Research article

Genetic determinants of mammographic density

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Abstract

Background: Changes in breast density are highly correlated with steroid hormone exposure.

Materials and methods: In a cross-sectional study of 396 Caucasian and African-American women, we evaluated whether polymorphisms in genes involved in steroid hormone biosynthesis and metabolism, *CYP17* (T27C), *COMT* (Val158Met), *17HSDB1* (Ser312Gly) and *3HSDB1* (Asn367Thr), predict mammographic density. We also evaluated whether associations vary by menopausal and hormone replacement therapy status.

Results: We found no strong consistent relationships between polymorphisms in these genes and breast density. African-American women homozygous for the Thr allele of *3HSDB1* had increased density (the absolute difference versus the Asn/Asn genotype was +19.7%; Ptrend = 0.02), while Caucasian homozygous women had decreased density (−5.1%; Ptrend = 0.04). Among premenopausal women, carriers of the Ser allele had (not significantly) greater density (versus Gly/Gly genotype: +7.1%; Ptrend = 0.07). In addition, among current users of hormone replacement therapy, we observed that women with the low-activity Met/Met genotype of *COMT* had greater breast density (versus the Val/Val genotype: +11.7%; Ptrend = 0.01).

Conclusion: Additional large studies evaluating these and other candidate breast cancer genes will be required to determine what proportion, if any, of the interindividual differences in breast density are due to underlying genetic variation in genes involved in steroid hormone biosynthesis or metabolism.

Keywords: breast cancer, genetic susceptibility, mammographic density, molecular epidemiology

Introduction

Breast density is one of the strongest independent predictors of breast cancer risk [1–5], and the extent of breast densities measured on a mammogram is correlated with a woman's menopausal status [6,7]. Premenopausal women have a considerably larger proportion of dense breast tissue than postmenopausal women, and in some women, breast density is greater during the luteal phase of the menstrual cycle when breast tissue is highly exposed to estradiol and progesterone [8]. Reducing circulating steroid hormone levels with a gonadotropin releasing hormone agonist [9,10], or antagonizing effects of estrogens on breast tissue with tamoxifen [11], result in substantial reductions in breast density. Exogenous steroid hormones have also been shown to influence breast density, and recent studies provide evidence that postmenopausal hormone replacement therapy (HRT) composed of an estrogen plus a progestin (estrogen plus

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CARE study = National Institute of Child Health and Human Development Women's Contraceptive and Reproductive Experiences study; *COMT* = catechol-*O*-methyltransferase gene; *CYP17* = cytochrome p450c17α gene; EPRT = estrogen plus progestin replacement therapy; ERT = estrogen replacement therapy; HRT = hormone replacement therapy; *HSD3B1* = 3β-hydroxysteroid dehydrogenase type I gene; *HSD17B1* = 17β-hydroxysteroid dehydrogenase type I gene; SD = standard deviation.

progestin replacement therapy [EPRT]) increases breast density to a much greater extent than postmenopausal estrogen replacement therapy (ERT) alone [12,13].

Based on the epidemiologic evidence linking steroid hormones with changes in breast density, we decided to examine whether variation in genes that regulate steroid hormone biosynthesis (*CYP17, HSD17B1, HSD3B1*) and metabolism (*COMT*) may partially explain interindividual differences in mammographic density. Polymorphisms in these genes have been proposed to represent biomarkers of long-term exposure to endogenous steroid hormones and potential risk factors for breast cancer [14–16]. We assessed the association between polymorphisms in these candidate genes and mammographic density in a cross-sectional study of African-American and Caucasian women who were part of a national, multicenter, population-based, case-control study of breast cancer. We also evaluated whether associations between variant alleles of these genes and breast density differed by menopausal status and HRT use.

Materials and methods

Women in this study were African-American and Caucasian breast cancer patients who participated in the National Institute of Child Health and Human Development Women's Contraceptive and Reproductive Experiences (CARE) study, which is a multicenter, population-based, case-control study designed to evaluate risk factors for breast cancer [17]. US-born African-American and Caucasian women, aged from 35 to 64 years, were eligible to participate in the CARE study. Participants were enrolled between July 1994 and April 1998, and were interviewed in person using a standardized questionnaire to collect detailed demographic information as well as information regarding established and suspected breast cancer risk factors, including oral contraceptive use, HRT use, family history of breast cancer, age at menarche, number of pregnancies, physical activity, and mammogram history (for the five years preceding a reference date, which was the date of diagnosis for patients). Breast cancer patients participating in the CARE study in Los Angeles County were identified by the Los Angeles County Cancer Surveillance Program, which is a Surveillance, Epidemiology and End Results registry program. These women were eligible to be in the Radiographic Densities and Breast Cancer Prevention study at the University of Southern California if they had undergone a mammogram within five years of their breast cancer diagnosis. The Radiographic Densities study was designed to examine the association between mammographic densities and breast cancer risk. As part of this study, mammograms of the contralateral (cancer free) breast were obtained from 242 African-American and 312 Caucasian breast cancer cases (73% of the total eligible African-American and Caucasian cases in the Los Angeles section of the CARE study, $n = 755$). Of these,

blood samples had been collected as part of the CARE Study from 436 of the 554 (78.7%) women for whom we obtained a mammogram. The study protocol was approved by the Institutional Review Board at the University of Southern California.

Mammographic density assessment

Mammograms were digitized using a Cobrascan CX312.T scanner (Radiographic Digital Imaging, Compton, CA, USA). All assessments were done using a computer-assisted method on the digitized cranio-caudal mammographic image that we have previously described and validated [10]. The mammographic image is first digitized and displayed on the computer screen. Next, the reader outlines the breast and the software calculates the total area of the breast. The reader then uses a tinting tool to apply a yellow tint to gray levels within the breast above the threshold X, where all pixels equal to or greater than X are considered to represent mammographic densities. The reader excludes artefactual dense areas such as the pectoralis muscle, prominent veins, and fibrous strands. The software counts the number of tinted pixels within the defined breast area. The percentage of the breast area with densities is taken as the ratio of the tinted area within the region of interest : total area of the breast (×100). This breast density assessment method has been found to be highly reproducible and correlates well with the subjective classification method [10]. A subset of the mammograms in this study were interpreted by an expert reader (IMS). The correlation between these expert readings and the computer-assisted readings was 0.90. A subset of the readings were reread blindly with the computer-assisted method, and correlation between these readings was also 0.90.

Genotyping analysis

DNA was extracted from buffy coat fractions using the Qiagen Blood DNA Extraction Kit (Qiagen, Valencia, CA, USA). We genotyped one polymorphism in the transcribed, but untranslated, region of *CYP17* (T27C; alleles A1, A2, respectively), and missense polymorphisms in *COMT (*Val158Met), *17HSDB1* (Ser312Gly), and *3HSDB1* (Asn367Thr). All genotyping was performed by the Taqman Allelic Discrimination Method [18]. Reaction conditions, primers and probes are available from the corresponding author.

Statistical analysis

Generalized linear models were used to estimate least-squares arithmetic and geometric mean percentages of breast density for each genotype. Both arithmetic and log-transformed percentages gave similar results and the arithmetic means are provided. We also report the absolute differences in percent breast density between genotypes. Gene dosage was evaluated by including genotype as an ordinal variable in multivariate models. We adjusted for the following predictors of breast density:

1 Age at mammogram (years): <40; 40–44; 45–49; 50–54; 55–59; >59.
2 Body mass index (kg/m^2): <22; 22–24.9; 25–29.9; ≥30.
3 Age at menarche (years): <12; 12; 13; >13.
4 Parity (children)/age at first birth (years): nulliparous; 1–2/< 24; 1–2/≥24; ≥3<24; ≥3/≥24.
5 First degree family history of breast cancer: yes; no; not known.
6 Menopausal status/HRT use status at mammography: premenopausal; postmenopausal/never used HRT or past HRT user; postmenopausal/current ERT user; postmenopausal/current EPRT user; unknown status.
7 Race: African-American; Caucasian.

We also evaluated associations between genotype and mammographic density by menopausal and HRT status. Interactions between genotype and HRT status were evaluated by including multiplicative interaction terms between HRT status (past user or never used versus current user) and genotype (ordinal). Data were analyzed using SAS software [19].

Results

A total of 152 African-American and 244 Caucasian women, with mean ages at the time of their mammogram of 48.4 years (standard deviation [SD] 7.7) and 48.2 years (SD 8.9), respectively, participated in the study. The average duration between the mammogram and breast cancer diagnosis was 5.7 months (range 0–63 months). After adjustment for body mass index, differences in mammographic density did not differ between African-American and Caucasian women (Table 1). The percentage of breast density among current users of ERT or EPRT (HRT users) was slightly greater than that of premenopausal women, while density was substantially lower among women with no HRT use. Associations between percent breast density and established breast cancer risk factors were in the expected directions, except for age at menarche where breast density was significantly greater for women with a later age at menarche ($P = 0.04$).

CYP17 genotype was not a strong predictor of breast density among African-American or Caucasian women (Table 2). The direction and magnitude of the associations between *COMT, 17HSDB1,* and *3HSDB1* genotypes, and breast density, were not consistent for African-American and Caucasian women. African-American women homozygous for the low-activity allele (Met) of *COMT* had greater breast density (the absolute difference compared to the Val/Val genotype was +5.4% density); however, a significant gene-dosage effect was not observed (Ptrend = 0.19). This modest association with *COMT* genotype was not observed among Caucasian women. Among Caucasian women, carriers of the Ser allele of *17HSDB1* had greater breast density (Ser/Ser versus Gly/Gly genotype: +4.1% density), while the Thr allele of

Table 1

Mean percentage of breast density by descriptive characteristics ($n = 396$)

Characteristic	n (%)	Mean % density	$P^\S$
Race*			
African-American	152 (38)	34.8	
Whites	244 (68)	34.8	0.98
Menopausal status/ HRT use**,†			
Premenopausal	176 (44)	35.4	
Postmenopausal			
Never used or past user of HRT	67 (17)	30.4	
Current user of ERT	53 (13)	39.3	
Current user of EPRT	51 (13)	39.8	0.08
Age at mammogram (years)			
≤40	91 (23)	45.6	
41–45	84 (21)	41.2	
46–50	41 (10)	38.3	
51–55	74 (19)	34.4	
56–60	69 (17)	28.3	
>60	37 (9)	28.9	<0.0001
Body mass index (kg/m^2)**			
<22	114 (29)	49.8	
22–24.9	107 (27)	38.3	
25–29.9	111 (28)	29.2	
≥30	64 (16)	21.8	<0.0001
Parity**			
0	83 (21)	45.1	
1–2	175 (44)	38.0	
3+	138 (35)	29.1	<0.0001
Age at first birth (years)**			
<25	191(61)	30.9	
25–30	69 (22)	35.1	
≥30	53 (17)	43.8	0.0004
Age at menarche (years)**			
<12	106 (27)	32.6	
12	105 (27)	36.5	
13	97 (24)	34.7	
>13	88 (22)	41.3	0.04
First-degree family history of breast cancer**,‡			
No	256 (65)	35.0	
Yes	120 (30)	39.3	0.12

*Adjusted for age and body mass index. **Adjusted for age. †$n = 49$ missing menopausal/ hormone replacement therapy (HRT) status. ‡$n = 20$ missing family history information. §Analysis of covariance. EPRT, estrogen plus progestin replacement therapy; ERT, estrogen replacement therapy.

3HSDB1 was associated with lower breast density (Thr/Thr versus Asn/Asn genotype: −5.1% density). Among African-Americans, the Thr allele was less common (prevalence, 15%), and women homozygous for this allele had greater breast density (versus Asn/Asn genotype: +19.7% density).

Among premenopausal women, carriers of the Ser allele had (not significantly) greater density (versus the Gly/Gly

Table 2

Least-squares mean percentage breast density by genotype

Genotype	All women* mean (n)	Caucasians** mean (n)	African-Americans** mean (n)
CYP17			
A1/A1	34.1 (144)	33.7 (87)	34.6 (57)
A1/A2	34.8 (204)	34.6 (127)	35.0 (77)
A2/A2	37.2 (48)	39.1 (30)	34.6 (18)
*P*trend	0.40	0.26	0.95
COMT			
Val/Val	33.4 (139)	34.3 (76)	32.3 (63)
Val/Met	35.8 (175)	35.6 (104)	36.2 (71)
Met/Met	34.9 (82)	34.2 (64)	37.7 (18)
*P*trend	0.46	0.99	0.19
17HSDB1			
Gly/Gly	32.4 (109)	32.0 (71)	33.3 (38)
Gly/Ser	35.8 (191)	35.9 (121)	35.6 (70)
Ser/Ser	35.6 (96)	36.1 (52)	34.9 (44)
*P*trend	0.24	0.22	0.73
3HSDB1			
Asn/Asn	35.4 (240)	37.4 (131)	32.9 (109)
Asn/Thr	33.6 (126)	31.5 (88)	38.4 (38)
Thr/Thr	35.1 (27)	32.3 (23)	52.6 (4)
*P*trend	0.58	0.04	0.02

*Adjusted for race, age, body mass index (BMI), menopausal status/ hormone replacement therapy (HRT) use, parity, age at first birth, age at menarche and first-degree family of breast cancer. **Adjusted for age, BMI, menopausal status/HRT use, parity, age at first birth, age at menarche and first-degree family of breast cancer.

Table 3

Least-squares mean percentage breast density by menopausal and HRT status

		Postmenopausal/HRT status*		
Genotype	Premeno- pausal* mean (n)	Never and past use mean (n)	Current use** mean (n)	P***
CYP17				
A1/A1	34.0 (58)	31.9 (26)	37.9 (39)	
A1/A2	35.7 (94)	30.5 (33)	38.6 (52)	
A2/A2	37.9 (24)	24.9 (8)	48.6 (13)	
P trend	0.39	0.42	0.16	0.13
COMT				
Val/Val	36.9 (62)	29.7 (20)	33.7 (39)	
Val/Met	34.4 (70)	31.6 (40)	42.3 (46)	
Met/Met	34.9 (44)	25.3 (7)	45.4 (19)	
P trend	0.56	0.80	0.01	0.10
17HSDB1				
Gly/Gly	32.0 (52)	24.0 (13)	41.1 (32)	
Gly/Ser	35.5 (76)	33.7 (40)	39.4 (47)	
Ser/Ser	39.1 (48)	26.9 (14)	38.2 (25)	
P trend	0.07	0.77	0.57	0.65
3HSDB1				
Asn/Asn	35.5 (106)	32.8 (42)	40.6 (61)	
Asn/Thr	33.4 (56)	25.8 (23)	40.8 (32)	
Thr/Thr	42.7 (14)	31.8 (2)	28.9 (9)	
P trend	0.55	0.27	0.22	0.95

*Adjusted for race, age, body mass index, parity, age at first birth, age at menarche and first-degree family history of breast cancer. **Current use of estrogen replacement therapy or estrogen plus progestin replacement therapy. ***P value for interaction between hormone replacement therapy (HRT) status (never used and past use versus current use) and genotype (ordinal).

genotype: +7.1%; *P*trend = 0.07) (Table 3). We observed little evidence of a relationship between *CYP17, COMT,* or *3HSDB1* genotypes, and breast density among pre-menopausal women. For postmenopausal women, we did observe suggestive evidence that the direction of the associations between *CYP17* and *COMT* genotypes, and breast density differed by HRT status (for interactions, $P = 0.13$ and $P = 0.10$, respectively). Among current HRT users, women with A2/A2 genotype of *CYP17* had greater density (versus A1/A1 genotype: +10.7% density), while in the group of women who either had never used HRT or were past users, those with the A2/A2 genotype had lower density (versus A1/A1 genotype: −7.0% density). In analyses among current HRT users, women with the Met/Met *COMT* genotype had greater breast density (versus Val/Val genotype: +11.7% density; *P*trend = 0.01). We had limited power to assess associations among the women who either had never used HRT or were past users, due to the small number of post-menopausal women in this study who were not currently using HRT.

Discussion

CYP17, *17HSDB1* and *3HSDB1* encode key enzymes involved in steroid hormone biosynthesis, and common polymorphisms in these genes may account for part of the observed interindividual variation in mammographic density. The single nucleotide polymorphism (T → C) in the 5′-transcribed but untranslated region of *CYP17* has been studied extensively in relation to breast cancer risk (for review, see [20]). Most, but not all of these studies, have been null. However, some data suggest that women with the A2/A2 genotype have modestly higher levels of circulating estrogens [21,22]. In another study of genetic variation in relation to mammographic density among Caucasian women ($n = 538$; Haiman *et al.*, unpublished observations), *CYP17* genotype was not a strong predictor of breast density. The results from our study are compatible with studies suggesting that this polymorphism is not a strong breast cancer risk factor. Two studies have examined the Ser312Gly polymorphism in exon 6 of *17HSDB1* in relation to breast cancer risk and both reported a positive association with the Ser allele [14,23]. Although we found slightly greater density among Caucasian women with the Ser/Ser genotype, this result was not statistically significant. It is currently not known whether the missense variant (Asn367Thr [24]) in exon 4 of *3HSDB1* alters the normal activity of the enzyme,

steroid hormone levels, and breast cancer risk. Our finding of higher density with the Thr allele in African-Americans is based on small numbers (Thr/Thr genotype: $n = 4$) and may be due to chance.

Catechol metabolites of estrogen (i.e. 2-OH and 4-OH estrogens) have been demonstrated to have cancer-promoting properties [25]. One route of catechol estrogen inactivation is by *O*-methylation, which is catalyzed by the enzyme catechol-*O*-methyltransferase (COMT). COMT activity is polymorphic in humans and the missense variant we evaluated (Met allele) has been associated with lower enzymatic activity [26,27]. However, studies have been inconsistent in linking the Met allele with breast cancer risk [16,28–30]. In another study of genetic determinants of mammographic density (Haiman *et al.*, unpublished observations), COMT genotypes were associated with mammographic density, but the association went in the opposite direction for premenopausal and postmenopausal women. Premenopausal women homozygous for the 'low-activity' Met allele of *COMT* had (not significantly) greater breast density (Met/Met versus Val/Val genotypes: +9.2% density; $P = 0.18$), while among postmenopausal women who had never used or were past users of HRT, the Met allele was associated with lower density (Met/Met versus Val/Val genotypes: −5.3% density; $P = 0.04$). In contrast, among postmenopausal women in the present study who were current HRT users, we observed carriers of the Met/Met genotype to have greater density than those with the Val/Val genotype. Our data support the observation that breast cancer risk associated with the Met allele may be greater among long-term HRT users [30].

The women evaluated in this study were originally selected based on their diagnosis with breast cancer, and thus, it is possible that the associations we observed may not reflect associations among unaffected women in the general population. The mammograms in our study were collected, on average, only six months prior to diagnosis. It is possible that invasive breast cancer alters breast density in the contralateral breast prior to diagnosis; however, among the women for whom we had collected several mammograms at different times, there was little indication that such a change took place. Although there may have been some nondifferential misclassification of the mammographic density readings that would have biased the results towards the null, we think any such misclassification is likely to have been minimal, given the high correlations between duplicate blinded readings and the high correlation between the readings used and those of an expert reader.

Conclusion
In conclusion, in this study we did not observe compelling evidence of an association between variant alleles of

CYP17, *COMT*, *17HSDB1* and *3HSDB1* and breast density. Modest associations between these genes and breast cancer risk have been hypothesized (relative risks approximately 1.2–1.5), however, conclusive data linking any of the four variants we examined with breast cancer risk are lacking. If these variants do have a moderate biological effect on steroid hormone biosynthesis or metabolism, then the genotype differences in percent mammographic density we are attempting to discern are most likely to be small. Additional data from large mammographic density and breast cancer studies of these and other candidate genes are required before we can accurately assess the impact of these allelic variants.

References
1. Saftlas AF, Szklo M: **Mammographic parenchymal patterns and breast cancer risk.** *Epidemiol Rev* 1987, **9**:146-174.
2. Oza AM, Boyd NF: **Mammographic parenchymal patterns: a marker of breast cancer risk.** *Epidemiol Rev* 1993, **15**:196-208.
3. Boyd NF, Lockwood GA, Martin LJ, Knight JA, Byng JW, Yaffe MJ, Tritchler DL: **Mammographic densities and breast cancer risk.** *Breast Disease* 1998, **10**:113-126.
4. Boyd NF, Byng J, Jong R, Fishell E, Little L, Miller AB, Lockwood G, Tritchler D, Yaffe M: **Quantitative classification of mammographic densities and breast cancer risks: results from the Canadian National Breast Screening Study.** *J Natl Cancer Inst* 1995, **87**:670-675.
5. Byrne C, Schairer C, Wolfe J, Parekh N, Salane M, Brinton LA, Hoover R, Haile R: **Mammographic features and breast cancer risk: effects with time, age and menopause status.** *J Natl Cancer Inst* 1995, **87**:1622-1629.
6. Brisson J, Sadowsky NL, Twaddle JA, Morrison AS, Cole P, Merletti F: **The relation of mammographic features of the breast to breast cancer risk factors.** *Am J Epidemiol* 1982, **115**:438-443.
7. Grove JS, Goodman MJ, Gilbert Fl, Mi MP: **Factors associated with mammographic pattern.** *Br J Radiol* 1985, **58**:21-25.
8. Ursin G, Parisky TR, Pike MC, Spicer DV: **Mammographic density changes during the menstrual cycle.** *Cancer Epidemiol Biomarkers Prev* 2001, **10**:141-142.
9. Spicer DV, Ursin G, Parisky YR, Pearce JG, Shoupe D, Pike A, Pike MC: **Changes in mammographic densities induced by a hormonal contraceptive designed to reduce breast cancer risk.** *J Natl Cancer Inst* 1994, **86**:431-436.
10. Ursin G, Astrahan MA, Salane M, Parisky YR, Pearce JG, Daniels JR, Pike MC, Spicer DV: **The detection of changes in mammographic densities.** *Cancer Epidemiol Biomarkers Prev* 1998, **7**:43-47.
11. Brisson J, Brisson B, Coté G, Maunsell E, Bérubé S, Robert J: **Tamoxifen and mammographic densities.** *Cancer Epidemiol Biomarkers Prev* 2000, **9**:911-915.
12. Greendale GA, Reboussin BA, Sie A, Singh R, Olson LK, Gatewood O, Bassett LW, Wasilauskas C, Bush T, Barrett-Conner E for the Postmenopausal Estrogen/Progestin Interventions (PEPI) investigators: **Effects of estrogen and estrogen-progestin on mammographic parenchymal density.** *Ann Intern Med* 1999, **130**:262-269.

13. Laya MB, Gallagher JC, Schreiman JS, Larson EB, Watson P, Weinstein L: **Effect of postmenopausal hormonal replacement therapy on mammographic density and parenchymal pattern.** *Radiology* 1995, **196**:433-437.
14. Feigelson HS, McKean-Cowdin R, Coetzee GA, Stram DO, Kolonel LN, Henderson BE: **Building a multigenic model of breast cancer susceptibility:** *CYP17* **and** *HSD17B1* **are two important candidates.** *Cancer Res* 2001, **61**:785-789.
15. Haiman CA, Hankinson SE, Spiegelman D, Colditz GA, Willett WC, Speizer FE, Kelsey KT, Hunter DJ: **The relationship between a polymorphism in** *CYP17* **with plasma hormone levels and breast cancer.** *Cancer Res* 1999, **59**:1015-1020.
16. Lavigne JA, Helzlsouer KJ, Huang H-Y, Strickland PT, Bell DA, Selmin O, Watson MA, Hoffman S, Comstock GW, Yager JD: **An association between the allele coding for a low activity variant of catechol-***O***-methyltransferase and the risk for breast cancer.** *Cancer Res* 1997, **57**:5493-5497.
17. Marchbanks PA, McDonald JA, Wilson HG, Burnett NM, Daling JR, Bernstein L, Malone KE, Strom BL, Norman SA, Weiss LK, Liff JM, Wingo PA, Burkman RT, Folger SG, Berlin JA, Deapen DM, Ursin G, Coates RJ, Simon MS, Press MF, Spirtas R: **The NICHD Women's Contraceptive and Reproductive Experiences Study: methods and operational results.** *Ann Epidemiol*, in press.
18. Livak KT: **Allelic discrimination using fluorogenic probes and the 5′ nuclease assay.** *Genet Anal* 1999, **14**:143-149.
19. SAS Institute, Inc: *SAS/STAT User's Guide* (version 6, edition 4). Cary: SAS Institute, Inc; 1989.
20. Dunning AM, Healey CS, Pharoah PDP, Teare MD, Ponder BAJ, Easton DF: **A systematic review of genetic polymorphisms and breast cancer risk.** *Cancer Epidemiol Biomarkers Prev* 1999, **8**:843-854.
21. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FS, Henderson BE: **Cytochrome P450c17α (***CYP17***) polymorphism is associated with serum estrogen and progesterone concentrations.** *Cancer Res* 1998, **58**:585-587.
22. Haiman CA, Hankinson SE, Colditz GA, Hunter DJ, De Vivo I: **A polymorphism in** *CYP17* **and endometrial cancer risk.** Cancer Res 2001, **61**:3955-3960.
23. Mannermaa A, Peltoketo H, Winqvist R, Ponder BAJ, Kiviniemi H, Easton DF, Poutanen M, Isomaa V, Vihko R: **Human familial and sporadic breast cancer: analysis of the coding regions of the 17β-hydroxysteroid dehydrogenase 2 gene (EDH17B2) using single-strand conformation polymorphism assay.** *Hum Genet* 1994, **93**:319-324.
24. Rhéaume E, Sirois I, Labrie F, Simard J: **Codon 367 polymorphism of the human type I 3-hydroxysteroid dehydrogenase/isomerase gene (HSD3BI).** *Nucleic Acids Res* 1991, **19**:6060.
25. Van Aswege CH, Purdy RH, Wittliff JL: **Binding of 2-hydroxyestradiol and 4-hydroxyestradiol to estrogen receptors from human breast cancers.** *J Steroid Biochem* 1989, **32**:485-492.
26. Syvanen AC, Tilgmann C, Rinne J, Ulmanen I: **Genetic polymorphism of catechol-***O***-methyltransferase (COMT): correlation of genotype with individual variation of S-COMT activity and comparison of the allele frequencies in the normal population and Parkinsonian patients in Finland.** *Pharmacogenetics* 1997, **7**:65-71.
27. Dawling S, Roodi N, Mernaugh RL, Wang X, Parl FF: **Catechol-***O***-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms.** *Cancer Res* 2001, **61**:6716-6722.
28. Thompson PA, Shields PG, Freudenheim JL, Stone A, Vena JE, Marsha JR, Graham S, Laughlin R, Nemoto T, Kadlubar FF, Ambrosone CB: **Genetic polymorphism in catechol-***O***-methyltransferase, menopausal status, and breast cancer risk.** *Cancer Res* 1998, **58**:2107-2110.
29. Millikan RC, Pittman GS, Tse CK, Duell E, Newman B, Savitz D, Moorman PG, Biossy RJ, Bell DA: **Catehchol-***O***-methyltransferase and breast cancer risk.** *Pharmacogenetics* 1998, **19**: 1943-1947.
30. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S, Kang D, Vainio H, Uusitupa M, Hirvonen A: **Polymorphic catechol-***O***-methyltransferase gene and breast cancer risk.** *Cancer Epidemiol Biomarkers Prev* 2001, **10**:635-640.