KU ScholarWorks

ASSOCIATIONS BETWEEN POLYMORPHISMS AND ABNORMAL RPFNA CYTOMORPHOLOGY IN HIGH-RISK POSTMENOPAUSAL WOMEN TAKING HRT

Item Type	Thesis
Authors	Michaels, Whitney Lee
Publisher	University of Kansas
Rights	This item is protected by copyright and unless otherwise specified the copyright of this thesis/dissertation is held by the author.
Download date	2024-08-03 15:25:46
Link to Item	https://hdl.handle.net/1808/15774

ASSOCIATIONS BETWEEN POLYMORPHISMS AND ABNORMAL RPFNA
CYTOMORPHOLOGY IN HIGH-RISK POSTMENOPAUSAL WOMEN TAKING HRT

BY WHITNEY L. MICHAELS

Submitted to the graduate degree program in Clinical Research
and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the
degree of Master of Science.

_____
Chairperson, Brooke L. Fridley, PhD

_____
Bruce F. Kimler, PhD

_____
Carol J. Fabian, MD

Date Defended: May 16th, 2014

The Thesis Committee for WHITNEY L. MICHAELS certifies that this is the approved version of the following thesis:

ASSOCIATIONS BETWEEN POLYMORPHISMS AND ABNORMAL RPFNA CYTOMORPHOLOGY IN HIGH-RISK POSTMENOPAUSAL WOMEN TAKING HRT

_____

Chairperson, Brooke L. Fridley, PhD

Date approved: June 2nd, 2014

Abstract

Introduction: Hormone replacement therapy (HRT) is an effective treatment option for women experiencing symptoms of menopause but long-term use is associated with an increased risk of breast cancer. HRT-related breast cancer risk is dependent on many other factors including age at menopause, age at initiation of therapy, duration of use, dose and method of delivery. Differences in genetic factors, specifically single nucleotide polymorphisms (SNP) may identify those women whose breast tissue is likely or unlikely to be seriously affected by HRT. **Methods:** Post-menopausal women at increased risk for breast cancer underwent breast tissue sampling by random periareolar fine needle aspiration (RPFNA) and epithelial cells were characterized as to whether they exhibited cytologic evidence of hyperplasia with atypia. DNA was isolated from buccal cells for assessment of candidate genes involved in steroid metabolism, receptor function, cell cycle control, DNA repair and/or carcinogen metabolism. Associations between each SNP and RPFNA atypia were examined by unconditional logistic regression in three different genetic models: a log-additive, dominant, and co-dominant. Linear regression was used to assess the association between each SNP and worsening cytomorphology while taking systemic HRT for ≥ 6 months vs. off systemic HRT. The adjusted significance level, $p<0.001$ was used to account for multiple testing. **Results:** *RAD54* Gln^{929}Glu, *TFR* Gly^{142}Ser, *VEGF* 3'UTR and *ACE16* (I/D) were associated with RPFNA atypia in all women at $p<0.05$ and *TFR* Gly^{142}Ser reached borderline significance ($p=0.0025$). Interestingly, the cohort of women with RPFNA cytomorphology both while taking HRT and off HRT showed the most significant results. *RAD23B* Ala249Val was significantly associated with worsening cytomorphology while on HRT vs. off HRT ($p=0.0009$) and *ERCC1* 3'UTR was borderline ($p=0.0015$). SNPs associated with worse cytomorphology showed similar effects when we defined the outcome variable as evidence of atypia while on systemic HRT with no atypia while off systemic HRT. **Conclusion:** Promising associations exist between polymorphisms involved in DNA repair and worse RPFNA cytomorphology as a consequence of HRT use. However, given the probability of chance finding due to multiple testing, these results will need to be validated in an independent cohort.

TABLE OF CONTENTS

Introduction

Hormone replacement therapy (HRT) is an effective treatment option for women experiencing symptoms of menopause. However, long-term use is associated with an increased risk of breast cancer in post-menopausal women [Beral 2003, Rossouw 2002, Chlebowski 2009]. It is well-established that breast cancer risk associated with HRT differs by the type of therapy, with combined estrogen-progestin therapy posing a greater risk than estrogen monotherapy [Rossouw 2002; Beral 2003; Li 2003; Anderson 2004]. However, HRT-related breast cancer risk is dependent on many other factors including age at menopause, age at initiation of therapy, duration of use, dose and method of delivery [Sood 2014, Manson 2013, Li 2003, Modena 2005] and it is likely that for the majority of women there is little to no increase in risk from taking low-dose HRT for 5 years or less during the menopause transition [Johansen 2008, North American Menopause Society 2012].

Women who are already at increased risk for breast cancer, due to family history or other risk factors, may be particularly anxious about initiating HRT despite the adverse effects of estrogen deprivation on their quality of life. While a 15-50% increase in relative risk would not appreciably elevate absolute risk in the average 50 year old whose baseline five year risk is 1%, a high risk woman with a baseline five year risk of 5% could have her absolute risk estimate increased to as much as 7.5% with more than five years of use of estrogen and progestin [Rossouw 2002]. However, high risk women who undergo oophorectomy for risk reduction before age 45, resulting in early or pre-mature menopause are at increased risk for osteoporosis, heart disease or cognitive impairment if they do not take HRT until the age of natural menopause [Shuster 2009]. Given that the quality of life symptoms during the menopause transition and the long-term consequences of estrogen deprivation can be severe, it would be advantageous to develop a simple way to identify those women whose breast tissue is unlikely to be seriously affected by HRT.

A research program was started at The University of Kansas Medical Center (KUMC) in the late 1980s aimed at predicting the short-term risk of developing breast cancer in women who were already at increased risk on the basis of family and personal history. Given the principle that histologic hyperplasia

with atypia on a diagnostic biopsy is known to increase 5 year risk of breast cancer by 4 fold [Hartmann 2005; Dupont 1985; Pearlman 2010] and reported evidence that proliferative changes in the breast are seen in a multi-focal and multi-centric pattern [Neilson 1987, Hoogerbrugge 2003], CJF developed the procedure of random per-areolar fine needle aspiration (RPFNA). This minimally invasive, non-lesion directed technique assesses multiple areas of benign breast tissue in order to detect a field effect in the entire breast. The rationale was that women with evidence of atypia upon random sampling of the entire breast are likely to have multiple areas of proliferative tissue and increased likelihood that one of these areas will progress to invasive cancer. The RPFNA technique was assessed in a cohort of 480 high-risk women. The results of this prospective study indicated that evidence of atypia by RPFNA strongly predicts the development of subsequent DCIS or invasive breast cancer and that cytomorphology along with the Gail model [Gail 1989] could be used to identify a cohort of women at a very high short-term risk (Fabian 2000). RPFNA is currently used in the Breast Cancer Prevention Center at KUMC for risk stratification and as a surrogate end point biomarker in chemoprevention trials (Fabian 2007).

Estrogen and its metabolic compounds are involved in breast carcinogenesis indirectly through their influence on cell growth and proliferation [Horwitz 1998, Zumoff 1998] and directly via oxidative reactions with DNA, forming adducts which generate mutations [Yue 2003, Yager 2006, Cavalieri 2006]. Progesterone can influence breast carcinogenesis through increased formation of estradiol by induction of 17β-hydroxysteroid dehydrogenase. [Poutanen 1995] Progesterone metabolites regulate estrogen receptor levels in breast epithelial cells [Pawlak 2007] as well as proliferation, apoptosis and cell adhesion in tumor and normal breast cells [Weibe 2006, Wiebe 2006]. The effects of exogenous hormones on benign breast tissue are likely to be modulated by genes important in steroidogenesis, catabolism and elimination of estrogens, transcriptional activation in response to hormones and growth factors, cell cycle control, differentiation, and genes involved in carcinogen metabolism and DNA repair. Common polymorphisms in these genes have been associated with increased breast cancer risk in prior studies [Haiman 2003; Tworoger 2004; Lisgarten 2004; Brandt 2004; Liede 2003; Haiman 2002; Zheng 2001; Aston 2004; Coughlin 1999] and some have been shown to influence hormone levels [Feigelson 1998, Dunning 2004,

2

Tworoger 2004]. However, associations with HRT-related breast cancer risk have been inconsistent [Campa 2011, Kawase 2009, Prentice 2009, Travis 2010, Hein 2012, MARIE-GENICA 2010 (1-4), Nickels 2013, Rebbeck 2007, Reding 2009, Conway 2007, Lee 2011, Andersen 2013, Diergaarde 2008] and few validated interactions between single nucleotide polymorphisms (SNP) and HRT use exist to date [Hein 2012, Justenhoven 2011, Obazee 2013].

To our knowledge, no study has associated candidate polymorphisms with risk biomarkers, such as benign breast tissue cytology. Since risk biomarkers may be subject to modulation, they are ideally suited to monitor change in breast cancer susceptibility with to the use of HRT. Additionally, breast tissue cytology has the advantage of being directly related to the underlying neoplastic process [Fabian 2005]. Thus, high risk women who develop abnormal cytomorphology as a consequence of HRT are likely more susceptible to the carcinogenic effects of exogenous hormones. In order to examine the relationship between relevant polymorphisms, HRT use and RPFNA cytomorphology, we have conducted a genetic association study in post-menopausal women at increased risk for breast cancer.

Materials and Methods

Study Subjects

High-risk post-menopausal women were selected from those being followed at the KUMC Breast Cancer Prevention Center as part of the High Risk Breast Clinic (HRBC) cohort. The HRBC database was queried to identify potential subjects with prior RPFNA (performed by CJF between January 2000 and October 2004) or those planning RPFNA for short-term risk assessment or for ongoing clinical trials between October 2004 and January 2006. The cohort was later expanded to include women (at high risk for breast cancer) with RPFNA performed between January 2000 and March 2009. In order to meet the criteria for the RPFNA procedure as previously described [Fabian 2007, Fabian 2013], subjects were required to be at increased risk for breast cancer by virtue of one or more of the following: (1) five-year Gail predicted probability of breast cancer ≥1.67% [Gail 1989]; (2) prior biopsy showing atypical hyperplasia or lobular carcinoma in situ; (3) prior treated contralateral DCIS or invasive cancer; (4)

known BRCA1/2 mutation carrier. Potential subjects were post-menopausal at the time of aspiration, defined as complete surgical (a prior hysterectomy and bilateral oophorectomy), partial surgical (a prior hysterectomy with ovaries intact and age >50 years) or natural menopause (no period for at least 12 months prior to RPFNA), and were either not using hormone replacement therapy or had been on a stable dose of estrogen alone or estrogen plus a progestin for at least 6 months prior to RPFNA. Upon entry into the HRBC cohort and before each RPFNA procedure, women were asked to sign a consent form which stated that the RPFNA was still considered a research procedure and outlined any additional specimen collection procedures, including buccal cell collection, to be conducted for future studies in the HRBC cohort. Potential subjects for our study were contacted either in-person or by phone and asked to provide a buccal cell sample for SNP genotyping. Women who agreed to participate were either mailed or given a buccal cell collection kit and asked to sign a separate consent form. At the time of buccal cell sample collection, women were asked to verify information in the HRBC database corresponding to their baseline risk and reproductive history factors. A separate database was created to record baseline data, RPFNA history and HRT use at the time of each RPFNA for our study cohort. HRT use was divided into four categories: (1) on well-absorbed systemic HRT (oral, patch, troches, vaginal creams or gels, estrogen + progestin +/- testosterone, estrogen alone, estrogen + testosterone); (2) on poorly-absorbed vaginal estrogen (estring, vagifem tabs); (3) on phytoestrogens (flaxseed, soy, black cohash); (4) off HRT (none of these). Only those women with RPFNA while on well-absorbed systemic HRT for at least six months prior to aspiration or off HRT for at least six months prior to aspiration were considered for this study. RPFNA data and HRT information was updated prospectively for each subject from the time of entry into the study database until March 2009, when data was extracted for analysis.

RPFNA and cytomorphology

RPFNA was performed under local anesthesia from two sites (upper outer and upper inner quadrant) and cells were pooled from both breasts [Fabian 2000] in a single 15 cc tube containing 9 cc of CytoLyt™ and 1 cc of 10% neutral buffered formalin. Cells were spun, washed and re-suspended in PreservCyt™ after approximately 24 h in CytoLyt™. At least three aliquots were processed to slides using a

ThinPrep™ (Cytyc Corporation, Malborough, MA) non-Gyn protocol. Slides for cytomorphology were Papanicolaou-stained using an RNase-free technique [Fabian 2000, Fabian 2005]. All slides were assessed by a single cytopathologist (Carolla M. Zalles) who assigned a categorical assessment of non-proliferative, hyperplasia, borderline hyperplasia with atypia, or hyperplasia with atypia [Zalles 1995, Zalles 2006]; a Masood semi-quantitative index score [Masood 1990], and a Consensus Panel Designation [Uniform 1997].

Sample collection, DNA isolation and genotyping assays

Buccal cells collected via an oral rinse with Scope mouthwash were obtained either by mail or in person from each participant and shipped to Intergenetics, Inc. (Oklahoma City, OK). Genomic DNA was isolated using the Gentra PureGene DNA extraction kit (Gentra, Minneapolis, MN). Genotyping was done by high throughput microsphere-based, allele-specific primer extension (ASPE) assays followed by analysis on the Luminex 100 flow cytometer (Luminex, Austin, TX) as previously described [Ashton 2005, Diergaarde 2008]. DNA was pre-amplified by multiplex PCR using HotStar Taq DNA polymerse (Qiagen Inc., Valencia, CA).

One-hundred and seventeen common single nucleotide polymorphisms (SNPs) were selected in collaboration with Intergenetics, Inc. [Jupe 2007, Ralph 2007, Diergaarde 2008, Jupe 2008]; a summary of these SNPs can be found in the supplementary materials (**Supp. Table 1**). The SNPs were located in breast cancer candidate genes involved in steroid hormone metabolism (CYP17, CYP19, CYP1A1, CYP1B1, COMT, SULT1A1, UGT1A1) and receptor function (AR, A1B1, ESR1, PGR), cell cycle control (PHB, CCDN1, EGFR), DNA repair (ERCC2, RAD23B) and/or carcinogen metabolism (SULT1A1, NQ01, GSTP1) [Haiman 2003; Tworoger 2004; Lisgarten 2004; Brandt 2004; Liede 2003; Haiman 2002, 2003; Zheng 2001; Aston 2004; Coughlin 1999].

We identified 361 potential subjects from the HRBC database; of these potential subjects, 258 were genotyped and had at least one RPFNA performed by March 2009. Following quality-control, 218 women & 79 SNPs passed with >95% call rate. A flow-chart of the exclusion of subjects and SNPs can

be found in the supplementary materials (**Supp. Table 2**). To note, no SNP deviated from Hardy-Weinberg equilibrium (HWE) with a p-value <0.05.

Statistical Analysis

Descriptive statistics were generated using mean and standard deviation for continuous variables and frequency and percent for categorical variables. Distribution of risk, reproductive, RPFNA history and hormone use factors among women with RPFNA atypia and women without RPFNA atypia were compared using t-tests for continuous variables and X^2 test for categorical variables. Genotype frequencies were tested for Hard-Weinberg equilibrium (HWE) using X^2 goodness-of-fit test. All analyses were performed using R 3.0.2 [R Core Team 2013].

To examine associations between each SNP and RPFNA atypia we used three different genetic models: a log-additive (genotype coded in terms of the number of minor alleles), dominant (carrier vs non-carrier of minor allele), and co-dominant (genotype) genetic models. These analyses were completed in all women regardless of HRT use and then stratified by HRT use. Analysis of each SNP with evidence of RPFNA atypia was computed using unconditional logistic regression models with odds ratio (OR) and 95% confidence interval (CI) estimated using Wald statistics. In addition to the SNP association analysis stratified by HRT use, we also completed tests for gene-environment interaction with logistic regression models including each SNP, the HRT use variable (stable dose for ≥6 months at time of RPFNA) and a gene-environment (GE) interaction term. P-values for the interaction effects were obtained using likelihood-ratio tests (i.e. Ho: interaction effect = 0).

Women with RPFNA performed both on and off HRT were excluded from the stratified analysis (described above) and made up the group of women for which we assessed the change in RPFNA cytomorphology while on HRT versus off HRT. Linear regression was used to evaluate the correlation between each SNP and change in cytomorphology (worst on HRT minus worst off HRT) based on the numeric cytomorphology categorization index (1=no cells, 2=normal, 3=normal borderline EH, 4=apocrine metaplasia, 5=EH, 6=EH borderline atypia, 7=atypia, 8=suspicious for cancer). Most changes in RPFNA cytomorphology categorization index occurred between category 5 and category 7 (EH to AH)

6

although a few women developed atypia (category 7) on HRT with normal cytology (category 2) off HRT. Logistic regression was used to evaluate the association between each SNP and the development of RPFNA atypia only while on HRT (with no atypia while off HRT). All significance tests were two-sided and p-value <0.001 was considered significant when we adjusted for multiple testing; however, p<0.05 was reported due to the exploratory nature of this analysis.

Results

Risk and reproductive history

The study population consisted of 218 post-menopausal women at increased risk for breast cancer. Of these women, 95 (43.6 %) had evidence of atypia by RPFNA and 123 (56.4 %) did not have evidence of atypia by RPFNA. Baseline characteristics for the study population are presented in **Table 1**. In general, the cohort consisted of young post-menopausal women, mean age at menopause = 45.3 years and age at entry < 60 years (data not shown), who were at increased risk for breast cancer compared to the general population, mean 5-yr GAIL risk = 3.80% (data not shown). Women with evidence of atypia by RPFNA do not differ from women without evidence of atypia by most risk or reproductive history factors. However, those with evidence of RPFNA atypia were slightly younger at baseline (54.5 ± 6.53 vs. 56.48 ± 7.38, p=0.042) and more likely to have a negative family history, although this did not reach statistical significance (p=0.052). These findings are consistent with other studies using the high risk breast clinic (HRBC) post-menopausal cohort.

Women with evidence of atypia had an average of 4.66 ± 2.15 aspirations throughout the duration of this study while women without evidence of atypia had an average of 2.68 ± 1.78 aspirations (p<0.001). Two factors contribute to this finding: (1) the RPFNA procedure involves random, non-lesion directed sampling of the breast tissue and thus cytologic atypia is more likely to be recognized in those women with increased sampling and (2) those with evidence of atypia by RPFNA are more likely to enroll in chemoprevention trials and continue to receive aspirations as part of the HRBC cohort. Women with evidence of atypia as part of this study were also more likely to have had evidence of atypia prior to 2000.

Evidence of atypia in random sampling of benign breast tissue is indicative of widespread proliferative changes in the breast [Fabian 2005] and the high-risk density is not removed as part of the RPFNA procedure.

Characteristic	Never Atypia (n=123)[a]	Ever Atypia (n=95)[a]	p-value[b]
Table 1: Description of risk and reproductive history for 218 women by evidence of atypia on RPFNA			
Breast Cancer Risk Information[c]			
Age (yrs), mean (SD)	56.48 (7.38)	54.5 (6.53)	0.042
BMI (kg/m^2), mean (SD)	27.02 (6.65)	27.48 (6.68)	0.62
Mammographic Breast Density, n (%)			0.27
<5%	14 (11.48%)	8 (8.42%)	
5-25%	40 (32.79%)	25 (26.32%)	
26-50%	35 (28.69%)	25 (26.32%)	
51-75%	19 (15.57%)	16 (16.84%)	
>75%	10 (8.2%)	11 (11.58%)	
Number of affected 1st Degree Relatives			0.064
None	30 (24.4%)	36 (37.9%)	
1	61 (49.6%)	43 (45.3%)	
At least 2	32 (26.0%)	16 (16.8%)	
5-yr Gail Risk (%), mean (SD)	3.84 (2.3)	3.74 (2.64)	0.76
Prior AH or LCIS biopsy, n (%)	22 (17.89%)	21 (22.11%)	0.55
Prior contralateral breast cancer, n (%)	14 (11.38%)	13 (13.68%)	0.76
Reproductive History Information[c]			
Parity (0 vs. ≥1), n (%)	15 (12.2%)	16 (16.84%)	0.44
Age at Menopause (yrs), mean (SD)	45.42 (7.24)	45.05 (7.11)	0.71
Interval from Menopause (yrs), mean (SD)	11.11 (8.39)	9.45 (7.57)	0.13
Type of Menopause			0.17
Natural	50 (41.0%)	30 (31.9%)	
Surgical	72 (59.0%)	64 (68.1%)	
RPFNA History in High Risk Breast Clinic (HRBC)[d]			
Number of RPFNAs since 2000, mean (SD)	2.68 (1.78	4.66 (2.15)	<0.001

| RPFNA atypia prior to 2000, n (%) | 17 (14.05%) | 26 (27.66%) | 0.021 |

a. Numbers do not add up to the total for never atypia (n=123) and ever atypia (n=95) due to missing data for some participants
b. Two-sided t-test p-value for continuous variable; chi-square p-value for categorical variables
c. Risk information and reproductive history were collected prospectively upon entry and at each follow-up visit with CJF as part of the High Risk Breast Clinic (HRBC); information stored in the HRBC database was verified at the time of buccal sample collection
d. RPFNA data was collected prospectively at each aspiration visit and entered into the HRBC database; relevant information was collected for each subject throughout the duration of this study and entered in a separate database

Hormone Replacement Therapy (HRT) use history

As shown in **Table 2**, most women in this post-menopausal cohort had used hormone replacement at some point in their personal history to control symptoms of menopause (n=172/218, 78.9%). The proportion women with a history of HRT use appears to be slightly greater among those women with evidence RPFNA atypia (85.3% vs. 74.0%, p=0.064). In order to examine this further, we considered three important factors which contribute to HRT-related breast cancer risk: duration of use, type of therapy and whether dose was expected to increase risk.

Most women in our study cohort, with or without evidence of RPFNA atypia, were likely advised to initiate low-dose and/or estrogen alone hormone replacement therapy; especially since this cohort consisted of young post-menopausal women (mean age at menopause = 45.3, data not shown) and more than 50% had undergone partial or complete surgical removal of their uterus and/or both ovaries. Consistent with this, combined estrogen + progesterone therapy use was low in our cohort (37.5%). However, many women were taking doses of estrogen that have been associated with increased risk of breast cancer ($\geq$ 0.625 mg oral estrogen, 0.05 mg transdermal estradiol or 1 mg oral estradiol) and there appears that more women with evidence of RPFNA atypia were on these higher doses compared to women without evidence of RPFNA atypia (74.5% vs 60.7%, p=0.046).

Duration of HRT use appears to differ among those with and without evidence of RPFNA atypia (p=0.0014); the greatest proportion of women with evidence of atypia were on HRT for $\leq$ 5 years while the greatest proportion of women without evidence of atypia were on HRT for > 5 years. This difference indicates that certain women in our cohort may have continue on hormone replacement long-term, either

for symptom relief or until the age of natural menopause (if <40 years), without affecting their breast cancer risk as predicted by benign breast tissue cytomorphology.

Table 2: Description of Hormone Replacement Therapy (HRT) for 218 women by evidence of atypia on RPFNA				
Post-menopausal Hormone Use[e]	**Entire Cohort (n=218)[a]**	**Never Atypia (n=123)[a]**	**Ever Atypia (n=95)[a]**	**p-value[b]**
On Systemic HRT, n (%)				0.064
Never	46 (21.1%)	32 (26.02%)	14 (14.7%)	
Ever	172 (78.9%)	91 (74.0%)	81 (85.3%)	
Duration of systemic HRT (years)				0.0014
Never	46 (21.1%)	32 (26.02%)	14 (14.7%)	
≤ 5 years	78 (35.8%)	31 (25.2%)	47 (49.5%)	
>5 years	94 (43.1%)	60 (48.8%)	34 (35.8%)	
Estrogen Dose Increased Risk[c], n (%)				0.046
No	72 (33.3%)	48 (39.3%)	24 (25.5%)	
Yes	144 (66.7%)	74 (60.7%)	70 (74.5%)	
On Combined (E + P) HRT[d], n (%)				0.99
Never	141 (65.3%)	80 (65.6%)	61 (64.9%)	
Ever	75 (34.7%)	42 (34.4%)	33 (35.1%)	

a. Numbers do not add up to the total for never atypia (n=123) and ever atypia (n=95) due to missing data for some participants
b. Two-sided t-test p-value for continuous variable; chi-square p-value for categorical variables
c. Estrogen dosages expected to increase risk include: ≥ 0.625 mg oral estrogen, 0.05 mg transdermal estradiol or 1 mg oral estradiol
d. Use of systemic Estrogen + Progesterone (or Progestin) hormone replacement therapy
e. Post-menopausal hormone use was collected prospectively at each BCPC clinic visit and recorded in the HRBC database. The drug name, dose, type, dose-class, method of delivery, start and end date of HRT use was collected at each RPFNA throughout the duration of this study and entered in a separate database

SNPs associated with RPFNA atypia

Polymorphisms in four genes were associated with evidence of RPFNA atypia with a p-value < 0.05.

However, these polymorphisms did not remain significant when we adjust for multiple testing, p<0.001.

Results for select polymorphisms (p<0.05) are shown in **Table 3** and for all polymorphisms in the

supplementary materials (Supp. Table 3). Carriers of the variant allele for *RAD54* Gln[929]Glu, *TFR*

Gly[142]Ser or *VEGF* 3'UTR polymorphisms showed the highest frequency of RPFNA atypia (OR_{dom}=

1.74, 1.98 and 1.86, p_{dom}=0.046, 0.026, 0.022). Log-additive genetic models for both *RAD54*

Gln[929]Glu and *TFR* Gly[142]Ser showed an increase in the frequency of RPFNA atypia in relationship

to the number of minor alleles a woman carried (OR_{ord}=1.71, 1.81 and p_{ord}=0.013, 0.0025). The

VEGF 3'UTR polymorphism was not associated with RPFNA atypia in this model and this was most likely due to a very small number of women with two minor alleles (T/T=2). The D/D genotype for the ACE16 I/D polymorphism is considered to be rare in the general population and associated with high expression of the ACE16 gene. However, in our cohort the deletion genotype was most common, with genotype frequencies D/D=33.2%, D/I=45.8%, I/I=21.03%, and DD was significantly associated with RPFNA atypia (OR_{dom}=0.55, p_{dom}=0.036).

Table 3: SNPs associated with RPFNA atypia (n=218) (p < 0.05)

SNP ID	Gene (var)	Genotype	RPFNA atypia / total[a] (%)	P_{2df}	OR (95% CI)	P_{dom}	OR (95% CI)	P_{ord}
rs4646994	ACE16 (I/D)	D/D D/I I/I	38/71 (53.5%) 38/98 (38.8%) 17/45 (37.8%)	0.11	0.55 (0.31, 0.96)	0.036	0.7 (0.48, 1.02)	0.067
rs3088074	RAD54 (Q929E)	C/C C/G G/G	37/103 (35.9%) 44/94 (46.8%) 13/20 (65.0%)	0.050	1.74 (1.01, 2.99)	0.046	1.71 (1.12, 2.62)	0.013
rs3817672	TFR (S142G)	A/A A/G G/G	21/67 (31.3%) 43/100 (43.0%) 30/50 (60.0%)	0.013	1.98 (1.09, 3.61)	0.026	1.81 (1.23, 2.65)	**0.0025***
rs3025039	VEGF (3'UTR)	C/C C/T T/T	62/162 (38.3%) 30/50 (60.0%) 0/2 (0%)	0.037	2.12 (1.13, 3.99)	0.022	1.86 (1.02, 3.39)	0.042

*borderline statistically significant when we adjust for multiple testing using Bonferroni type-1 error correction, p<0.001
a. total may not add to 218 due to incomplete genotype data for certain polymorphisms

SNPs associated with RPFNA atypia and HRT use

In order to determine whether the associations between candidate polymorphisms and RPFNA atypia would be modified by HRT, we stratified our cohort by use of systemic hormone replacement therapy at the time of RPFNA. This analysis excluded women with RPFNA results both on and off HRT (n=55) so that gene-environment interaction could be assessed using a single RPFNA for each subject's worst benign breast tissue cytomorphology. Results for select polymorphisms (p<0.05)

are shown in **Table 4** and for all polymorphisms in the supplementary materials (**Supp. Table 4**).

We found 13 polymorphisms associated with RPNFA atypia in only one of the two strata (p<0.05),

indicating a gene-environment effect or a modifying effect of HRT use on genetic risk.

			OFF Systemic HRT (n=77)					**ON Systemic HRT (n=86)**				
SNP ID	**Gene (var)**	**Genotype**	**RPFNA atypia / total[b] (%)**	**OR (95% CI)**	**P_{dom}**	**OR (95% CI)**	**P_{ord}**	**RPFNA atypia / total[b] (%)**	**OR (95% CI)**	**P_{dom}**	**OR (95% CI)**	**P_{ord}**
rs16260	ECAD (nt-160)	C/C C/A A/A	14/32 (43.8%) 12/36 (33.3%) 3/9 (33.3%)	0.64 (0.25, 1.64)	0.35	0.74 (0.37, 1.51)	0.41	12/43 (27.9%) 17/32 (53.1%) 4/10 (40.0%)	2.67 (1.09, 6.54)	0.032	1.65 (0.87, 3.13)	0.12
rs3088074	RAD54 (Q929E)	C/C C/G G/G	26/70 (37.1%) 3/7 (42.9%) NONE	0.81 (0.32, 2.05)	0.66	0.93 (0.43, 2.03)	0.86	31/79 (39.2%) 2/6 (33.3%) NONE	2.28 (0.93, 5.58)	0.07	1.89 (1.01, 3.53)	0.046
rs3817672	TFR (S142G)	A/A A/G G/G	11/30 (36.7%) 10/32 (31.3%) 8/15 (53.3%)	1.07 (0.42, 2.76)	0.89	1.31 (0.7, 2.44)	0.4	5/23 (21.7%) 14/38 (36.8%) 14/25 (56.0%)	0.36 (0.14, 0.93)	0.034	0.47 (0.25, 0.87)	0.017
rs1139793	TXNRD2 (I340T)	C/C C/T T/T	11/30 (36.7%) 14/38 (36.8%) 4/9 (44.4%)	1.07 (0.42, 2.76)	0.89	1.12 (0.56, 2.26)	0.75	24/29 (49.0%) 7/29 (24.1%) 1/7 (14.3%)	0.29 (0.11, 0.75)	0.011	0.37 (0.16, 0.83)	0.016
rs3025039	VEGF (3'UTR)	C/C C/T T/T	18/53 (34.0%) 11/23 (47.8%) 0/1 (0.0%)	1.65 (0.62, 4.4)	0.32	1.42 (0.57, 3.57)	0.45	22/68 (32.4%) 10/15 (66.7%) 0/1 (0.0%)	3.41 (1.1, 10.53)	0.033	2.57 (0.91, 7.29)	0.076
rs25487	XRCC1 (Q399R)	G/G G/A A/A	13/37 (35.1%) 13/34 (38.2%) 3/6 (50.0%)	1.23 (0.49, 3.1)	0.66	1.26 (0.61, 2.62)	0.53	14/27 (51.9%) 17/45 (37.8%) 2/14 (14.3%)	0.44 (0.17, 1.12)	0.085	0.45 (0.22, 0.9)	0.024
rs700519	CYP19 (R264C)	C/C C/T T/T	23/66 (34.8%) 6/9 (66.7% NONE	3.91 (0.9, 17.09)	0.07	3.74 (0.86, 16.35)	0.08	32/80 (40.0%) 1/6 (16.7%) NONE	0.3 (0.03, 2.69)	0.28	0.3 (0.03, 2.69)	0.28
rs10012	CYP1B1 (R48G)	C/C C/G G/G	13/44 (29.5%) 12/25 (48.0%) 4/7 (57.1%)	2.46 (0.96, 6.34)	0.062	1.92 (0.94, 3.93)	0.072	13/38 (34.2%) 13/37 (35.1%) 5/9 (55.6%)	1.07 (0.45, 2.56)	0.88	1.37 (0.7, 2.66)	0.36
rs5918	ITGB3 (L33P)	T/T T/C C/C	19/60 (31.7%) 10/17 (58.8%) NONE	3.08 (1.02, 9.34)	0.047	3.08 (1.02, 9.34)	0.047	23/81 (37.7%) 10/22 (45.5%) 0/2 (0.0%)	1.21 (0.46, 3.17)	0.7	0.98 (0.42, 2.32)	0.97

Table 4: SNPs associated with RPFNA atypia in cohort (n=163[a]) stratified by systemic HRT use at time of aspiration (p < 0.05)

SNP ID	Gene (var)											
rs198977	KLK2 (G226W)	C/C C/T T/T	21/45 (46.7%) 6/28 (21.4%) 2/4 (50.0%)	0.38 (0.14, 1.03)	0.056	0.55 (0.24, 1.27)	0.16	14/43 (32.6%) 3/10 (30.0%) 15/36 (41.7%)	1.64 (0.68, 3.94)	0.27	1.71 (0.84, 3.47)	0.14
rs6917	PHB (3'UTR)	C/C C/G G/G	16/24 (33.3%) 12/35 (34.3%) 6/12 (50.0%)	0.29 (0.1, 0.9)	0.031	0.29 (0.1, 0.88)	0.029	13/35 (37.1%) 12/33 (36.4%) 7/10 (70.0%)	0.47 (0.16, 1.35)	0.16	0.68 (0.33, 1.37)	0.28
rs1799977	MLH1 (I219V)	A/A A/G G/G	19/40 (47.5%) 9/34 (26.5%) 1/3 (33.3%)	0.41 (0.16, 1.06)	0.067	0.48 (0.2, 1.15)	0.099	14/44 (31.8%) 18/41 (43.9%) 1/1 (100%)	1.77 (0.74, 4.26)	0.2	1.88 (0.81, 4.38)	0.14
rs4072037	MUC1 (E2)	A/A A/G G/G	7/24 (29.2%) 13/37 (35.1%) 9/16 (56.3%)	1.72 (0.61, 4.86)	0.30	2.64[c] (0.86, 8.1)	0.091[c]	7/26 (26.9%) 18/36 (50.0%) 6/21 (28.6%)	1.62 (0.63, 4.16)	0.32	0.56[c] (0.19, 1.64)	0.29[c]

a. Certain women (n=55) were excluded due to cross-over (multiple RPFNAs were performed both on and off systemic HRT); these women were analyzed separately (table 6, 7)

b. Total may not add to total women in each strata due to incomplete genotype data for some polymorphisms

c. Interaction most significant using the recessive model (those homozygous for the variant allele used as the reference group)

Gene-environment (GE) interaction was formally assessed by incorporating an interaction term into the logistic model. For this analysis, we used a stable dose of systemic HRT for at least 6 months prior to aspiration as our hormone environment variable. Again, we excluded women with RPFNA results both on and off HRT (n=55). The GE interaction results can be found in the supplementary materials (**Supp. Table 5**). GE interactions for ECAD (nt-160), TXNRD2 (I340T), CYP19 (R264C), KLK2 (G226W), MLH1 (I219V) were significant at p<0.05 but did not remain significant when we adjusted for multiple testing, p<0.001 (**Table 5**).

Table 5: Gene-Environment[b] interaction in cohort (n=163[a]) stratified by systemic HRT use at the time of aspiration (p < 0.05)

SNP ID	Gene (var)	Dominance model			Log-additive model		
		OR$_{OFF HRT}$ (95% CI)	OR$_{SYS HRT}$ (95% CI)	P$_{int}$	OR$_{OFF HRT}$ (95% CI)	OR$_{SYS HRT}$ (95% CI)	P$_{int}$
rs16260	ECAD (nt-160)	0.64 (0.25, 1.64)	2.67 (1.09, 6.54)	0.03	0.74 (0.37, 1.51)	1.65 (0.87, 3.13)	0.1
rs1139793	TXNRD2 (I340T)	1.07 (0.42, 2.76)	0.29 (0.11, 0.75)	0.05	1.12 (0.56, 2.26)	0.37 (0.16, 0.83)	0.04

rs25487	XRCC1 (Q399R)	1.23 (0.49, 3.1)	0.44 (0.17, 1.12)	0.12	1.26 (0.61, 2.62)	0.45 (0.22, 0.9)	0.04
rs700519	CYP19 (R264C)	3.91 (0.9, 17.09)	0.3 (0.03, 2.69)	0.04	3.74 (0.86, 16.4)	0.3 (0.03, 2.69)	0.04
rs198977	KLK2 (G226W)	0.38 (0.14, 1.03)	1.64 (0.68, 3.94)	0.03	0.55 (0.24, 1.27)	1.71 (0.84, 3.47)	0.04
rs1799977	MLH1 (I219V)	0.41 (0.16, 1.06)	1.77 (0.74, 4.26)	0.03	0.48 (0.2, 1.15)	1.88 (0.81, 4.38)	0.02
rs4072037	MUC1 (E2)	1.72 (0.61, 4.86)	1.62 (0.63, 4.16)	0.29	2.64[c] (0.86, 8.1)	0.56[c] (0.19, 1.64)	0.05[c]

a. Certain women (n=55) were excluded due to cross-over (multiple RPFNAs were performed both on and off systemic HRT); these women were analyzed separately (table 6, 7)

b. The environment variable considered for this analysis was on or off a stable dose of systemic HRT for $\geq$ 6 months prior to aspiration

c. Interaction most significant using the recessive model (those homozygous for the variant allele used as the reference group)

Given the lack of significant GE effect when we examined SNPs in two separate hormone environment strata, we were interested in examining the association between candidate polymorphisms and individual change in cytomorphology as a consequence of HRT use. For approximately ¼ of our cohort (n=55), RPFNA cytomorphology was assessed under multiple hormone environments. In this subset of women, we were able to identify SNPs associated with worse cytomorphology while on a stable dose of systemic HRT for $\geq$6 months compared to cytomorphology while off HRT for $\geq$6 months. **Table 6** shows select associations between polymorphisms ERCC1 (3'UTR), RAD23B (A249V), RAD51D (E233G), XRCC3 (T241M), INS-IGF2 (nt1107), MMP2 (nt-1306), NBS1 (E185Q) and worse RPFNA cytomorphology while on HRT vs. off HRT at the significance level p <0.05. For this outcome variable, RPFNA cytomorphology is treated as a numeric index with more abnormal cytomorphology (ex. Hyperplasia with Atypia) receiving as higher score than normal or less abnormal cytomorphology (ex. Epithelial Hyperplasia). When we adjusted for multiple testing, RAD23B (A249V) remained significant with p=0.0009, while ERCC1 (3'UTR) was considered borderline significant with p=0.0015. Many SNPs associated with worse cytomorphology in Table 5 showed similar associations when we defined the

outcome variable as evidence of atypia while on systemic HRT with no atypia while off systemic HRT. **Table 7** shows select associations between polymorphisms, MMP2 (nt-1306), RAD23B (A249V), XRCC3 (T241M) and CYP19A1 (3'UTR), and evidence of RPFNA atypia which were significant at p<0.05. None of the associations presented in Table 6 remained significant when we adjust for multiple testing, p<0.001. However, the two outcomes are highly correlated and it is likely that we were under-powered to detect a significant association between the binary outcome variable (evidence of RPFNA atypia on vs. off HRT) in this smaller subset of women.

Table 6: SNPs associated with worse cytomorphology categorization on HRT vs. off HRT (n=55[a]) (p < 0.05)

SNP ID	Gene (var)	r	Beta (SE)	P_{ord}	Beta (SE)	P_{dom}	Beta (SE)	P_{rec}[b]
rs3212986	ERCC1 (3'UTR)	-0.43	-0.95 (0.28)	**0.0015***	-0.85 (0.37)	0.024	-1.67 (0.70)	0.022
rs1805329	RAD23B (A249V)	-0.45	-1.20 (0.34)	**0.0009****	-1.11 (0.36)	0.0035	-2.59 (1.39)	0.068
rs28363284	RAD51D (E233G)	0.34	1.75 (0.68)	0.013	3.52 (1.35)	0.012	3.52 (1.35)	0.012
rs861539	XRCC3 (T241M)	0.32	0.76 (0.31)	0.019	0.62 (0.39)	0.12	1.84 (0.70)	0.011
rs243865	MMP2 (nt-1306)	-0.25	-0.55 (0.30)	0.076	-0.45 (0.39)	0.25	-1.40 (0.71)	0.056

* Borderline significant when we adjust for multiple testing using Bonferroni type-1 error correction, p<0.001
** Statistically significant association when we adjust for multiple testing
a. Select cohort (n=55) with multiple RPFNAs performed both on and off systemic HRT
b. Recessive model indicates association with worse (more abnormal) cytomorphology categorization on sHRT in those homozygous for the variant allele vs. those with at least one common allele

Table 7: SNPs associated with RPFNA atypia on sHRT vs. no RPFNA atypia off HRT (n=55[a]) (p < 0.05)

SNP ID	Gene (Var)	Geno-type	RPFNA atypia / total[b] (%)	OR (95% CI)	P_{ord}	OR (95% CI)	P_{dom}	OR (95% CI)	P_{rec}[c]

rs1805329	RAD23B (A249V)	C/C C/T T/T	18/31 (58.1%) 6/20 (30.0%) 0/1 (0.0%)	0.29 (0.09, 0.91)	0.035*	0.36 (0.11, 1.14)	0.081	NA	0.99
rs861539	XRCC3 (T241M)	C/C C/T T/T	6/19 (31.6%) 14/31 (45.2%) 4/4 (100%)	2.94 (1.06, 8.14)	0.038*	2.47 (0.77, 7.91)	0.13	NA	0.99
rs10046	CYP19A1 (3' UTR)	C/C C/T T/T	5/12 (41.7%) 11/34 (32.4%) 6/7 (85.7%)	1.32 (0.52, 3.32)	0.56	0.71 (0.21, 2.39)	0.58	10 (1.1, 90)	0.04
rs243865	MMP2 (nt-1306)	C/C C/T T/T	17/31 (54.8%) 7/18 (38.9%) 0/4 (0%)	0.36 (0.14, 0.98)	0.045*	0.44 (0.14, 1.36)	0.15	NA	0.99

*similar association for worse cytomorphology (table 6) & for evidence of atypia (table 7) on HRT vs. off HRT
a. Select cohort (n=55) with multiple RPFNAs performed both on and off systemic HRT
b. Total may not add to total women in each strata due to incomplete genotype data for some polymorphisms
c. Recessive model indicates association with worse (more abnormal) cytomorphology categorization on sHRT in those homozygous for the variant allele vs. those with at least one common allele

Discussion

This study evaluated associations between 79 polymorphisms in candidate genes important for breast carcinogenesis and the risk biomarker of cytomorphology of breast epithelial cells obtained by RPFNA in postmenopausal women either taking a stable dose of hormone replacement for ≥6 months at the time of cytomorphology assessment or not taking HRT. We were able to identify many promising associations between genetic variation and RPFNA atypia (1) in all women regardless of hormone use and (2) stratified by HRT use at the time of RPFNA which reached significance at p<0.05; however these did not remain significant when we adjust for multiple testing. Our most significant findings were for the cohort of women with RPFNA results both on and off systemic HRT. Given the probability of chance finding due to multiple testing, these results will need to be validated in an independent cohort.

None of the polymorphisms we examined were significantly associated with RPFNA atypia using the adjusted p-value, <0.001. However, the top association in this analysis, TFR (Gly^{142}Ser),

reached a borderline significant level (p=0.0025). This polymorphism is a missense variation located in codon 142 of the transferrin receptor gene (TFR) leading to a Glycine (GGC) to Serine (AGC) amino acid change [Evans and Kemp 1997]. The NCBI dbSNP database indicates that the G allele (Glycine) is more common in the general population with genotype frequencies: A/A=16.1%, G/A=54.8%, G/G=29.2%. However, the A allele (Serine) was more common in our cohort with genotype frequencies: A/A=30.7%, G/A=45.9% and G/G=22.9%. We found a higher frequency of RPFNA atypia with increasing numbers of the minor allele or G (Gly) in our cohort. A recent study in type 2 diabetes patients found that increased prevalence of the G allele ran in parallel to increase serum ferritin and increased soluble transferrin receptor levels (Fernández-Real 2010). Transferrin is the chief glycoprotein responsible for iron transport in mammalian blood. It binds to transferrin receptors leading to iron uptake by the cell [Elliot 1993]. Increased levels of transferrin receptors has been reported in proliferating and malignant cells, including breast cancer cells, [Shindelman 1981, Cavanaugh et al. 1999, Hogemann-Savellano et al 2003] with more malignant and aggressive tumors showing higher levels of transferrin receptors (Elliot 1993, Hogeman-Savellano 2003). Most published studies to date have found no associations of TFR genotypes with breast cancer risk when considered separately; however, in combination with the *HFE* C[282]Y polymorphism, *TFR* Ser[142] was associated with increased risk for breast cancer (Beckman et al. 1999).

We observed that the genetic associations with RPFNA atypia differed when we stratified by systemic HRT use, with most SNP showing association in only one of the two strata. However, none of these associations reached statistical significance at $p<0.001$. Given that HRT use differed among those with and without evidence of RPFNA atypia, we would expect significantly different SNP effects (estimated by odds-ratios) for groups stratified by use of systemic HRT at the time of RPFNA. This difference was statistically assessed using gene-environment interaction models. In this study, gene-environment interactions for ECAD (nt-160), TXNRD2 (I340T), CYP19 (R264C), KLK2 (G226W), MLH1 (I219V) were significant at $p<0.05$ but did not remain significant when we adjusted for multiple testing, $p<0.001$.

Interestingly, the most significant genes associated with worse cytomophology and many genes associated with RPFNA atypia as a consequence of systemic HRT use were genes involved in DNA repair pathways. Exogenous estrogens are metabolized by phase 1 enzymes to form catechol estrogens and estrogen quinones [Spink 1992, Zhang 2007] and these metabolites have been shown to form base adducts in DNA [Ding 2003]. These adducts can either be removed by the nucleotide excision repair pathway or can spontaneously depurinate to produce apurinic sites that are then repaired by base excision repair [Cavalier 2006]. Thus, estrogens metabolites directly produce many of the base lesions that ERCC1, XRCC3, RAD51D and RAD23B proteins help repair. It is possible that polymorphisms in DNA repair genes may contribute to individual variation in the ability to recover from estrogen-mediated DNA damage.

The RAD23B Ala[249]Val polymorphism was significantly associated with worse cytomorphology (p=0.0009) and non-significantly associated with evidence of RPFNA atypia as a consequence of HRT use (p=0.035). The RAD23B protein forms a complex with XPC and this complex acts as the earliest DNA damage detector to initiate nucleotide excision repair (NER); it is also specifically involved in global genome NER [Sugasawa 1998]. This pathway is the main mechanism for the repair of bulky DNA adducts and non-bulky DNA lesions that result from oxidative damage [Hutsell 2005]. Several studies have associated genetic variants in RAD23B gene, including the Ala249Val polymorphism, with breast cancer [Mechanic 2006, Perez-Mayoral 2013]. In our cohort, we found that frequency of atypia by RPFNA with the use of systemic HRT was decreased for carriers of the variant T allele and further reduced in women homozygous for the T allele (OR = 0.29, p=0.035). We also identified an inverse correlation between number of homozygous alleles and worse cytomorphology by RPFNA in this cohort (OR = 0.30, p=0.0009). These findings are unexpected, considering a recent study by Perez-Mayoral et al. which showed that women carrying the T allele (Valine) at codon position 249 had decreased DNA repair capacity compared to women with the wild-type C allele (Alanine). If these findings are validated, it would be interesting to

assess the effects of this variation on RAD23B protein function and DNA repair capacity in our cohort.

This study revealed promising associations between certain polymorphisms and worse cytomorphology while on a stable dose of systemic HRT compared to off HRT. However, due to the increased probability of chance findings due to multiple testing, all associations need to be validated in an independent cohort. We have identified 48 potential subjects by querying the HRBC database for post-menopausal women with multiple RPFNA both while currently using systemic HRT and while not currently using systemic HRT. These women have been contacted by phone and asked to submit a saliva sample for genotyping with the original 107 SNPs [excluding 10 SNPs never genotyped] and 57 new SNPs which have been associated with HRT-related breast cancer risk in recent literature [Campa 2011, MARIE 2010, Reding 2009, Hein 2012, Justenhoven 2012, Conway 2007, Prentice 2010, Andersen 2013, Obazee 2013, Cerne 2012, Rebbeck 2009, Lee 2011]. These new candidate polymorphisms include genes related to hormone metabolism (CYP19A1, CYP2C19, CYP3A4, HSD17B1, SRD5A1, AKR1C3), transport (ABCB1, SLCO1B1) and receptor function (FGFR2, KRAS, MAP3K1, ESR1). The original cohort of women (n=55) and the independent cohort (n=48) will be genotyped for the new SNPs. This will serve to update our study to account for relevant findings from recent breast cancer GWAS and candidate gene studies.

The strengths of our study include the use of prospectively collected data regarding hormone therapy use and the use of an outcome variable, evidence of atypia by RPFNA, which divides our cohort into roughly two equal groups (43.58% had evidence of atypia by RPFNA and 56.42% did not have evidence of atypia by RPFNA). The RPFNA procedure allows for both continued monitoring of short-term breast cancer risk with changes in hormonal milieu and is ideally suited to monitor the biological effects of exogenous hormones on post-menopausal breast tissue. We tested a large number of polymorphisms in multiple candidate genes thought to be involved in breast cancer development; and many of these SNPs were suspected to affect protein function [Feigelson 1998, Dunning 2004, Tworoger 2004].Thus, we were well positioned to identify biologically relevant associations between SNPs and increased breast cancer risk

due to HRT use, predicted by the development of cytomorphologic atypia. One limitations of our study was the relatively small sample size and it is possible that some associations and/or interactions were not detected due to insufficient power. It must also be noted that multiple comparisons may have led to chance findings; with 80 SNPs tested in each analysis we would expect approximately one chance finding at $p<0.05$ for each analysis conducted. It is important to consider adjustment for multiple testing ($p< 0.001$) in the interpretation of these study results and to consider that certain associations will be confirmed in an independent cohort.

References:

1. Andersen SW, Trentham-Dietz A, Figueroa JD, Titus LJ, Cai Q, Long J, Hampton JM, Egan KM, Newcomb PA. Breast cancer susceptibility associated with rs1219648 (fibroblast growth factor receptor 2) and postmenopausal hormone therapy use in a population-based United States study. Menopause. 2013 Mar;20(3):354-8. doi: 10.1097/GME.0b013e318268ca46.

2. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, Chlebowski R, Curb D, Gass M, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell A, Jackson R, Johnson KC, Judd H, Kotchen JM, Kuller L, LaCroix AZ, Lane D, Langer RD, Lasser N, Lewis CE, Manson J, Margolis K, Ockene J, O'Sullivan MJ, Phillips L, Prentice RL, Ritenbaugh C, Robbins J, Rossouw JE, Sarto G, Stefanick ML, Van Horn L, Wactawski-Wende J, Wallace R, Wassertheil-Smoller S; Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA. 2004 Apr 14;291(14):1701-12

3. Aston CE1, Ralph DA, Lalo DP, Manjeshwar S, Gramling BA, DeFreese DC, West AD, Branam DE, Thompson LF, Craft MA, Mitchell DS, Shimasaki CD, Mulvihill JJ, Jupe ER. Oligogenic combinations associated with breast cancer risk in women under 53 years of age. Hum Genet. 2005 Feb;116(3):208-21. Epub 2004 Dec 21.

4. Beckman LE, Van Landeghem GF, Sikström C, Wahlin A, Markevärn B, Hallmans G, Lenner P, Athlin L, Stenling R, Beckman L. Interaction between haemochromatosis and transferrin receptor genes in different neoplastic disorders. Carcinogenesis. 1999 Jul;20(7):1231-3.

5. Beral V; Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2003 Aug 9;362(9382):419-27. Lancet. 2003 Oct 4;362(9390):1160.

6. Brandt B, Hermann S, Straif K, Tidow N, Buerger H, Chang-Claude J. Modification of breast cancer risk in young women by a polymorphic sequence in the egfr gene. Cancer Res. 2004 Jan 1;64(1):7-12.

7. Campa D, Kaaks R, Le Marchand L, Haiman CA, Travis RC, Berg CD, Buring JE, Chanock SJ, Diver WR, Dostal L, Fournier A, Hankinson SE, Henderson BE, Hoover RN, Isaacs C, Johansson M, Kolonel LN, Kraft P, Lee IM, McCarty CA, Overvad K, Panico S, Peeters PH, Riboli E, Sanchez MJ, Schumacher FR, Skeie G, Stram DO, Thun MJ, Trichopoulos D, Zhang S, Ziegler RG, Hunter DJ, Lindström S, Canzian F. Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. J Natl Cancer Inst. 2011 Aug 17;103(16):1252-63. doi: 10.1093/jnci/djr265. Epub 2011 Jul 26.

8. Cavalieri E, Rogan E. Catechol quinones of estrogens in the initiation of breast, prostate, and other human cancers: keynote lecture.Ann N Y Acad Sci. 2006 Nov;1089:286-301.

9. Cavanaugh PG1, Jia L, Zou Y, Nicolson GL. Transferrin receptor overexpression enhances transferrin responsiveness and the metastatic growth of a rat mammary adenocarcinoma cell line. Breast Cancer Res Treat. 1999 Aug;56(3):203-17.

10. Cerne JZ, Stegel V, Gersak K, Novakovic S. KRAS rs61764370 is associated with HER2-overexpressed and poorly-differentiated breast cancer in hormone replacement therapy users: a case control study. BMC Cancer. 2012 Mar 22;12:105. doi: 10.1186/1471-2407-12-105.

11. Chlebowski RT, Kuller LH, Prentice RL, Stefanick ML, Manson JE, Gass M, Aragaki AK, Ockene JK, Lane DS, Sarto GE, Rajkovic A, Schenken R, Hendrix SL, Ravdin PM, Rohan TE,

Yasmeen S, Anderson G; WHI Investigators. Breast cancer after use of estrogen plus progestin in postmenopausal women. N Engl J Med. 2009 Feb 5;360(6):573-87. doi:10.1056/NEJMoa0807684.

12. Conway K, Parrish E, Edmiston SN, Tolbert D, Tse CK, Moorman P, Newman B, Millikan RC. Risk factors for breast cancer characterized by the estrogen receptor alpha A908G (K303R) mutation. Breast Cancer Res. 2007;9(3):R36.

13. Coughlin SS1, Piper M. Genetic polymorphisms and risk of breast cancer. Cancer Epidemiol Biomarkers Prev. 1999 Nov;8(11):1023-32.

14. Diergaarde B, Potter JD, Jupe ER, Manjeshwar S, Shimasaki CD, Pugh TW, DeFreese DC, Gramling BA, Evans I, White E. Polymorphisms in genes involved in sex hormone metabolism, estrogen plus progestin hormone therapy use, and risk of postmenopausal breast cancer. Cancer Epidemiology Biomarkers and Prevention 2008;17:1751-1759

15. Ding S1, Shapiro R, Geacintov NE, Broyde S. Conformations of stereoisomeric base adducts to 4-hydroxyequilenin. Chem Res Toxicol. 2003 Jun;16(6):695-707.

16. Dunning AM, Dowsett M, Healy CS, et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. J Natl Cancer Inst 2004;96:936–945.

17. Dunning AM, Healy CS, Pharaoh PDP, Teare MD, Ponder BAJ, Easton DF. A systematic overview of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:843– 854.

18. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. N Engl J Med. 1985 Jan 17;312(3):146-51

19. Elliott RL1, Elliott MC, Wang F, Head JF. Breast carcinoma and the role of iron metabolism. A cytochemical, tissue culture, and ultrastructural study. Carcinogenesis 1993 Nov 30;698:159-66.

20. Evans P, Kemp J. Exon/intron structure of the human transferrin receptor gene. Gene. 1997 Oct 15;199(1-2):123-31.

21. Fabian CJ, Kimler BF, Donnelly JE, Sullivan DK, Klemp JR, Petroff BK, Phillips TA, Metheny T, Aversman S, Yeh HW, Zalles CM, Mills GB, Hursting SD. Favorable modulation of benign breast tissue and serum risk biomarkers is associated with > 10 % weight loss in postmenopausal women. Breast Cancer Res Treat. 2013 Nov;142(1):119-32. doi: 10.1007/s10549-013-2730-8. Epub 2013 Oct 19.

22. Fabian CJ, Kimler BF, Mayo MS, Khan SA. Breast-tissue sampling for risk assessment and prevention. Endocr Relat Cancer. 2005 Jun;12(2):185-213. Review.

23. Fabian CJ, Kimler BF, Zalles CM, Khan QJ, Mayo MS, Phillips TA, Simonsen M, Metheny T, Petroff BK. Reduction in proliferation with six months of letrozole in women on hormone replacement therapy. Breast Cancer Res Treat. 2007 Nov;106(1):75-84. Epub 2007 Jan 13.

24. Fabian CJ, Kimler BF, Zalles CM, Klemp JR, Kamel S, Zeiger S, Mayo MS. Short-term breast cancer prediction by random periareolar fine-needle aspiration cytology and the Gail risk model. J Nat Can Inst. 2000 Aug; 92(15); 1217-1227.

25. Fabian CJ, Kimler BF. Use of biomarkers for breast cancer risk assessment and prevention. J Steroid Biochem Mol Biol. 2007 Aug-Sep;106(1-5):31-9. Epub 2007 May 24.

26. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17α gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. Cancer Res 1998;58:585–587. [PubMed: 9485002]

27. Fernández-Real JM1, Mercader JM, Ortega FJ, Moreno-Navarrete JM, López-Romero P, Ricart W. Ann N Y Acad Sci. Transferrin receptor-1 gene polymorphisms are associated with type 2 diabetes. Eur J Clin Invest. 2010 Jul;40(7):600-7. doi: 10.1111/j.1365-2362.2010.02306.x. Epub 2010 May 23.

28. Fletcher O, Johnson N, Orr N, Hosking FJ, Gibson LJ, Walker K, Zelenika D, Gut I, Heath S, Palles C, Coupland B, Broderick P, Schoemaker M, Jones M, Williamson J, Chilcott-Burns S, Tomczyk K, Simpson G, Jacobs KB, Chanock SJ, Hunter DJ, Tomlinson IP, Swerdlow A, Ashworth A, Ross G, dos Santos Silva I, Lathrop M, Houlston RS, Peto J. Novel breast cancer

susceptibility locus at 9q31.2: results of a genome-wide association study. J Natl Cancer Inst. 2011 Mar 2;103(5):425-35. doi: 10.1093/jnci/djq563. Epub 2011 Jan 24.

29. Gaikwad NW, Yang L, Muti P, Meza JL, Pruthi S, Ingle JN, et al. The molecular etiology of breast cancer: evidence from biomarkers of risk. Int J Cancer. 2008;122:1949-57.

30. Gaikwad NW, Yang L, Pruthi S, Ingle JN, Sandhu N, Rogan EG, et al. Urine biomarkers of risk in the molecular etiology of breast cancer. Breast Cancer (Auckl). 2009;3:1-8.

31. Gail MH1, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, Mulvihill JJ. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst. 1989 Dec 20;81(24):1879-86.

32. Haiman CA, Brown M, Hankinson SE, Spiegelman D, Colditz GA, Willett WC, Kantoff PW, Hunter DJ. The androgen receptor CAG repeat polymorphism and risk of breast cancer in the Nurses' Health Study. Cancer Res. 2002 Feb 15;62(4):1045-9.

33. Haiman CA, Hankinson SE, De Vivo I, Guillemette C, Ishibe N, Hunter DJ, Byrne C. Polymorphisms in steroid hormone pathway genes and mammographic density. Breast Cancer Res Treat. 2003 Jan;77(1):27-36.

34. 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 Feb;11(2):215-6. No abstract available

35. Hartmann LC1, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C,Tlsty T, Vachon CM, Melton LJ 3rd, Visscher DW. Benign breast disease and the risk of breast cancer. N Engl J Med. 2005 Jul 21;353(3):229-37.

36. Hein R, Abbas S, Seibold P, Salazar R, Flesch-Janys D, Chang-Claude J. Polymorphism Thr160Thr in SRD5A1, involved in the progesterone metabolism, modifies postmenopausal breast cancer risk associated with menopausal hormone therapy. Breast Cancer Res Treat. 2012 Jan;131(2):653-61. doi: 10.1007/s10549-011-1772-z. Epub 2011 Sep 23

37. Hersh AL1, Stefanick ML, Stafford RS. National use of postmenopausal hormone therapy: annual trends and response to recent evidence. JAMA. 2004 Jan 7;291(1):47-53.

38. Högemann-Savellano D1, Bos E, Blondet C, Sato F, Abe T, Josephson L, Weissleder R, Gaudet J, Sgroi D, Peters PJ, Basilion JP. The transferrin receptor: a potential molecular imaging marker for human cancer. Neoplasia. 2003 Nov-Dec;5(6):495-506.

39. Hoogerbrugge N, Bult P, deWidt-Levert LM, et al. High prevalence of premalignant lesions in prophylactically removed breasts from women at hereditary risk for breast cancer. JClin Oncol 2003;21:41^5.

40. Horwitz K1, Clarke C. Estrogens and progestins in mammary development and neoplasia. Introduction. J Mammary Gland Biol Neoplasia. 1998 Jan;3(1):1-2.

41. Hutsell SQ1, Sancar A. Nucleotide excision repair, oxidative damage, DNA sequence polymorphisms, and cancer treatment. Clin Cancer Res. 2005 Feb 15;11(4):1355-7.

42. Johansen OE1, Qvigstad E. Rationale for low-dose systemic hormone replacement therapy and review of estradiol 0.5 mg/NETA 0.1 mg. Adv Ther. 2008 Jun;25(6):525-51. doi: 10.1007/s12325-008-0070-6.

43. Jupe ER, Knowlton NS, Pugh TW, Ralph DA, Manjeshwar S, DeFreese DC, Blaylock L, Gramling BA, Shimasaki CD. Individualized breast cancer risk prediction using the OncoVue(R) model. AACR Meeting Abstracts 2008:1913. Presented at: 2008 Annual Meeting of the American Association for Cancer Research in San Diego, CA, April 2008.

44. Jupe ER, Ralph DA, Manjeshwar S, Knowlton NS, Pugh TW, DeFreese DC, Gramling BA, Shimasaki CD. The OncoVue® model for predicting breast cancer risk. Presented at: 30th Annual San Antonio Breast Cancer Symposium in San Antonio, TX, December 2007

45. Justenhoven C, Obazee O, Winter S, Couch FJ, Olson JE, Hall P, Hannelius U, Li J, Humphreys K, Severi G, Giles G, Southey M, Baglietto L, Fasching PA, Beckmann MW, Ekici AB, Hamann U, Baisch C, Harth V, Rabstein S, Lotz A, Pesch B, Brüning T, Ko YD, Brauch H. The postmenopausal hormone replacement therapy-related breast cancer risk is decreased in women

carrying the CYP2C19*17 variant. Breast Cancer Res Treat. 2012 Jan;131(1):347-50. doi: 10.1007/s10549-011-1827-1. Epub 2011 Oct 26.

46. Kawase T, Matsuo K, Suzuki T, Hiraki A, Watanabe M, Iwata H, Tanaka H, Tajima K. FGFR2 intronic polymorphisms interact with reproductive risk factors of breast cancer: results of a case control study in Japan. Int J Cancer. 2009 Oct 15;125(8):1946-52. doi: 10.1002/ijc.24505.

47. Lee E, Schumacher F, Lewinger JP, Neuhausen SL, Anton-Culver H, Horn-Ross PL, Henderson KD, Ziogas A, Van Den Berg D, Bernstein L, Ursin G. The association of polymorphisms in hormone metabolism pathway genes, menopausal hormone therapy, and breast cancer risk: a nested case-control study in the California Teachers Study cohort.

48. Li CI, Daling JR, Haugen KL, Tang MT, Porter PL, Malone KE. Use of menopausal hormone therapy and risk of ductal and lobular breast cancer among women 55-74 years of age. Breast Cancer Res Treat. 2014 Jun;145(2):481-9.

49. Li Z1, Zhang Y2, Ying X1, Song J3, Zhang R4, Li Z3, Chen H1, Ye P1, Shen Y1, Pan W1, Chen Z1. The association between RAD23B Ala249Val polymorphism and cancer susceptibility: evidence from a meta-analysis. PLoS One. 2014 Mar 18;9(3):e91922.

50. Liede A1, Zhang W, De Leon Matsuda ML, Tan A, Narod SA. Androgen receptor gene polymorphism and breast cancer susceptibility in The Philippines. Cancer Epidemiol Biomarkers Prev. 2003 Sep;12(9):848-52.

51. Listgarten J1, Damaraju S, Poulin B, Cook L, Dufour J, Driga A, Mackey J, Wishart D, Greiner R, Zanke B. Predictive models for breast cancer susceptibility from multiple single nucleotide polymorphisms. Clin Cancer Res. 2004 Apr 15;10(8):2725-37.

52. Manson JE, Chlebowski RT, Stefanick ML, Aragaki AK, Rossouw JE, Prentice RL, Anderson G, Howard BV, Thomson CA, LaCroix AZ, Wactawski-Wende J, Jackson RD, Limacher M, Margolis KL, Wassertheil-Smoller S, Beresford SA, Cauley JA, Eaton CB, Gass M, Hsia J, Johnson KC, Kooperberg C, Kuller LH, Lewis CE, Liu S, Martin LW, Ockene JK, O'Sullivan MJ, Powell LH, Simon MS, Van Horn L, Vitolins MZ, Wallace RB. Menopausal hormone

therapy and health outcomes during the intervention and extended post-stopping phases of the Women's Health Initiative randomized trials. JAMA. 2013 Oct 2;310(13):1353-68. doi: 10.1001/jama.2013.278040.

53. MARIE-GENICA Consortium on Genetic Susceptibility for Menopausal Hormone Therapy Related Breast Cancer Risk. Polymorphisms in genes of the steroid receptor superfamily modify postmenopausal breast cancer risk associated with menopausal hormone therapy. Int J Cancer. 2010 Jun 15;126(12):2935-46. doi: 10.1002/ijc.24892.

54. MARIE-GENICA Consortium on Genetic Susceptibility for Menopausal Hormone Therapy Related Breast Cancer Risk. Postmenopausal estrogen monotherapy-associated breast cancer risk is modified by CYP17A1_-34_T>C polymorphism. Breast Cancer Res Treat. 2010 Apr;120(3):737-44. doi: 10.1007/s10549-009-0490-2. Epub 2009 Aug 12.

55. MARIE-GENICA Consortium on Genetic Susceptibility for Menopausal Hormone Therapy Related Breast Cancer Risk. Genetic polymorphisms in phase I and phase II enzymes and breast cancer risk associated with menopausal hormone therapy in postmenopausal women. Breast Cancer Res Treat. 2010 Jan;119(2):463-74. doi: 10.1007/s10549-009-0407-0. Epub 2009 May 8.

56. Masood S. Assessment of prognostic factors in breast fine-needle aspirates. Am J Clin Pathol. 2000 May;113(5 Suppl 1):S84-96. Review.

57. Mechanic LE, Millikan RC, Player J, de Cotret AR, Winkel S, et al. (2006) Polymorphisms in nucleotide excision repair genes, smoking and breast cancer in African Americans and whites: a population-based case-control study. Carcinogenesis 27: 1377–1385.

58. Mizuiri S, Hemmi H, Kumanomidou H, Iwamoto M, Miyagi M et al. (2001) Angiotensin-converting enzyme (ACE) I/D genotype and renal ACE gene expression. Kidney Int 60: 1124–1130.10.1046/j.1523-1755.2001.0600031124.x PubMed: 11532108.

59. Modena MG1, Sismondi P, Mueck AO, Kuttenn F, Lignières Bd, Verhaeghe J, Foidart JM, Caufriez A, Genazzani AR; TREAT. New evidence regarding hormone replacement therapies is

urgently required transdermal postmenopausal hormone therapy differs from oral hormone therapy in risks and benefits. Maturitas. 2005 Sep 16;52(1):1-10.

60. Nickels et al. Evidence of gene-environment interactions between common breast cancer susceptibility loci and established environmental risk factors. PLoS Genet. 2013;9(3):e1003284. doi: 10.1371/journal.pgen.1003284. Epub 2013 Mar 27.

61. Nielsen M,Thomsen JL, Primdahl S, Dyreborg U,Andersen JA. Breast cancer and atypia among young and middle-aged women: a study of 110 medicolegal autopsies. BrJCancer1987;56:814^9.

62. North American Menopause Society. The 2012 hormone therapy position statement of: The North American Menopause Society. Menopause. 2012 Mar;19(3):257-71.

63. Pawlak KJ1, Wiebe JP. Regulation of estrogen receptor (ER) levels in MCF-7 cells by progesterone metabolites. J Steroid Biochem Mol Biol. 2007 Nov-Dec;107(3-5):172-9.

64. Pearlman MD1, Griffin JL. Benign breast disease. Obstet Gynecol. 2010 Sep;116(3):747-58.

65. Perez-Mayoral J, Pacheco-Torres AL, Morales L, Acosta-Rodriguez H, Matta JL, et al. (2013) Genetic polymorphisms in RAD23B and XPC modulate DNA repair capacity and breast cancer risk in Puerto Rican women. Mol Carcinog 52. Suppl 1: 127–138.

66. Poutanen M1, Isomaa V, Peltoketo H, Vihko R. Role of 17 beta-hydroxysteroid dehydrogenase type 1 in endocrine and intracrine estradiol biosynthesis. J Steroid Biochem Mol Biol. 1995 Dec;55(5-6):525-32.

67. Prentice RL, Huang Y, Hinds DA, Peters U, Pettinger M, Cox DR, Beilharz E, Chlebowski RT, Rossouw JE, Caan B, Ballinger DG. Variation in the FGFR2 gene and the effects of postmenopausal hormone therapy on invasive breast cancer. Cancer Epidemiol Biomarkers Prev. 2009 Nov;18(11):3079-85. doi: 10.1158/1055-9965.EPI-09-0611.

68. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. < http://www.R-project.org/>

69. Ralph, DA, Zhao, LP, Aston, CE, Manjeshwar, S, Pugh, TW, DeFreese, DC, Gramling, BA, Shimasaki, CD, Jupe, ER. Age-specific association of steroid hormone pathway gene polymorphisms with breast cancer risk. CANCER (2007) May 15; 109 (10):1940-1948.

70. Rebbeck TR, Troxel AB, Shatalova EG, Blanchard R, Norman S, Bunin G, Demichele A, Schinnar R, Berlin JA, Strom BL. Lack of effect modification between estrogen metabolism genotypes and combined hormone replacement therapy in postmenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2007 Jun;16(6):1318-20.

71. Reding KW, Li CI, Weiss NS, Chen C, Carlson CS, Duggan D, Thummel KE, Daling JR, Malone KE. Genetic variation in the progesterone receptor and metabolism pathways and hormone therapy in relation to breast cancer risk. Am J Epidemiol. 2009 Nov 15;170(10):1241-9. doi: 10.1093/aje/kwp298. Epub 2009 Oct 21.

72. Reding KW, Weiss NS, Chen C, Li CI, Carlson CS, Wilkerson HW, Farin FM, Thummel KE, Daling JR, Malone KE. Genetic polymorphisms in the catechol estrogen metabolism pathway and breast cancer risk.Cancer Epidemiol Biomarkers Prev. 2009 May;18(5):1461-7. doi: 10.1158/1055-9965.EPI-08-0917. Epub 2009 Apr 21.

73. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002 Jul 17;288(3):321-33.

74. Shindelman JE, Ortmeyer AE, Sussman HH. Demonstration of the transferrin receptor in human breast cancer tissue. Potential marker for identifying dividing cells. Int J Cancer. 1981 Mar 15;27(3):329-34.

75. Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature menopause or early menopause: long-term health consequences. Maturitas. 2010 Feb;65(2):161-6. doi: 10.1016/j.maturitas.2009.08.003. Epub 2009 Sep 5. Review.

76. Sood R, Faubion SS, Kuhle CL, Thielen JM, Shuster LT. Prescribing menopausal hormone therapy: an evidence-based approach. Int J Womens Health. 2014 Jan 11;6:47-57. eCollection 2014. Review.

77. Spink DC, Eugster HP, Lincoln DW 2nd, Schuetz JD, Schuetz EG, Johnson JA, Kaminsky LS, Gierthy JF. 17 beta-estradiol hydroxylation catalyzed by human cytochrome P450 1A1: a comparison of the activities induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in MCF-7 cells with those from heterologous expression of the cDNA. Arch Biochem Biophys. 1992 Mar;293(2):342-8.

78. Sugasawa K1, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP, Hanaoka F, Bootsma D, Hoeijmakers JH. Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Mol Cell. 1998 Aug;2(2):223-32.

79. Travis RC, Reeves GK, Green J, Bull D, Tipper SJ, Baker K, Beral V, Peto R, Bell J, Zelenika D, Lathrop M; Million Women Study Collaborators. Gene-environment interactions in 7610 women with breast cancer: prospective evidence from the Million Women Study. Lancet. 2010 Jun 19;375(9732):2143-51. doi: 10.1016/S0140-6736(10)60636-8. Epub 2010 Jun 3.

80. Tworoger SS, Chubak J, Aiello EJ, et al. Association of CYP17, CYP19, CYP1B1, and COMT polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:94–101. [PubMed: 14744739]

81. Wiebe JP, Souter L, Zhang G. Dutasteride affects progesterone metabolizing enzyme activity/expression in human breast cell lines resulting in suppression of cell proliferation and detachment.J Steroid Biochem Mol Biol. 2006 Aug;100(4-5):129-40. Epub 2006 Jun 27.

82. Wiebe JP. Progesterone metabolites in breast cancer. Endocr Relat Cancer. 2006 Sep;13(3):717-38. Review.

83. Yager JD1, Davidson NE. Estrogen carcinogenesis in breast cancer. N Engl J Med. 2006 Jan 19;354(3):270-82.

84. Yue W, Santen RJ, Wang JP, Li Y, Verderame MF, Bocchinfuso WP, Korach KS, Devanesan P, Todorovic R, Rogan EG, Cavalieri EL. Genotoxic metabolites of estradiol in breast: potential mechanism of estradiol induced carcinogenesis. J Steroid Biochem Mol Biol. 2003 Sep;86(3-5):477-86. Review.

85. Zalles CM1, Kimler BF, Simonsen M, Clark JL, Metheny T, Fabian CJ. Comparison of cytomorphology in specimens obtained by random periareolar fine needle aspiration and ductal lavage from women at high risk for development of breast cancer. Breast Cancer Res Treat. 2006 May;97(2):191-7. Epub 2005 Dec 2.

86. Zhang Y, Gaikwad NW, Olson K, Zahid M, Cavalieri EL, Rogan EG. Cytochrome P450 isoforms catalyze formation of catechol estrogen quinones that react with DNA. Metabolism. 2007 Jul;56(7):887-94.

87. Zhao M, Lewis R, Gustafson DR, Wen WQ, Cerhan JR, Zheng W. No apparent association of GSTP1 A(313)G polymorphism with breast cancer risk among postmenopausal Iowa women. Cancer Epidemiol Biomarkers Prev. 2001 Dec;10(12):1301-2.

88. Zheng T, Holford TR, Zahm SH, Owens PH, Boyle P, Zhang Y, Zhang B, Wise JP Sr, Stephenson LP, Ali-Osman F. Glutathione S-transferase M1 and T1 genetic polymorphisms, alcohol consumption and breast cancer risk. Br J Cancer. 2003 Jan 13;88(1):58-62.

89. Zumoff B. Does postmenopausal estrogen administration increase the risk of breast cancer? Contributions of animal, biochemical, and clinical investigative studies to a resolution of the controversy. Proc Soc Exp Biol Med. 1998 Jan;217(1):30-7.