%)	0.02
Missing	14	31	
Ever used oral contraceptives, n (%)	108 (30%)	241 (36%)	0.04
Missing	30	46	
Ever used hormone replacement therapy, n (%)	155 (47%)	201 (33%)	<0.0001
Missing	64	102	
Ever smoked, n (%)	146 (40%)	294 (43%)	0.38
Missing	27	27	
History of diabetes, n (%)	28 (8%)	25 (4%)	0.004
Missing	50	62	

Table 2

Odds ratios for endometrial cancer associated with gene variants

Gene/SNP (aliases)	Genotype	Cases n (%)	Controls n (%)	OR[a] (95% CI)	P_{trend}	OR[b] (95%CI)	P_{trend}
CYP19 rs4775936 (5'Flank, G to A in exon 1.6)	GG	117 (30%)	238 (34%)	1.0		1.00	
	GA	185 (47%)	357 (50%)	1.03 (0.78, 1.37)		1.08 (0.80, 1.46)	
	AA	89 (23%)	114 (16%)	1.51 (1.05, 2.16)		1.55 (1.06, 2.27)	
	Per allele			1.20 (1.01, 1.44)	0.04	1.22 (1.01, 1.47)	0.04
CYP19 rs10046 (3'UTR, C to T in intron 4)	CC	96 (25%)	206 (29%)	1.0		1.00	
	CT	200 (51%)	365 (52%)	1.15 (0.85, 1.55)		1.19 (0.86, 1.64)	
	TT	95 (24%)	138 (19%)	1.42 (0.99, 2.05)		1.44 (0.98, 2.12)	
	Per allele			1.19 (0.99, 1.43)	0.06	1.20 (0.99, 1.45)	0.06
CYP1B1 rs1800440 (A4390G, Asn453Ser)	AA	283 (73%)	496 (70%)	1.00		1.00	
	AG/GG	106 (27%)	213 (30%)	0.88 (0.66, 1.15)	0.34	0.91 (0.68, 1.21)	0.51
CYP1B1 rs1056836 (*3, C4326G, Leu432Val)	CC	126 (32%)	221 (31%)	1.0		1.00	
	CG	189 (48%)	352 (50%)	0.96 (0.72, 1.28)		0.99 (0.73, 1.34)	
	GG	76 (20%)	131 (19%)	1.11 (0.77, 1.60)		1.08 (0.73, 1.61)	
	Per allele			1.04 (0.87, 1.25)	0.66	1.03 (0.85, 1.25)	0.75
UGT1A1 rs8175347 (*28 and *33, ≥7 TA repeats in TATA box of the promoter)	6/6	171 (45%)	311 (45%)	1.0		1.00	
	6/7	163 (43%)	296 (43%	0.96 (0.74, 1.26)		0.91 (0.68, 1.22)	
	7/7	46 (12%)	82 (12%)	1.01 (0.65, 1.57)		0.95 (0.59, 1.53)	
	Per allele			0.99 (0.81, 1.21)	0.92	0.95 (0.77, 1.18)	0.65
SHBG rs6259 (G5790A)	GG	307 (81%)	548 (81%)	1.0		1.00	
	GA/AA	73 (19%)	131 (19%)	1.03 (0.75, 1.42)	0.86	1.02 (0.72, 1.45)	0.90
PGR rs1042838 (V660L)	GG	281 (72%)	540 (76%)	1.00		1.00	
	GT	96 (24%)	147 (21%)	1.21 (0.90, 1.62)		1.28 (0.93, 1.76)	
	TT	14 (4%)	18 (3%)	1.38 (0.67, 2.86)		1.42 (0.65, 3.09)	
	Per allele			1.19 (0.94, 1.52)	0.14	1.25 (0.96, 1.61)	0.09
ESR1 rs2234693 (T397C, Pvull in intron 1, IVS1-401)	TT	116 (30%)	194 (27%)	1.0		1.00	
	TC	184 (47%)	369 (52%)	0.86 (0.64, 1.16)		0.89 (0.65, 1.23)	
	CC	91 (23%)	146 (21%)	1.06 (0.74, 1.51)		1.12 (0.77, 1.64)	
	Per allele			1.02 (0.85, 1.22)	0.84	1.05 (0.87, 1.27)	0.61

[a] Adjusted for race (Caucasian, African-American, Hispanic, Other/Unknown) and controlled (through matching) for age, menopausal status at blood donation and biospecimen storage duration.

[b] Adjusted, age at menarche (<12, 12, 13, >13, missing), parity (ever, never, missing), oral contraceptive use (never, ever, missing), hormone replacement therapy use (never, ever, missing), and BMI (<25, 25–30, >30, missing), in addition to factors in [1].

Table 3

Geometric mean (95% CI) of circulating sex hormones and SHBG in postmenopausal healthy women, adjusted for cohort, assay, race, age, and BMI

	N (%)	Estradiol, pmol/l	Estrone, pmol/l	SHBG, nmol/l	Free Estradiol, pmol/l
CYP19 rs4775936					
GG	188 (32%)	64.2 (58.0, 71.0)	74.7 (67.2, 83.0)	45.0 (39.9, 50.7)	1.53 (1.37, 1.71)
GA	300 (50%)	67.5 (61.1, 74.6)	76.6 (69.0, 85.0)	44.6 (39.6, 50.2)	1.62 (1.45, 1.81)
AA	107 (18%)	66.6 (59.0, 75.0)	77.3 (68.2, 87.6)	48.6 (42.1, 56.1)	1.55 (1.35, 1.77)
p for trend		0.35	0.48	0.27	0.64
CYP79 rs10046					
CC	155 (26%)	63.9 (57.6, 71.0)	74.3 (66.6, 82.9)	44.1 (38.9, 50.0)	1.53 (1.36, 1.72)
CT	304 (51%)	66.9 (60.6, 73.6)	76.3 (68.8, 84.5)	45.9 (40.8, 51.6)	1.59 (1.42, 1.78)
TT	135 (23%)	67.4 (60.0, 75.7)	78.4 (69.5, 88.5)	45.4 (39.5, 52.1)	1.60 (1.40, 1.82)
p for trend		0.26	0.29	0.60	0.41
CYP1B1 rs1800440					
AA	119 (64%)	65.9 (53.6, 80.9)	77.0 (62.3, 95.2)	55.8 (45.1, 69.1)	1.46 (1.17, 1.83)
AG/GG	67 (36%)	56.3 (45.1, 70.4)	73.3 (58.2, 92.4)	55.4 (44.0, 69.7)	1.26 (0.98, 1.60)
p-value		0.03	0.49	0.91	0.04
CYP1B1 rs1056836					
CC	52 (28%)	57.0 (45.3, 71.8)	71.6 (56.2, 91.1)	58.2 (45.8, 74.0)	1.26 (0.98, 1.61)
CG	95 (51%)	62.1 (50.1, 76.8)	74.4 (59.6, 92.7)	55.0 (44.1, 68.7)	1.38 (1.09, 1.73)
GG	38 (21%)	67.7 (53.7, 85.1)	81.0 (63.9, 102.8)	55.2 (43.5, 70.1)	1.52 (1.19, 1.95)
p for trend		0.07	0.22	0.56	0.07
UGT1A1 rs8175347					
6/6	264 (45%)	63.0 (57.0, 69.7)	72.4 (65.2, 80.4)	46.6 (41.3, 52.6)	1.48 (1.32, 1.66)
6/7	230 (39%)	67.6 (60.9, 74.9)	80.1 (71.9, 89.3)	44.2 (39.0, 50.1)	1.62 (1.44, 1.82)
7/7	92 (16%)	67.9 (60.3, 76.4)	77.1 (68.2, 87.3)	44.1 (38.2, 50.8)	1.64 (1.43, 1.87)
p for trend		0.05	0.05	0.22	0.03
SHBG rs6259					
GG	487 (83%)	65.3 (59.5, 71.7)	75.4 (68.4, 83.1)	44.9 (40.1, 50.1)	1.56 (1.41, 1.53)
GA/AA	103 (17%)	72.0 (63.9, 81.1)	78.1 (68.9, 88.5)	49.7 (43.1, 57.3)	1.65 (1.44, 1.89)
p-value		0.03	0.43	0.05	0.26
PGR-12 rs1042838					

	N (%)	Estradiol, pmol/l	Estrone, pmol/l	SHBG, nmol/l	Free Estradiol, pmol/l
GG	417 (70%)	65.9 (60.0, 72.3)	76.0 (68.9, 83.7)	45.6 (40.8, 51.0)	1.57 (1.41, 1.74)
GT	156 (26%)	66.1 (59.0, 74.1)	74.1 (65.7, 83.5)	42.7 (37.2, 48.9)	1.60 (1.41, 1.82)
TT	22 (4%)	58.1 (47.9, 70.6)	73.5 (60.0, 90.0)	43.3 (34.4, 54.7)	1.43 (1.15, 1.78)
p for trend		0.42	0.51	0.17	0.88
ESR1 rs2234693					
TT	171 (29%)	63.7 (57.1, 71.1)	74.1 (66.1, 83.1)	45.7 (40.0, 52.1)	1.50 (1.33, 1.70)
TC	302 (51%)	66.9 (60.8, 73.6)	76.6 (69.4, 84.6)	44.9 (40.1, 50.3)	1.60 (1.44, 1.78)
CC	123 (20%)	64.1 (57.1, 72.0)	74.0 (65.6, 83.6)	46.4 (40.4, 53.4)	1.51 (1.33, 1.72)
p for trend		0.79	0.92	0.81	0.80