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Association of genetic polymorphisms in CYP19A1 and blood levels of sex hormones among postmenopausal Chinese women

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Abstract

Objective—Circulating estrogen levels have been related to the risk of several female cancers. Blood levels of estrogen are controlled by estrogen synthesis enzymes. Genetic variation of estrogen genes thus may influence circulating estrogen levels. We investigated the associations of genetic polymorphisms in *CYP19A1*, a critical gene involved in estrogen synthesis, with plasma levels of sex hormones among postmenopausal Chinese women.

Methods—Included in this study were 345 postmenopausal community controls from a population-based case-control study conducted in Shanghai. Fasting blood samples from those women were measured for blood estradiol, estrone, estrone sulphate and testosterone. A total of 19 genetic polymorphisms in *CYP19A1* were genotyped using ABI7900 or PCR-RFLP methods. Differences in plasma levels of hormones by genotype were examined using variance analysis.

Result—The geometric means of plasma levels of estradiol, estrone, estrone sulphate and testosterone were 10.1 pg/ml, 16.8 pg/ml, 969.0 pg/ml and 202.9 pg/ml, respectively, for this study population. We found that plasma levels of estrone were associated with rs28566535 ($P=0.0180$), rs730154 ($P=0.0141$) and rs936306 ($P=0.0274$) in block 2. In the same block, the haplotype CGCTA was related to level of estrone ($P=0.0064$). SNP rs1902584 in block 1 was associated with estradiol only in overweight postmenopausal women. No clear association with sex hormones was noted for the other genetic polymorphisms evaluated in the study.

Conclusion—SNPs in block 1 and block 2 of the *CYP19A1* gene are related to plasma levels of estrogen among postmenopausal Chinese women and may therefore play an important role in the etiology of hormone-related cancers.

Keywords

association; genetic polymorphism; *CYP19A1*; sex hormone; synthesis; BMI

Introduction

Endogenous sex-steroid hormones play an important role in the etiology of female cancers, including breast and endometrial cancer [1,2]. Sex-steroid hormones are synthesized and

eliminated through complex metabolic pathways. Polymorphisms in genes coding for key enzymes involved in these pathways could modulate endogenous hormone levels and through this mechanism, influence the risk of hormone-related cancers [2,3].

In postmenopausal women, the most abundant circulating estrogens are estrone, and to a lesser extent, estradiol, both derived from the extragonadal aromatisation of androstenedione and testosterone [4-6]. It is generally believed that genetic polymorphisms in hormone-related genes could explain a portion of breast cancer susceptibility through modulating the level of endogenous hormones. The effect of polymorphisms on circulating levels of hormones, however, has been evaluated only in few studies with no consistent evidence of substantial effect [2,3,7-10].

The *CYP19A1* gene codes for aromatase, which catalyzes the conversion of testosterone to estradiol and androstenedione to estrone [11]. In this study, we evaluated 19 polymorphisms in the *CYP19A1* gene for associations with levels of several sex hormones in the blood of Chinese women who were involved as controls in a population-based, case-control study, the Shanghai Breast Cancer Study.

Materials and methods

Study subjects

The current study was conducted in a subset of controls recruited as a part of the Shanghai Breast Cancer Study (SBCS). The study design and participant recruitment have been described in detail elsewhere [12]. Briefly, from August 1996 to March 1998 a population-based case-control study was conducted in Shanghai, China. 1724 healthy control women aged 25–64 years were enrolled in the study. They were randomly selected from the general population in Shanghai, using the resident registration information provided by the population-based Shanghai Resident Registry. An in-person interview was conducted by a trained interviewer for all eligible controls. Of those who were eligible for our study, 90.3% completed an in-person interview (refusals: n=166, 9.6%; death or prior cancer diagnosis: n=2, 0.1%).

The in-person interview was conducted with the use of a structured questionnaire, which elicited information on demographic features, menstrual and reproductive history, use of sex steroid hormones, medical history, physical activity, dietary habits and family history of cancer. Of the 1556 controls, morning fasting blood samples were collected from 1310 controls (84.2%). These blood sample were processed to separate plasma within six hours of collection, and the plasma specimens were immediately stored at -70°C .

Genetic marker selection and genotyping assays

The genotyping assays were conducted in 2004 before the release of HapMap data. Therefore, the genetic markers of the *CYP19A1* gene included in this study were selected according to a report from the Multiethnic Cohort Study [13]. Based on haplotype analyses of 74 densely spaced SNPs across the gene, 19 haplotype tagging SNPs (htSNPs) were identified in a Japanese population. Because of the similarity in linkage disequilibrium patterns between Chinese and Japanese populations [14], we used these 19 htSNPs in the present study.

Genomic DNA was extracted from buffy coats using a Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. SNP rs1004984 was genotyped using a Masscode assay at BioServe Biotechnologies, Ltd (Laurel, MD). The nonsynonymous SNP, rs700519, was genotyped using PCR-RFLP and confirmed by direct sequencing using BigDye Terminator Chemistry on an ABI 3700 (ABI, Applied Biosystems, Inc, Foster City, CA, USA). Genotyping of the other 17 SNPs was performed by running the

5' nuclease TaqMan allelic discrimination assay using an ABI 7900 (ABI). Details concerning assays, primers, probes, and procedures are available upon request.

Laboratory staff were blind to the identity of the subjects. Quality control samples were included in the genotyping assays. Each 96-well plate of genomic DNA contained multiple quality controls, including one water, two samples of CEPH 1347-02, two known duplicates, and two blinded duplicates. The average agreement of the genotypes for these 19 markers determined for the blinded quality control samples was 98.7%.

Sex hormone measurement

Measurement of steroids in our study was performed in a reference laboratory at Diagnostic Systems Laboratories, Inc. (DSL, Webster, TX). The reference laboratory specializes in *in vitro* diagnostic testing of endocrine markers and is certified by the Clinical Laboratory Improvement Amendments (CLIA) and International Standard ISO 9002. Each sample was tested in duplicate. Commercial radioimmunoassays (RIA) from DSL were used for the measurement of steroids. The intra- and inter-assay precisions expressed as coefficient of variation (CV) were 3.2–5.3% and 8.1–9.3% for estradiol; 4.4–9.4% and 6.0–11.1% for estrone; 4.6–9.2% and 5.1–8.8% for estrone sulfate and 6.7–8.1% and 5.7–10.5% for testosterone, respectively. The assay's standard range and sensitivity (in brackets) were 20–6000 pg/ml (4.7 pg/ml) for estradiol; 15–2000 pg/ml (12 pg/ml) for estrone; 0.025–15 ng/ml (0.01 ng/ml) for estrone sulfate and 0.1–25 ng/ml (0.05 ng/ml) for testosterone.

Statistical analysis

The program PHASE, which is based on a Bayesian statistical model [15], was used to reconstruct haplotypes within each block of the *CYP19A1* gene. The SNPs used for haplotype reconstruction were arranged according to their chromosomal locations in the order of rs2446405, rs2445765, rs2470144, rs1004984 and rs1902584 for block 1; rs28566535, rs12900137, rs730154, rs936306 and rs1902586 for block 2; rs749292, rs6493494 and rs1008805 for block 3; rs727479, rs2414096, rs700519, rs10046 and rs4646 for block 4. Because the measured values of the hormones were not normally distributed, all values of hormones were log-transformed in the statistical analysis. Variance analysis was used to evaluate the differences of levels of hormones among genotypes of *CYP19A1* with adjustment for age, age at menarche, BMI and batch (because hormone fractions were measured in two laboratory batches). Adjusted means of sex hormones and their confidence intervals were calculated. All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) and all tests were based on two-tailed probability.

Results

There were 438 postmenopausal control women in the case-control study. Among them 93 women who had either used estrogen replacement therapy or smoked or consumed alcohol regularly or had non-natural menopause were excluded. The remaining 345 women were included in this study. The geometric means of plasma levels of estradiol, estrone, estrone sulfate and testosterone were 10.1 pg/ml, 16.8 pg/ml, 969.0 pg/ml and 202.9 pg/ml, respectively, for the study population.

Table 1 presents characteristics of the study subjects. All of the women were between 39 and 64 years of age (57.1 ± 4.8). Their average age at menopause was 48.3 years (48.3 ± 3.9). Most were married (86.4%). The mean BMI and WHR were 24.2 and 0.82, respectively. Genotype and allele distributions for the 19 SNPs are summarized in Table 2. With the exception of rs2445765 and rs12907866, all other SNPs were consistent with Hardy-Weinberg equilibrium (HWE) distribution ($P > 0.05$).

Table 3 presents the relationship between levels of sex hormones (estradiol and estrone), estradiol:testosterone ratio and SNPs among four blocks in the *CYP19A1* gene. Only genotypes in block 2 were associated with estrone levels. The mean estrone concentration was 15.7 pg/ml among women with the rs28566535 AA or rs730154 TT genotype, which was significantly lower ($P=0.0180$ and $P=0.0141$) than that of women with genotype AC/CC or TC/CC at these loci (means were 17.8 and 18.0 pg/ml, respectively). Also, the geometric means of estrone among women with rs936306 CC was 15.6 pg/ml, which was significantly lower ($P=0.0274$) than that of women with genotype CT/TT (geometric mean was 17.6 pg/ml). There were no significant associations between levels of estradiol, estradiol:testosterone ratio, estrone sulphate and testosterone and other SNPs (data not shown).

Haplotype analyses were conducted to evaluate the combined effect of the SNPs located within each haplotype block. Because the genotype distributions of rs2445765 and rs12907866 deviated from HWE (Table 2), they were eliminated from the haplotype reconstruction. As shown in Table 4, an association between haplotype and level of estrone was observed for the CGCTA haplotype (minor alleles for rs28566535, rs730154, rs936306, and rs1902586 in block 2) with statistical significance ($P=0.0064$); women with one or two copies of this haplotype had an 18% higher level of estrone. Estrone sulfate was 22% lower among women having one or two copies of another haplotype composed of minor alleles of 5 SNPs CCCTA. Haplotypes in the other three blocks were, in general, not related to blood levels of estrogen (data not shown).

As aromatase expression in adipose tissue occurs primarily via promoter I.4 in block 1, we explored relationships of SNPs in block 1 with estrogen levels in overweight women ($BMI \geq 25 \text{ kg/m}^2$), who have higher levels of adiposity. We found that overweight women with the rs1902584 AT/TT genotype had 34% higher levels of estradiol, compared to women with the AA genotype ($P=0.0312$). We also found a significant interaction between rs1902584 and BMI ($P=0.0201$) after adjustment for age, age at menarche and batch. Figure 1 presents the 'dose-response' relationship between estradiol concentrations and BMI by rs1902584 genotype. Estradiol levels did not change materially with increasing BMI in women with the AA genotype. In contrast, estradiol levels increased markedly with increasing BMI in women with the AT/TT genotype. The peak in estradiol levels in this group was about 15 pg/ml, which occurred at a BMI of approximately 27 kg/m^2 . The association between estradiol and SNPs in block 1 was confirmed by the TCAT haplotype; overweight women with the TCAT haplotype had a higher level of estradiol (geometric mean=14.1 pg/ml) when compared to women with a non-TCAT haplotype (geometric mean=10.5, $P=0.0411$) (data not shown in the table). The association of estrone and CGCTA in block 2 remained ($P=0.0358$) in overweight women. We did not find relationships in the remaining SNPs with estradiol levels among overweight women nor did we observe relationships between SNPs in block 1 and estrogen levels in non-obese women.

Discussion

This is the first study to comprehensively evaluate the associations of 19 tagging SNPs in the *CYP19A1* gene with levels of sex hormones among postmenopausal Chinese women. We found that rs1902584 located in haplotype block 1 was associated with estradiol levels among overweight postmenopausal women, whereas polymorphisms in haplotype block 2 were associated with estrone levels among all postmenopausal women.

It is widely accepted that estrogens are involved in the development of breast cancer, and longer lifetime exposure to endogenous estrogen or to high levels of estrogen are known to increase breast cancer risk [16]. Among estrogen biosynthesis genes, *CYP19A1* is the most important [17]. The *CYP19A1* gene, mapped to chromosome 15q21.1, spans about 123 kb with translated

exons II-X (about 30 kb) and a 93 kb regulatory region (exon I). The regulatory region contains at least 190 distinct promoters that regulate in a tissue- or signal pathway- specific manner [18]. Among these, promoters I.3, I.4 and II are expressed in adipose tissue and may also participate in the pathogenesis of malignant breast transformation, since different promoters in exon I were found to control aromatase expression in normal breast adipocytes (exon I.4) and during malignant breast transformation (exon I.3 and promoter II) [19]. Downstream of the transcriptional start site for promoter I.4, there is a specificity protein-1 (Sp1) binding site; upstream of the transcriptional start site of promoter I.4 there is a gamma interferon activating sequence (GAS) element, downstream of which is a glucocorticoid response element (GRE) [20]. All of these three elements are required for expression from promoter I.4. The SNPs located in haplotype block 1 are very close to promoter I.4. Among the five SNPs of haplotype block 1 in our study, rs1902584 has the shortest distance to the three response elements, 493 bp upstream of GAS, 646 bp upstream of GRE and 915 bp upstream of Sp1 [20]. Therefore this SNP may have an effect on regulating gene transcription and may affect the activity of aromatase in adipose tissue. We have previously reported a positive association between this genetic polymorphism (rs1902584) and obesity among Chinese women [21]. In postmenopausal women estrogen is produced primarily through the conversion of androgens to estrogens catalyzed by aromatase in the adipose tissue [22,23], and the genetic effect of *CYP19A1*, thus, may be more evident among postmenopausal women with high BMI. Indeed, we found interactions between haplotype TCAT in block 1 and BMI. Overweight women with the TCAT haplotype had 34% higher levels of estradiol compared to women without the TCAT haplotype. We further observed that in this block only rs1902584 AA was related to low levels of estradiol among overweight postmenopausal women. This may indicate that rs1902584 AA is the main predictor of low estrogen levels in this population.

We observed associations between each SNP in haplotype block 2 and levels of estrone and these associations were confirmed by haplotype analysis. Women with the minor allele at four of the five SNPs tended to have higher levels of estrone and estrone sulphate, compared to those with the major alleles. These associations may be related to the function of promoter I.7 in this block. Promoter I.7, recently cloned by Sebastian et al [24], is located approximately 36 kb upstream of the coding region. It is unique in that it is a GATA-2-regulated endothelial promoter of the human P450arom and may increase estrogen biosynthesis in the vascular endothelial cells of breast cancer [24]. The activity of this promoter may also be important for the intracrine and paracrine effects of estrogen on blood vessel physiology [18]. In the Multiethnic Cohort Study [13] a significant association was observed between our risk haplotype, CGCTA, and breast cancer with an odds ratio (95% CI) of 1.2 (1.1–1.4) in the entire study population. Interestingly, the association was strongest among the Japanese women (OR: 1.4; 95% CI 1.1–1.8), a group genetically more similar to the Chinese women included in the present analysis. Our findings thus support the possibility that this promoter may have a potential role in breast cancer pathogenesis [25,26]. In line with these results, we have previously reported that patients homozygous for the minor alleles in the five SNPs located in haplotype block 2 had a lower 5-year disease-free survival rate compared with those carrying the major allele [27].

In a study among 1747 postmenopausal women that examined two polymorphisms in the *CYP19A1* gene, Dunning et al. found rs10046 to be a modest predictor of estradiol and estrone levels (about 6% increase per allele, $P < 0.01$) [28]. In a more comprehensive evaluation of common variations spanning the entire *CYP19A1* locus, Haiman et al. reported that the two-SNP haplotype rs749292-rs727479 (A-A) in *CYP19A1* is significantly associated with about a 15% increase in estrogen levels in postmenopausal women ($P < 0.01$) [29]. These SNPs, however, were not associated with estrogen levels in our study. The reasons for the inconsistencies in these studies remain unclear. It is possible that the association of these SNPs

with estrogen levels may be mediated through their LD with true functional SNPs, and the degree of LD of these SNPs may differ in these study populations.

Our study was based on an ethnically homogenous population among naturally postmenopausal women with no history of smoking, alcohol consumption or use of hormone replacement therapy. The results provide evidence that the variation of SNPs in haplotype block 1 and block 2 of the *CYP19A1* gene may be functionally relevant to circulating hormone concentrations. Selection bias was minimized given the population-based study design and high response rate. A potential concern for the study is the relatively small sample size, which may have reduced our power to detect minor variations in hormone levels determined by *CYP19A1* variants, especially in multivariate analysis and stratification.

In summary, our study suggests that SNPs in haplotype block 2 of the *CYP19A1* gene are associated with higher plasma levels of estrone in postmenopausal women, and rs1902584 may be associated with estradiol levels among overweight or obese postmenopausal women, contributing potentially to their greater breast cancer risk.

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Figure 1.
Relationship of estradiol level and BMI according to rs1902584 genotypes

Table1

Characteristics of 345 study participants

	N	$\bar{x} \pm s$
Age (years)	345	57.1 $\pm$ 4.8
BMI (kg/m ²)	345	24.2 $\pm$ 3.7
WHR	345	0.82 $\pm$ 0.06
Parity	336	2.29 $\pm$ 0.99
Age at menopause (years)	345	48.3 $\pm$ 3.9
Age at menarche (years)	345	15.0 $\pm$ 1.9
Marital status	Categories	No. (%)
	Never married	2 (0.6)
	Married/Living with partner	298 (86.4)
	Widowed	39 (11.3)
Education	Divorced/Separated	6 (1.7)
	<middle school	118 (34.2)
	=middle school	103 (29.9)
	>middle school	124 (35.9)

Table 2
Information for the studied markers in the *CYP19A1* gene in Chinese postmenopausal women

SNP	Alleles ^a	Location	Position ^b	Haplotype block ^c	Allele frequency ^d	Genotype frequency ^e			P value ^f
						AA	AB	BB	
rs2446405	A/T	exon 1	49434085	1	49.23	22.29	53.87	23.84	0.163
rs2445765	C/G	exon 1	49422190	1	28.33	5.57	45.51	48.92	0.030
rs2470144	T/C	exon 1	49409017	1	40.19	15.76	48.87	35.37	0.770
rs1004984	A/G	exon 1	49400821	1	31.68	10.25	42.86	46.89	0.859
rs1902584	T/A	exon 1	49398946	1	14.11	1.25	25.71	73.04	0.278
rs28566535	C/A	exon 1	49388433	2	35.61	12.42	46.36	41.21	0.841
rs12900137	C/G	exon 1	49386645	2	19.76	4.26	31.00	64.74	0.687
rs730154	C/T	exon 1	49378496	2	35.28	11.66	47.24	41.10	0.533
rs936306	T/C	exon 1	49366890	2	36.19	12.01	48.35	39.64	0.392
rs1902586	A/G	exon 1	49358145	2	36.45	13.08	46.73	40.19	0.877
rs749292	A/G	exon 1	49346023	3	44.91	21.60	46.60	31.79	0.295
rs6493494	A/G	exon 1	49337127	3	44.77	22.15	45.23	32.62	0.124
rs1008805	G/A	exon 1	49336891	3	28.66	8.72	39.88	51.40	0.656
rs12907866	A/G	exon 1	49332746	4	39.41	26.71	25.41	47.88	0.000
rs727479	C/A	intron 1	49321839	4	27.12	7.84	38.56	53.61	0.662
rs2414096	A/G	intron 1	49317071	4	45.26	20.80	48.93	30.28	0.821
rs700519	T/C	Cys264Arg (exon 8)	49295260	4	17.68	3.35	28.66	67.99	0.778
rs10046	G/A	3'UTR (exon 10)	49290278	4	44.41	18.32	52.17	29.50	0.309
rs4646	A/C	3'UTR (exon 10)	49290136	4	27.13	6.71	40.85	52.44	0.548

^aMinor allele is bolded.
^bChromosome position based on NCBI build 35.
^cHaplotype block that the SNPs belonged to, based on Haiman et al 2003 17.
^dMinor allele frequency for each SNP.
^eFor each SNP, AA, minor allele homozygote, AB, heterozygote, BB, major allele homozygote.
^fP value is the probability of the Chi-square test for Hardy-Weinberg disequilibrium.

Table 3
Geometric means and 95% CI of estrone and estradiol by SNPs^{1/}

	No.	No.	G(95%CI)	No.	G(95%CI)	P values
Block1	rs2446405					
	Estradiol (pg/ml)	77	TT	246	AT/AA	0.4069
	Estrone (pg/ml)	76	10.6(9.1–12.2)	246	9.8(9.0,10.7)	0.5025
	Estradiol: Testosterone	77	16.4(14.8–18.2)	246	17.1(16.2,18.1)	0.1910
	rs2445765					
	Estradiol (pg/ml)	158	5.5(4.6–6.5)	165	4.8(4.4–5.3)	0.3949
	Estrone (pg/ml)	157	GG	165	GC/CC	0.7799
	Estradiol: Testosterone	158	9.7(8.7–10.7)	165	10.3(9.3,11.4)	0.4403
	rs2470144					
	Estradiol (pg/ml)	110	16.9(15.7–18.2)	201	17.2(16.0,18.4)	0.1986
	Estrone (pg/ml)	109	5.1(4.5–5.8)	201	4.8(4.3–5.4)	0.0994
	Estradiol: Testosterone	110	CC	201	CT/TT	0.1094
	rs1004984					
Block2	Estradiol (pg/ml)	151	10.7(9.5–12.1)	171	9.7(8.9,10.6)	0.2361
	Estrone (pg/ml)	149	18.1(16.6–19.7)	171	16.5(15.4,17.6)	0.2815
	Estradiol: Testosterone	151	5.4(4.7–6.3)	171	4.7(4.2–5.2)	0.3236
	rs1902584					
	Estradiol (pg/ml)	233	GG	86	GA/AA	0.5032
	Estrone (pg/ml)	232	9.5(8.6–10.6)	86	10.4(9.4,11.5)	0.5847
	Estradiol: Testosterone	233	16.4(15.1–17.7)	86	17.4(16.1,18.7)	0.9810
	rs28566535					
	Estradiol (pg/ml)	136	4.7(4.1–5.3)	194	5.1(4.5–5.7)	0.6113
	Estrone (pg/ml)	135	AA	194	AT/TT	0.0180
	Estradiol: Testosterone	136	9.7(8.9–10.6)	194	10.3(9.0,11.8)	0.9294
	rs12900137					
	Estradiol (pg/ml)	213	16.8(15.9–17.8)	116	17.8(16.6,19.1)	0.4909
Block3	Estrone (pg/ml)	212	5.0(4.4–5.4)	115	4.9(4.2–5.8)	0.9399
	Estradiol: Testosterone	213	AA	116	4.5(3.9–5.3)	0.2284
	rs730154					
	Estradiol (pg/ml)	134	GG	192	GC/CC	0.4864
	Estrone (pg/ml)	133	10.1(9.3–11.1)	191	10.4(9.5,11.5)	0.0141
	Estradiol: Testosterone	134	15.7(14.5–17.1)	192	18.0(16.8,19.2)	0.9450
	rs936306					
	Estradiol (pg/ml)	132	4.9(4.3–5.6)	201	5.0(4.4–5.5)	0.8037
	Estrone (pg/ml)	131	CC	200	10.1(9.2,11.1)	0.0274
	Estradiol: Testosterone	132	9.9(8.9–11.1)	201	17.6(16.5,18.8)	0.8432
	rs1902586					
	Estradiol (pg/ml)	129	5.0(4.4–5.7)	192	4.9(4.4–5.5)	0.8277
	Estrone (pg/ml)	128	GG	192	10.2(9.3,11.2)	0.0675
Block4	Estradiol: Testosterone	129	10.0(8.9–11.2)	192	17.8(16.7,19.0)	0.8416
	rs749292					
	Estradiol (pg/ml)	103	5.0(4.4–5.7)	221	4.9(4.4–5.5)	0.1278
	Estrone (pg/ml)	102	GG	220	GA/AA	0.2069
	Estradiol: Testosterone	103	9.2(8.1–10.4)	221	17.2(16.1,18.4)	0.6724
	rs6493494					
	Estradiol (pg/ml)	106	16.0(14.5–17.6)	219	10.4(9.5,11.3)	0.4462
	Estrone (pg/ml)	105	4.8(4.1–5.6)	218	16.6(15.6,17.6)	0.2093
	Estradiol: Testosterone	106	GG	219	4.8(4.3–5.3)	0.3060
	rs1008805					
	Estradiol (pg/ml)	165	10.3(9.1–11.7)	156	AG/GG	0.1233
	Estrone (pg/ml)	164	17.8(16.3–19.4)	155	10.8(9.7,12.0)	0.2724
	Estradiol: Testosterone	165	5.3(4.5–6.1)	156	16.4(15.2,17.7)	0.2309
	rs12907866					
	Estradiol (pg/ml)		AA		5.3(4.7–5.9)	
	Estrone (pg/ml)		9.6(8.7–10.7)		GA/AA	
	Estradiol: Testosterone		17.4(16.2–18.8)			
			4.7(4.2–5.3)			
			GG			

	No.	G(95%CI)	No.	G(95%CI)	P values
Estradiol (pg/ml)	147	9.4(8.5–10.4)	160	10.2(9.2,11.3)	0.2580
Estrone (pg/ml)	146	16.7(15.5–17.9)	159	16.5(15.5,17.7)	0.8638
Estradiol: Testosterone	147	4.6(4.0–5.2)	160	5.1(4.5–5.7)	0.2397
rs727479		AA		AC/CC	
Estradiol (pg/ml)	171	9.8(8.8–10.8)	148	10.4(9.4,11.6)	0.3934
Estrone (pg/ml)	170	17.0(15.9–18.2)	147	16.8(15.6,18.1)	0.8472
Estradiol: Testosterone	171	4.8(4.3–5.4)	148	5.2(4.6–5.9)	0.3640
rs2414096		GG		GA/AA	
Estradiol (pg/ml)	99	10.5(9.2–11.9)	228	9.8(9.0,10.7)	0.3976
Estrone (pg/ml)	98	17.7(16.1–19.3)	227	16.6(15.7,17.7)	0.2862
Estradiol: Testosterone	99	5.5(4.8–6.5)	228	4.7(4.2–5.2)	0.0769
rs700519		CC		CT/TT	
Estradiol (pg/ml)	223	10.1(9.3–11.1)	105	9.8(8.6,11.1)	0.6149
Estrone (pg/ml)	222	16.9(15.9–18.0)	104	16.7(15.3,18.3)	0.8277
Estradiol: Testosterone	223	5.1(4.6–5.7)	105	4.6(3.9–5.3)	0.2085
rs10046		AA		AG/GG	
Estradiol (pg/ml)	95	9.7(8.5–11.1)	227	10.4(9.5,11.3)	0.4028
Estrone (pg/ml)	95	17.1(15.5–18.7)	226	17.1(16.1,18.1)	0.9581
Estradiol: Testosterone	95	4.8(4.1–5.6)	227	5.1(4.6–5.6)	0.4853
rs4646		CC		CA/AA	
Estradiol (pg/ml)	172	9.5(8.6–10.5)	156	10.8(9.7,12.1)	0.0742
Estrone (pg/ml)	171	17.1(15.9–18.4)	155	16.8(15.5,18.1)	0.7282
Estradiol: Testosterone	172	4.5(4.1–5.1)	156	5.4(4.8–6.1)	0.0544

¹ Adjusted for age, batch, age at menarche and BMI

CYP19A1 gene haplotypes in association with hormones^{1,2}

Table 4

Hormones	Haplotypes	Haplotype frequency	Number and Geometric mean (95%CI)				P values
			without copy of the haplotype		with one or two copies of the haplotype		
Estradiol (pg/ml)	AGTCG	0.632	47	9.6(8.0–11.7)	291	10.1(9.4–10.9)	0.6339
	CCCTA	0.190	220	10.2(9.3–11.1)	118	9.8(8.7–11.1)	0.6124
	CGCTA	0.148	247	9.8(9.0–10.6)	91	10.8(9.4–12.4)	0.2375
Estrone (pg/ml)	AGTCG	0.632	47	17.9(15.6–20.5)	289	16.6(15.7–17.5)	0.3270
	CCCTA	0.190	219	16.6(15.5–17.7)	117	17.1(15.7–18.6)	0.5714
	CGCTA	0.148	245	16.0(15.1–17.0)	91	18.8(17.1–20.8)	0.0064
Estrone Sulphate (pg/ml)	AGTCG	0.632	47	711.8(525.0–965.1)	291	1020.0(903.1–1152.0)	0.0532
	CCCTA	0.190	220	1061.4(922.9–1220.8)	118	820.6(677.9–993.3)	0.0339
	CGCTA	0.148	247	987.0(864.2–1127.3)	91	926.2(744.0–1153.1)	0.6274
Testosterone (pg/ml)	AGTCG	0.632	47	190.7(158.0–230.2)	291	204.7(189.8–220.6)	0.4952
	CCCTA	0.190	220	199.6(183.1–217.7)	118	208.4(185.2–234.5)	0.5670
	CGCTA	0.148	247	203.5(187.5–220.8)	91	200.5(175.2–229.3)	0.8521
Ratio ³	AGTCG	0.632	47	5.0(4.0–6.3)	291	5.0(4.5–5.4)	0.8662
	CCCTA	0.190	220	5.1(4.6–5.7)	118	4.7(4.1–5.4)	0.3641
	CGCTA	0.148	247	4.8(4.4–5.3)	91	5.4(4.6–6.3)	0.2485

¹ Adjusted for age, batch, age at menarche and BMI

² These haplotypes are in block 2.

³ Ratio= Estradiol / Testosterone *100

Table 5
Geometric means and 95% CI of sex hormones by SNPs in Block I for women with BMI>=25.0¹

	No.	G(95%CI)	No.	G(95%CI)	P values
rs2446405					
Estradiol (pg/ml)	29	TT	93	AT/AA	0.6725
Estrone (pg/ml)	28	11.7(9.2–14.9)	93	11.1(9.7,12.6)	0.2596
Estrone sulphate (pg/ml)	29	16.6(13.9–19.8)	93	18.6(16.9,20.5)	0.5009
Testosterone(pg/ml)	29	812.9(573.1–1153.1)	93	933.5(768.7,1133.5)	0.1783
Ratio ²	29	200.3(155.9–257.2)	93	244.2(212.5,280.6)	0.1811
rs2445765					
Estradiol (pg/ml)	29	5.9(4.2–8.1)	93	4.5(3.8–5.4)	
Estrone (pg/ml)	64	GG	58	GC/CC	0.4412
Estrone sulphate (pg/ml)	63	10.7(9.1–12.6)	58	11.7(9.9,13.9)	0.3405
Testosterone(pg/ml)	64	18.9(16.8–21.2)	58	17.4(15.4,19.6)	0.1692
Ratio ²	64	1012.2(801.9–1277.8)	58	796.7(623.6,1017.6)	0.8900
rs2470144					
Estradiol (pg/ml)	64	231.0(195.1–273.6)	58	235.1(196.8,280.8)	0.6493
Estrone (pg/ml)	44	4.7(3.7–5.8)	71	5.0(4.0–6.3)	
Estrone sulphate (pg/ml)	43	CC	71	CT/TT	0.4258
Testosterone(pg/ml)	44	11.6(9.6–14.1)	71	10.5(9.1,12.2)	0.1494
Ratio ²	44	19.9(17.2–23.0)	71	17.4(15.5,19.4)	0.3771
rs1004984					
Estradiol (pg/ml)	55	1014.3(770.0–1336.2)	71	864.8(696.6,1073.7)	0.2052
Estrone (pg/ml)	54	210.4(171.1–258.8)	71	249.9(212.4,294.0)	0.1190
Estrone sulphate (pg/ml)	55	5.5(4.2–7.2)	65	4.2(3.4–5.2)	
Testosterone(pg/ml)	55	GG	65	GA/AA	0.3077
Ratio ²	55	10.5(8.8–12.5)	65	11.9(10.1,14.0)	0.4400
rs1902584					
Estradiol (pg/ml)	90	17.0(14.7–19.7)	31	18.4(16.1,21.1)	0.9740
Estrone (pg/ml)	89	910.8(704.7–1177.2)	31	905.5(715.2,1146.5)	0.1839
Estrone sulphate (pg/ml)	90	214.0(178.8–256.0)	31	252.7(214.3,298.1)	0.8059
Testosterone(pg/ml)	90	4.9(3.9–6.2)	31	4.7(3.8–5.9)	
Ratio ²	90	AA	31	AT/TT	0.0312
rs1902584					
Estradiol (pg/ml)	90	10.3(9.0–11.8)	31	13.8(11.0,17.4)	0.6493
Estrone (pg/ml)	89	17.9(16.2–19.8)	31	18.8(15.8,22.2)	0.5106
Estrone sulphate (pg/ml)	90	973.8(798.8–1187.1)	31	853.3(608.4,1196.6)	0.7553
Testosterone(pg/ml)	90	232.1(201.4–267.3)	31	242.7(190.6–309.0)	0.1802
Ratio ²	90	4.4(3.7–5.3)	31	5.7(4.2–7.8)	

¹ Adjusted for age, batch and age at menarche.

² Ratio= Estradiol / Testosterone *100