Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2008 January ; 17(1): 27–32. doi:10.1158/1055-9965.EPI-07-0688.

Haplotype Analyses of *CYP19A1* Gene Variants and Breast Cancer Risk: Results from the Shanghai Breast Cancer Study

Qiuyin Cai[1,*], **Nobuhiko Kataoka**[1], **Chun Li**[2], **Wanqing Wen**[1], **Jeffrey R. Smith**[3], **Yu-Tang Gao**[4], **Xiao Ou Shu**[1], and **Wei Zheng**[1]

1*Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine and Vanderbilt-Ingram Cancer Center, Nashville, TN 37232, USA*

2*Department of Biostatistics, Vanderbilt University, Nashville, TN 37232, USA*

3*Department of Medicine, Vanderbilt University School of Medicine and Vanderbilt-Ingram Cancer Center, Nashville, TN 37232, USA*

4*Department of Epidemiology, Shanghai Cancer Institute, Shanghai, 200032, China*

Keywords

CYP19A1; breast cancer; haplotype; linkage disequilibrium; epidemiology

Introduction

A number of epidemiological studies, including prospective studies, have found a positive association between blood estrogen levels and breast cancer risk, supporting the notion that estrogen plays a central role in the pathogenesis of this common malignancy (1). The association between endogenous estrogen exposure and breast cancer risk could be explained, in part, by genetic factors that affect estrogen biosynthesis, metabolism, and signal transduction. The *CYP19A1* gene plays a central role in estrogen biosynthesis. The gene encodes aromatase, the enzyme that catalyses the conversion of androstenedione to estrone and testosterone to estradiol in both ovarian granulosa cells and peripheral adipose tissue. Several studies have described an overexpression of the *CYP19A1* gene in human breast tumors and surrounding tissue, suggesting that aromatase plays a role in the *in situ* production of estrogen in breast tissues. It has been hypothesized that *CYP19A1* gene polymorphisms may affect estrogen biosynthesis, and thus these polymorphisms may modify the risk of breast cancer. Several SNPs in the *CYP19A1* gene have been evaluated in relation to breast cancer risk with mixed results (2-11). In this study, we comprehensively evaluated the association between the *CYP19A1* gene polymorphisms and breast cancer risk among Chinese women using the data from the Shanghai Breast Cancer Study, a large-scale, population-based case-control study conducted among Chinese women in Shanghai.

Materials and Methods

Cases and controls in this study were participants of the Shanghai Breast Cancer Study. Detailed study methods have been published elsewhere (12,13). The study included 1,459 women between the ages of 25 and 64 and 1,556 age frequency-matched controls. Blood

*Correspondence to: Qiuyin Cai, M.D., Ph.D. Vanderbilt Epidemiology Center Vanderbilt University Medical Center B-2104 Medical Center North 1161 21st Avenue South Nashville, TN 37232-2400 Phone: (615) 936-1351 Fax: (615) 322-1754 E-mail: qiuyin.cai@vanderbilt.edu

samples were obtained from 1,193 (82%) cases and 1,310 (84%) controls who completed the in-person interviews. 1,140 cases and 1,244 controls were genotyped successfully in this study.

Haplotype-tagging SNP (htSNP) were selected based on the data provided in a study conducted by Haiman *et al.* (3). In that study, 25 htSNPs were identified to capture the variation of the *CYP19A1* gene. Among the 25 htSNPs, 2 SNPs had a minor allele frequency of <1% in the Japanese population and 4 SNPs were African-American-specific polymorphisms. Thus, 19 htSNPs were identified to capture the variations of the *CYP19A1* gene in the Japanese population (3). Because the pattern of genetic variation is similar in Japanese and Chinese populations (14), we used the 19 informative htSNPs reported in Haiman's study for the Japanese population to define haplotypes in our study. We also genotyped rs2304463 and included this SNP in the single SNP analyses. The SNP locus/position, LD block, and locations are shown in Appendix 1. In addition, we included the $(TTTA)_n$ repeat polymorphism in intron 4 in the study.

Two SNPs (rs1004984 and rs230463) were genotyped in 2004 by BioServe Biotechnologies, Ltd (Laurel, MD) using Masscode assay. One SNP (rs700519) was genotyped in 2002 using the PCR-RFLP method and the genotypes were confirmed by direct sequencing using BigDye Terminator Chemistry on an ABI PRISM® 3700 automated DNA Analyzer. Genotypes for the other 17 SNPs were conducted from 2003 to 2004 using the TaqMan genotyping assay in ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA). Details of the genotyping methods are described in Appendix I. The genotyping for the $(TTTA)_n$ repeat polymorphism in intron 4 was performed by detection of fluorescent amplimers on an ABI 3700 automated DNA sequencer as reported earlier (13) using the following primers: F: 5′-GAGGTTACAGTGAGCCAAG-3′ and R: 5′-gtgtcCAGGTACTTAGTTAGCTAC-3′. Sequenced alleles enabled distinction of amplimer size variation as a function of STR allele length and of the adjacent 3 bp insertion/deletion located approximately 50 bp upstream of the $(TTTA)_n$ repeat. Quality control (QC) samples were included in the genotyping assays. The consistency rate for QC samples was 98.7%. In addition, we genotyped rs1902584 in 45 DNA samples of the Chinese participants used in the International HapMap project (http://www.hapmap.org) and 24 DNA samples used in the Perlegen (http://genome.perlegen.com) database as an additional quality control. The concordance rates between the data generated in our lab and the data from the HapMap and Perlegen was 100%.

The Chi-squared test was used to evaluate case-control differences in the distributions of *CYP19A1* alleles and genotypes. The haplotype blocks were determined according to the method described by Haiman *et al.* (3). Haplotypes for the *CYP19A1* gene within each haplotype block were derived using the software PHASE (version 2.1), and the overall association between haplotypes within each block and breast cancer risk was evaluated with the permutation test (15,16). The risk of breast cancer associated with each haplotype as compared with the most common haplotype under different genetic modes (additive, dominant, and recessive) was estimated using logistic regression models with the HAPSTAT method recently developed by DY Lin *et al.*(17-19). The potential confounding effect of major demographic factors and known breast cancer risk factors were adjusted for using logistic models. Adjustments for these factors did not result in any appreciable changes in the risk estimates. Thus, we report results without adjustment for these factors.

Results

The distributions of selected demographic characteristics and major risk factors for breast cancer in the Shanghai Breast Cancer Study have been previously reported (12). The Hardy-Weinberg equilibrium (HWE) of all SNPs was examined in controls. The SNP rs12907866 was not in HWE ($p<10^{-10}$) and was excluded from subsequent analyses. The other 19 SNPs

were in HWE (p-values with Bonferroni correction>0.05). Overall, no apparent association of any SNP with breast cancer risk was observed. Similarly, no statistically significant association with any SNP was found in either pre- or post-menopausal women (data not shown).

The linkage disequilibrium plot is presented in Figure 1. Four haplotype blocks were identified in the *CYP19A1* gene among Chinese women. In each block, several common haplotypes with 5% or higher frequency accounted for between 91.0% to 99.9% of all haplotypes (Table 1). Also presented in Table 1 are the association results of breast cancer risk with common haplotypes in each haplotype block under additive models. No apparent association was found in the analysis including all women nor in analyses stratified by menopausal status. We performed a heterogeneity test by menopausal status, and found no statistically significant heterogeneity (p>0.05). Analyses under dominant or recessive models also showed no statistically significant associations of *CYP19A1* haplotypes with breast cancer risk either in the analyses including all women or in analyses conducted in pre- or post-menopausal women (data not shown). We also examined the interaction between BMI and *CYP19A1* haplotypes in relation to breast cancer risk under additive, dominant, and recessive models. No significant interactions were found either in the analyses including all women or in analyses stratified by menopausal status (data not shown).

We also evaluated the associations of the $(TTTA)_n$ repeat polymorphism with breast cancer risk. A total of 7 $(TTTA)_n$ repeat alleles were observed in our study population, ranking from 7 repeats to 13 repeats. Alleles with 7, 11, or 12 repeats were common. A 3-bp deletion polymorphism was reported approximately 50 bp upstream of the (TTTA)n polymorphic site. Virtually all alleles with this 3-bp deletion had 7 $(TTTA)_n$ repeats. No significant association with any repeat allele was found either in the analyses including all women or in analyses stratified by menopausal status (data not shown).

Discussion

In this study, we constructed common haplotyes from 19 SNPs in the *CYP19A1* gene for 1,140 breast cancer cases and 1,244 controls among Chinese women. Three to five common haplotypes accounted for >90% of the observed haplotypes in this Chinese population, which is consistent with observations in other ethnic groups (3).

Several tissue-specific promoters, including adipose and breast cancer tissue promoters, are located between promoter I.1 and exon 2 (approximately 89kb upstream of exon 2). Haplotype blocks 1 to 3 are located in this regulatory region. Few studies have evaluated the association of genetic polymorphisms in this region with breast cancer risk. In the report of Haiman *et al.* (3), four common haplotypes (1d, 2b, 2d, and 3c) in blocks 1 to 3 were significantly associated with increased breast cancer risk when analyses combined subjects in all ethnic groups. They also observed significant associations of breast cancer risk among Japanese subjects (347 cases and 420 controls) with four common haplotypes in block 1 (1d, OR=1.44; 95% CI, 1.07-1.93), block 2 (2b, OR=1.42; 95% CI: 1.13-1.80; 2c, OR=1.43; 95%CI: 1.03-1.98), and block 3 (3c, OR=1.40; 95% CI: 1.07-1.83). These positive associations, however, were not replicated in our study. Our results are supported by two very recent large-scale studies involving haplotype analyses (10,11). In a large-scale study conducted within the NCI Breast and Prostate Cancer Cohort Consortium, Haiman *et al.* (10) observed no significant associations with any SNPs or common haplotypes of the *CYP19A1* gene and breast cancer risk, although genetic variation in *CYP19A1* produces measurable differences in estrogen levels among post-menopausal women. Olson *et al.* (11) also failed to detect any association between the *CYP19A1* gene haplotype-tagging SNPs and breast cancer risk. Additionally, two recent studies reported that *CYP19A1* polymorphisms were not associated with breast density (20, 21).

Using the single polymorphism approach, several SNPs of *CYP19A1* have been studied to evaluate their association with breast cancer risk with conflicting results. The Arg/Cys or Cys/Cys genotypes of the $Arg^{264}Cys$ (rs700519) polymorphism in exon 7 were associated with increased risk of breast cancer when compared to the Arg/Arg genotype among Hawaiian and Japanese (3) and Korean women (4). Our study, along with several other studies (5-7), however, found a null association. Miyoshi *et al.* (6) found that carrying the Arg allele in the $Trp^{39}Arg$ polymorphism of exon 2 conferred significant protection against the development of breast cancer in Japanese women. This association, however, was not confirmed by another study (3) or by our study. A C-to-T polymorphism in the 3′UTR (rs10046) of exon 10 has also been associated with breast cancer risk (8). This finding, however, was not confirmed by another study (9). A 12-repeat allele in the tetranucleotide polymorphism $[(TTTA)_{12}]$ located in intron 4 was associated with increased breast cancer risk in a case-control study conducted among Norwegian women (22). In the Nurses' Health Study conducted in the United States, the $(TTTA)_{10}$ but not the $(TTTA)_{12}$ allele was associated with breast cancer risk (23). These findings, however, were not confirmed by other studies (24-26). Our data also showed a null association between the $(TTTA)_n$ repeat polymorphism and breast cancer risk. Many of the above studies had small sample sizes or used a hospital-based study design.

To our knowledge, this is the first large-scale study to comprehensively evaluate the association of *CYP19A1* polymorphisms with breast cancer risk in Chinese women. In addition, most previous studies have been conducted in post-menopausal women, while our study provides evidence that *CYP19A1* gene polymorphisms are not associated with breast cancer risk among pre-menopausal women. The participation rate of our study was high, minimizing the potential selection bias that is common to many case-control studies. Chinese women living in Shanghai are relatively homogenous in ethnic background, because more than 98% of them are classified in a single ethnic group (Han Chinese). The sample size of this study is large, which allowed for a careful analysis of *CYP19A1* gene polymorphisms and breast cancer risk. Our study includes a large number of loci (19 SNPs and the $(TTTA)_n$ repeat) and our estimates of haplotype frequencies should be accurate. Our study has 80% statistical power to detect an odds ratio of 1.41 for any genotype or haplotype with 10% frequency and an odds ratio of 1.29 for any genotype or haplotype with 20% frequency at a significance level of 0.05 under an additive genetic model.

In summary, our large-scale, comprehensive study failed to identify an overall association of breast cancer risk with common *CYP19A1* gene variants among Chinese women. However, we cannot rule out the possibility that the *CYP19A1* gene may interact with environmental exposure in the development of breast cancer. Further studies are needed to explore the *CYP19A1* gene-environment interaction in relation to breast cancer risk.

Acknowledgments

We thank Ms. Qing Wang and Ms. Regina Courtney for their excellent technical laboratory assistance and Ms. Bethanie Hull for technical assistance in manuscript preparation. We thank Dr. Christopher A. Haiman at the University of Southern California for sharing information about primers and probes of several SNPs. This study would not have been possible without the support of all of the study participants and research staff of the Shanghai Breast Cancer Study. This research was supported by research grants (R01CA64277 and R01CA90899) from the National Cancer Institute.

Sources of Support: This research was supported by research grants (R01CA64277 and R01CA90899) from the National Cancer Institute.

The abbreviations used are

SNP, single nucleotide polymorphism; LD, linkage disequilibrium; htSNP, haplotype-tagging SNP; UTR, untranslated region; OR, odds ratio; CI, confidence interval; BMI, body mass index; WHR, waist-to-hip ratio.

References

1. Colditz, GA.; Baer, H.; Tamimi, RM. Breast Cancer. In: Schottenfeld D, D.; Fraumeni, JF., Jr, editors. Cancer Epidemiology and Prevention. Oxford University Press; New York: 2006. p. 995-1012.
2. Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:843–54. [PubMed: 10548311]
3. Haiman CA, Stram DO, Pike MC, Kolonel LN, Burtt NP, Altshuler D, et al. A comprehensive haplotype analysis of CYP19 and breast cancer risk: the Multiethnic Cohort. Hum Mol Genet 2003;12:2679–92. [PubMed: 12944421]
4. Lee KM, Abel J, Ko Y, Harth V, Park WY, Seo JS, et al. Genetic polymorphisms of cytochrome P450 19 and 1B1, alcohol use, and breast cancer risk in Korean women. Br J Cancer 2003;88:675–8. [PubMed: 12618873]
5. Probst-Hensch NM, Ingles SA, Diep AT, Haile RW, Stanczyk FZ, Kolonel LN, et al. Aromatase and breast cancer susceptibility. Endocr Relat Cancer 1999;6:165–73. [PubMed: 10731105]
6. Miyoshi Y, Iwao K, Ikeda N, Egawa C, Noguchi S. Breast cancer risk associated with polymorphism in CYP19 in Japanese women. Int J Cancer 2000;89:325–8. [PubMed: 10956405]
7. Watanabe J, Harada N, Suemasu K, Higashi Y, Gotoh O, Kawajiri K. Arginine-cysteine polymorphism at codon 264 of the human CYP19 gene does not affect aromatase activity. Pharmacogenetics 1997;7:419–24. [PubMed: 9352581]
8. Kristensen VN, Harada N, Yoshimura N, Haraldsen E, Lonning PE, Erikstein B, et al. Genetic variants of CYP19 (aromatase) and breast cancer risk. Oncogene 2000;19:1329–33. [PubMed: 10713674]
9. Haiman CA, Hankinson SE, Spiegelman D, Brown M, Hunter DJ. No association between a single nucleotide polymorphism in CYP19 and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:215–6. [PubMed: 11867511]
10. Haiman CA, Dossus L, Setiawan VW, Stram DO, Dunning AM, Thomas G, et al. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. Cancer Res 2007;67:1893–7. [PubMed: 17325027]
11. Olson JE, Ingle JN, Ma CX, Pelleymounter LL, Schaid DJ, Pankratz VS, et al. A comprehensive examination of CYP19 variation and risk of breast cancer using two haplotype-tagging approaches. Breast Cancer Res Treat 2007;102:237–47. [PubMed: 17004113]
12. Gao YT, Shu XO, Dai Q, Potter JD, Brinton LA, Wen W, et al. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. Int J Cancer 2000;87:295–300. [PubMed: 10861490]
13. Cai Q, Gao YT, Wen W, Shu XO, Jin F, Smith JR, et al. Association of breast cancer risk with a GT dinucleotide repeat polymorphism upstream of the estrogen receptor-alpha gene. Cancer Res 2003;63:5727–30. [PubMed: 14522892]
14. Altshuler D, Brooks L, Chakravarti A, Collins F, Daly M, Donnelly P. A haplotype map of the human genome. Nature 2005;437:1299–320. [PubMed: 16255080]
15. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162–9. [PubMed: 14574645]
16. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89. [PubMed: 11254454]
17. Lin DY, Zeng D, Millikan R. Maximum likelihood estimation of haplotype effects and haplotype-environment interactions in association studies. Genet Epidemiol 2005;29:299–312. [PubMed: 16240443]

18. Zeng D, Lin DY, Avery CL, North KE, Bray MS. Efficient semiparametric estimation of haplotype-disease associations in case-cohort and nested case-control studies. Biostatistics 2006;7:486–502. [PubMed: 16500923]

19. Lin D, Zeng D. Likelihood-Based Inference on Haplotype Effects in Genetic Association Studies. J Am Stat Assoc 2006;101:89–118.

20. Haiman CA, Hankinson SE, De Vivo I, Guillemette C, Ishibe N, Hunter DJ, et al. Polymorphisms in steroid hormone pathway genes and mammographic density. Breast Cancer Res Treat 2003;77:27–36. [PubMed: 12602902]

21. Olson JE, Ma CX, Pelleymounter LL, Schaid DJ, Pankratz VS, Vierkant RA, et al. A comprehensive examination of CYP19 variation and breast density. Cancer Epidemiol Biomarkers Prev 2007;16:623–5. [PubMed: 17372263]

22. Kristensen VN, Andersen TI, Lindblom A, Erikstein B, Magnus P, Borresen-Dale AL. A rare CYP19 (aromatase) variant may increase the risk of breast cancer. Pharmacogenetics 1998;8:43–8. [PubMed: 9511180]

23. Haiman CA, Hankinson SE, Spiegelman D, De Vivo I, Colditz GA, Willett WC, et al. A tetranucleotide repeat polymorphism in CYP19 and breast cancer risk. Int J Cancer 2000;87:204–10. [PubMed: 10861475]

24. Siegelmann-Danieli N, Buetow KH. Constitutional genetic variation at the human aromatase gene (Cyp19) and breast cancer risk. Br J Cancer 1999;79:456–63. [PubMed: 10027313]

25. Healey CS, Dunning AM, Durocher F, Teare D, Pharoah PD, Luben RN, et al. Polymorphisms in the human aromatase cytochrome P450 gene (CYP19) and breast cancer risk. Carcinogenesis 2000;21:189–93. [PubMed: 10657957]

26. Baxter SW, Choong DY, Eccles DM, Campbell IG. Polymorphic variation in CYP19 and the risk of breast cancer. Carcinogenesis 2001;22:347–9. [PubMed: 11181459]

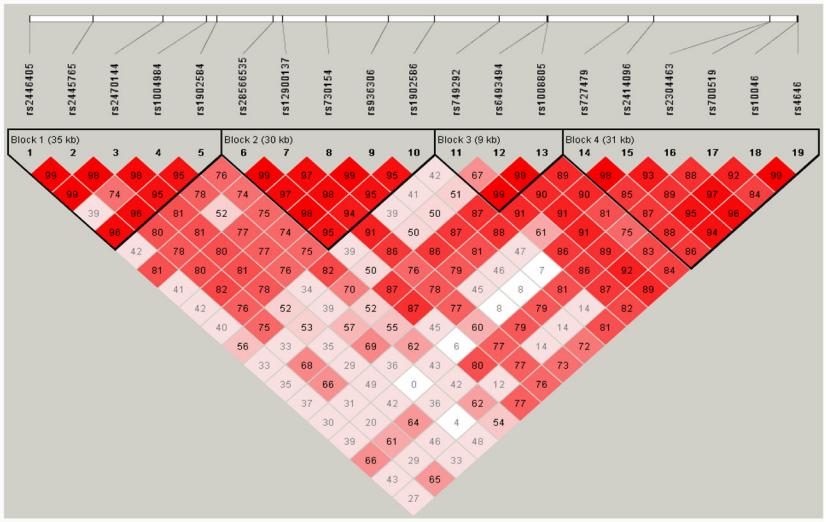

Figure 1.

LD plot for SNPs in the *CYP19A1* gene. The value within each diamond is D' between pairs of SNPs, estimated based on control subjects. The red-to-white gradient reflects higher to lower LD values (red=high, white=low).

Table 1

Association between breast cancer risk and *CYP19A1* haplotypes by blocks, the Shanghai Breast Cancer Study, 1996-1998

Haplotype	All Subjects			Pre-menopausal Women[a]			Post-menopausal Women[a]		
	Cases N=1140 (%)	Controls N=1244 (%)	OR (95% CI)	Cases N=760 (%)	Controls N=792 (%)	OR (95% CI)	Cases N=375 (%)	Controls N=448 (%)	OR (95% CI)
Block 1: rs2446405, rs2445765, rs2470144, rs1004984, rs1902584									
AGTGA	38.3	38.1	1.00 (reference)	37.1	37.0	1.00(reference)	40.0	39.8	1.00 (reference)
TGCGA	24.6	25.8	0.97 (0.84-1.12)	25.9	26.6	0.98(0.82-1.18)	22.0	24.0	0.93 (0.72-1.21)
TCCAT	14.1	13.5	1.06 (0.88-1.27)	14.0	13.0	1.10(0.88-1.37)	14.1	14.0	1.03 (0.76-1.39)
AGCAA	9.2	9.7	0.93 (0.75-1.14)	9.0	10.0	0.88(0.68-1.15)	9.6	9.0	1.03 (0.72-1.47)
TCCAA	8.4	7.7	1.06 (0.85-1.32)	8.3	8.0	1.03(0.78-1.35)	8.9	7.3	1.13 (0.77-1.64)
TCCGA	3.8	3.8	0.96 (0.70-1.31)	3.9	3.7	1.04(0.70-1.55)	3.7	4.4	0.86 (0.51-1.44)
		p^b=0.13			p=0.09			p=0.77	
Block 2: rs28566535, rs12900137, rs730154, rs936306, rs1902586									
AGTCG	66.5	64.1	1.00 (reference)	67.1	64.1	1.00(reference)	64.5	63.6	1.00 (reference)
CCCTA	16.0	17.1	0.92 (0.78-1.07)	15.6	16.1	0.95(0.78-1.16)	16.3	18.2	0.88 (0.67-1.15)
CGCTA	15.2	15.5	0.96 (0.82-1.13)	14.8	15.9	0.90(0.73-1.10)	16.7	15.4	1.08 (0.83-1.42)
		p=0.46			p=0.89			p=0.55	
Block 3: rs749292, rs6493494, rs1008805									
AAA	39.1	39.0	1.00 (reference)	37.0	38.2	1.00(reference)	41.2	38.7	1.00 (reference)
GGG	29.7	29.2	1.02 (0.89-1.18)	30.7	28.5	1.11(0.93-1.33)	26.7	29.6	0.85 (0.67-1.09)
GGA	17.2	16.8	1.01 (0.85-1.20)	16.2	15.9	1.05(0.85-1.31)	18.3	17.5	0.95 (0.71-1.26)
AGA	7.5	8.2	0.91 (0.72-1.15)	8.2	9.4	0.88(0.66-1.17)	7.9	7.3	0.96 (0.65-1.42)
GAA	6.4	6.8	0.95 (0.74-1.21)	7.6	7.6	1.01(0.75-1.37)	5.9	6.9	0.81 (0.53-1.24)
		p=0.71			p=0.80			p=0.55	
Block 4: rs727479, rs2414096, rs700519, rs10046, rs4646									
AACAC	45.0	45.1	1.00 (reference)	44.3	44.8	1.00(reference)	45.5	44.9	1.00(reference)
CGCGA	25.3	24.0	1.07 (0.93-1.24)	25.8	24.1	1.10(0.93-1.32)	23.9	23.4	1.01 (0.79-1.30)
AGTGC	14.3	13.3	1.09 (0.91-1.30)	14.3	12.8	1.16(0.93-1.44)	13.7	14.0	0.97 (0.72-1.31)

Haplotype	All Subjects			Pre-menopausal Women[a]			Post-menopausal Women[a]		
	Cases N=1140 (%)	Controls N=1244 (%)	OR (95% CI)	Cases N=760 (%)	Controls N=792 (%)	OR (95% CI)	Cases N=375 (%)	Controls N=448 (%)	OR (95% CI)
AGCAC	7.6	8.6	0.92 (0.74-1.14)	7.3	8.7	0.86(0.66-1.14)	8.4	8.3	0.98 (0.68-1.42)
AGCGA	2.5	2.3	1.12 (0.76-1.64)	2.9	2.6	1.23(0.78-1.94)	1.9	2.0	0.94 (0.45-1.95)
AGCGC	1.8	2.1	0.93 (0.61-1.41)	1.7	2.1	0.79(0.46-1.38)	2.7	2.1	1.18 (0.61-2.27)
CGCAC	1.1	1.7	0.69 (0.41-1.14)	1.2	1.6	0.78(0.41-1.50)	1.2	2.1	0.50 (0.22-1.17)
		p=0.16			P=0.12			p=0.58	

[a] Nine subjects without information on menopausal status were excluded from the stratified analyses.

[b] p: derived from permutation test for overall association.

[c] No interaction was statistically significant at p<0.05 for genetic variables and menopausal status.

Appendix I

SNPs included in the haplotype analysis of the *CYP19A1* gene and genotyping methods

SNP	Block	Position[a]	Location	Method	Primers or ABI Assay ID	Probes (VIC/FAM) or ABI assay ID
rs2446405	1	49225931	promoter	TaqMan	GGAGGGTGAATCATTCCAAGTACAG CTTCCTGACTTGCACCATTTTCATT	CTTGGCTCATATTATT TTGGCTCAAATTATT
rs2445765	1	49214036	promoter	TaqMan	GGGACGTCAATATGGTGCAATTTT CGCAGGTCCCATGTTAAGAAC	CTTTGACACTGCATTTT TTTGACAGTGCATTTT
rs2470144	1	49200863	intron	TaqMan	GGTATAATGGGAAGGCCAGCAA GGTGGTATTTGAAGGGAGTTCTCT	AAATTTCCCTCCATCAGTG AATTTCCCTCCGTCAGTG
rs1004984	1	49192667	intron	Masscode	gacgatgccttcagcacaCAGAGGGAGCAGGGTGAG ATAATTCAGGCCCTTCACAATC	gggacggtcggtagatATCCCCCATGACTGCCTACTGTTG gctggctcggtcaagaATCCCCCATGACTGCCTACTGTTA
rs1902584	1	49190792	intron	TaqMan	C 1664181 10	C 1664181 10
rs28566535	2	49180279	intron	TaqMan	C_1664178_10	C_1664178_10
rs12900137	2	49178491	intron	TaqMan	GAGCCAACGAAAGCAAACGT CCACCAATCCAAACAAAACCAAACA	CTACTAATCATGGATCTTCATG CTAATCATGGATGTTCATG
rs730154	2	49170342	promoter	TaqMan	GACCAGGCACCCCATCTG GCCGGTTCCAGCAAAACTTC	ACCCCCATGCTCCAT CCCCACGCTCCAT
rs936306	2	49158736	3′UTR	TaqMan	C 1664161 10	C 1664161 10
rs1902586	2	49149991	intron	TaqMan	C_11484670_1	C_11484670_1
rs749292	3	49137869	intron	TaqMan	TCTGCCAGTCCTTCTTCAAACC GGCTTAGGGCCTGATAGAAATTGTG	TCGGAGTCGAGGATT TCGGAGTCAAGGATT
rs6493494	3	49128972	intron	TaqMan	CTTGGCTTCCTGGACATTGTG CGCTGTGTGGGATTGATCCT	TCCAGCGCCTGAGC CTCCAGCACCTGAGC
rs1008805	3	49128737	intron	TaqMan	TTGGAAGTAATAGCAGGCCTAGGTA CCTTACCGAATCACTACCCTTCAC	CTTCCTGCGTCCTGC CTTCCTGTGTCCTGC
rs12907866	4	49124592	intron	TaqMan	GGGCCTTATCAGGTGTCTAGCATAT GCTCTTGCTGGAGACTGAATCATAA	CAAAACCTCAATAAAC CAAAACCTCGATAAAC
rs727479	4	49113685	intron	TaqMan	GTGGAATAAAGAGAAGGGATAAATACAAGACA TCTGGAACATCTTCTTCACTGCTTT	ACTTTGTTTCCGCCATGC CACTTTGTTTCCTCCATGC
rs2414096	4	49108917	intron	TaqMan	GGAGAATGTCCAATCCAAGAACATCT TTCAAAGACCCATTGCCTGACT	AAGACTCCGTTTAAGAAA AAAAGACTCCATTTAAGAAA
rs2304463	4	49087258	intron	Masscode	AGCTAACTCTGGCACCTTAACA gacgatgccttcagcacaAGTTTAGACATCTAGCGAAACAGA	gggacggtcggtagatTCACTTACTCATAAGCACCAATGT gctggctcggtcaagaTCACTTACTCATAAGCACCAATGG
rs700519	4	49087106	coding	RFLP	CGCTAGATGTCTAAACTGAG CATATGTGGCATGGGAATTA	Restrction enzyme; *HgaI*
rs10046	4	49082124	exon	TaqMan	C 8234731 1	C 8234731 1
rs4646	4	49081982	exon	TaqMan	C 8234730 1	C 8234730 1

[a] Chromosome position was based on NCBI build 35.