

Association of Variants in Candidate Genes with Lipid Profiles in Women with Early Breast Cancer on Adjuvant Aromatase Inhibitor Therapy

Cesar A. Santa-Maria¹, Amanda Blackford¹, Anne T. Nguyen², Todd C. Skaar², Santosh Philips², Steffi Oesterreich³, James M. Rae⁴, Zeruesenay Desta², Jason Robarge², Norah Lynn Henry⁴, Anna M. Storniolo², Daniel F. Hayes⁴, Roger S. Blumenthal¹, Pamela Ouyang¹, Wendy S. Post¹, David A. Flockhart², Vered Stearns¹, and Consortium on Breast Cancer Pharmacogenomics (COBRA)

Abstract

Purpose: Aromatase inhibitors can exert unfavorable effects on lipid profiles; however, previous studies have reported inconsistent results. We describe the association of single-nucleotide polymorphisms (SNP) in candidate genes with lipid profiles in women treated with adjuvant aromatase inhibitors.

Experimental Design: We conducted a prospective observational study to test the associations between SNPs in candidate genes in estrogen signaling and aromatase inhibitor metabolism pathways with fasting lipid profiles during the first 3 months of aromatase inhibitor therapy in postmenopausal women with early breast cancer randomized to adjuvant letrozole or exemestane. We performed genetic association analysis and multivariable linear regressions using dominant, recessive, and additive models.

Results: A total of 303 women had complete genetic and lipid data and were evaluable for analysis. In letrozole-treated patients, SNPs in *CYP19A1*, including rs4646, rs10046, rs700518, rs749292, rs2289106, rs3759811, and rs4775936 were significantly associated with decreases in triglycerides by 20.2 mg/dL and 39.3 mg/dL ($P < 0.00053$), respectively, and with variable changes in high-density lipoprotein (HDL-C) from decreases by 4.2 mg/dL to increases by 9.8 mg/dL ($P < 0.00053$).

Conclusions: Variants in *CYP19A1* are associated with decreases in triglycerides and variable changes in HDL-C in postmenopausal women on adjuvant aromatase inhibitors. Future studies are needed to validate these findings, and to identify breast cancer survivors who are at higher risk for cardiovascular disease with aromatase inhibitor therapy. *Clin Cancer Res*; 22(6); 1395–402. ©2015 AACR.

Introduction

Compared with tamoxifen, adjuvant aromatase inhibitors reduce the risk of recurrence and death in postmenopausal women with hormone receptor-positive breast cancer and are an integral component of adjuvant therapy, considered to be a standard of care (1, 2). At the same time, several studies are investigating the role of extended duration of endocrine therapy, particularly aromatase inhibitor therapy in postmenopausal

women, which may prolong aromatase inhibitor-associated toxicities (3).

While all three approved third-generation aromatase inhibitors (exemestane, anastrozole, letrozole) reduce the concentrations of effective circulating estrogens by inhibiting aromatase, and improve breast cancer outcomes, individual women may experience different toxicities with different aromatase inhibitors (4, 5). Among other detrimental effects, aromatase inhibitors may exert unfavorable effects on lipid profiles in treated women. In contrast, tamoxifen appears to exert a favorable effect on lipid profiles (6).

Previous studies investigating the effects of different aromatase inhibitors on lipid profiles have demonstrated mixed results (2, 7–14). Because of the intricate biologic relationship of estrogen with lipid profiles and metabolism, the mixed results from previous studies may be explained by heterogeneity in genes involved in estrogen signaling, and estrogen and aromatase inhibitor metabolism (15, 16). For example, certain polymorphisms in the estrogen receptor alpha (*ESR1*) gene have been shown to be associated with increased low density lipoprotein cholesterol (LDL-C) and triglyceride concentrations in women treated with aromatase inhibitors (17). Developing models for cardiovascular risk factors in breast cancer survivors is particularly relevant because many women are cured after a diagnosis of early-stage breast cancer and are expected to become long-term survivors (18). We report the results of a planned subset analysis

¹Johns Hopkins University School of Medicine, Baltimore, Maryland.

²Indiana University School of Medicine, Indianapolis, Indiana. ³University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania. ⁴University of Michigan Comprehensive Cancer Center, Ann Arbor, Michigan.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Current address for C.A. Santa-Maria: Department of Medicine, Division of Oncology, Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Corresponding Author: Vered Stearns, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Bldg., 1650 Orleans St., Room: 144, Baltimore, MD 21231-1000. Phone: 443-287-6489; Fax: 410-614-4073; E-mail: vstearn1@jhmi.edu

doi: 10.1158/1078-0432.CCR-15-1213

©2015 American Association for Cancer Research.

Translational Relevance

Aromatase inhibitors are a cornerstone of therapy for postmenopausal women with early breast cancer. However, there is a concern that negative effects on lipid profiles can potentially increase cardiovascular risk. While this relationship has not been clear to date, pharmacogenomics may help identify which patients may or may not be at risk. This study investigates whether single-nucleotide polymorphisms (SNP) in candidate genes related to estrogen and aromatase inhibitor signaling and metabolism predict changes to lipid profiles in aromatase inhibitor–treated women. Our findings that SNPs in the *CYP19A1* gene are associated with modulation of high-density lipoprotein and triglycerides demonstrate the powerful effects of pharmacogenomics. Future studies may ultimately lead to personalized and improved management of cardiovascular risk factors.

of a prospective randomized trial that evaluated the association of single-nucleotide polymorphisms (SNP) in candidate genes involved in estrogen and aromatase inhibitor metabolism with lipid profiles in postmenopausal women receiving adjuvant aromatase inhibitors.

Materials and Methods

Study design

The data in this report are derived from a planned sub-analysis of the Exemestane and Letrozole Pharmacogenomics (ELPh) study, a large prospective multicenter randomized observational open-label trial evaluating the effects of two years of therapy with either letrozole or exemestane in postmenopausal women with early-stage breast cancer on a variety of biomarkers of estrogen activity and potential aromatase inhibitor–related effects. The parent study has been described in detail previously (19).

Eligible participants were postmenopausal women with a biopsy-proven hormone receptor-positive ductal carcinoma *in situ* (DCIS) or stage I–III breast cancer either considering primary hormone therapy with aromatase inhibitors or in sequence with tamoxifen. Participants must have completed planned breast surgery, adjuvant or neoadjuvant chemotherapy, and adjuvant radiotherapy. Patients previously treated with aromatase inhibitors, with a history of bilateral mastectomy or radiotherapy to the contralateral breast, or with a history of gynecologic malignancies were excluded.

Participants were randomized in a stratified fashion based on prior chemotherapy, prior tamoxifen, and bisphosphonate use. Participants were randomly assigned to receive either exemestane (Aromasin; supplied by Pfizer, Inc., 25 mg orally per day) or letrozole (Femara; supplied by Novartis Pharmaceuticals Corporation, 2.5 mg orally per day) and followed for 2 years. Whole blood was collected at baseline and deoxyribonucleic acid (DNA) was isolated for SNP genotyping in candidate genes. Serum lipid profiles were collected at baseline prior to initiating aromatase inhibitor therapy and following 3 months of treatment.

Participants were excluded from the analysis if they did not undergo genotyping, if lipid data were not available either at baseline or 3 months, if they discontinued or crossed over to a different aromatase inhibitor during the first 3 months, or if they

were not fasting at both baseline and 3 months. Participants taking lipid-altering medications (including statins, fibrates, and/or ezetimibe) during the first 3 months were excluded from the primary analysis but included in a subanalysis. The derivation of the cohort is described in Fig. 1.

Participants were recruited from breast cancer clinics at participating sites including the University of Michigan Comprehensive Cancer Center, the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (Baltimore, MD), and the Melvin and Bren Simon Cancer Center at the Indiana University School of Medicine (Indianapolis, IN). The study protocol was approved by Institutional Review Boards at all sites and enrolled subjects provided signed written informed consent.

Sample collection

Sample collection and measurement of lipid profiles and estradiol. Venous blood samples for lipid panel analyses were collected at baseline and 3 months after fasting overnight for at least 12 hours.

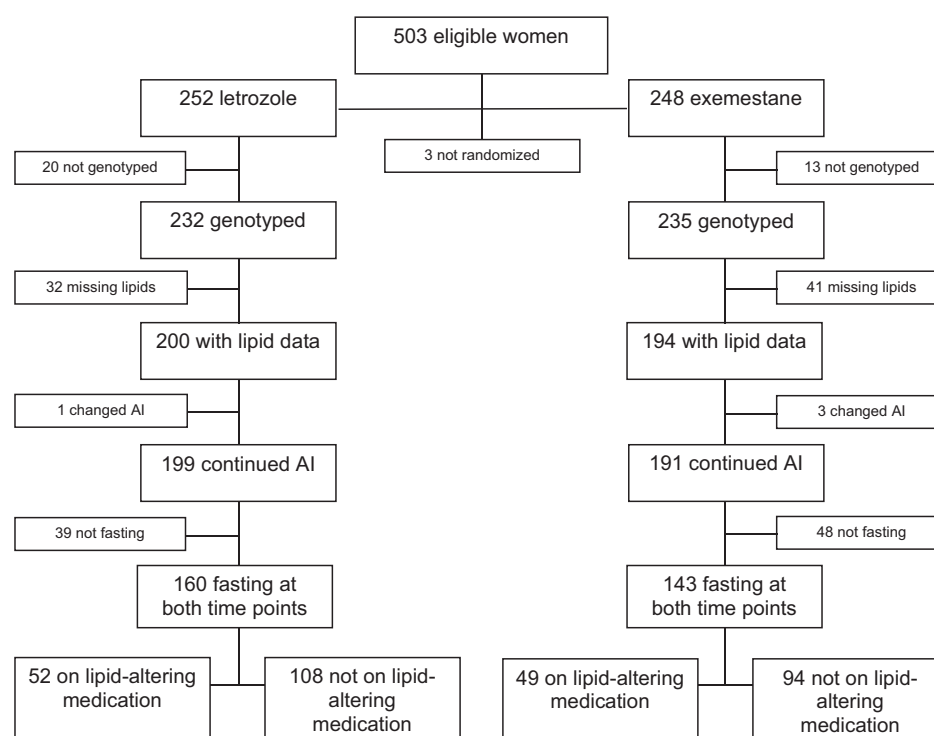
Serum total cholesterol, high density lipoprotein cholesterol (HDL-C), LDL-C, and triglycerides were analyzed by standardized enzymatic methodology at Clinical Laboratory Improvement Amendments (CLIA) certified laboratories at the University of Michigan Health System, the Johns Hopkins Medical Laboratories, and the Indiana University Health Pathology Laboratory.

Plasma estradiol was analyzed using an ultrasensitive gas chromatography and tandem mass spectrometry assay, as previously described (20). The lower limits of quantification were 1.25 or 0.625 pg/mL, as determined by calibration curves run with plasma sample batches. In the cohort analyzed, none of the baseline values were below the lower limit of quantification. As the majority of plasma estradiol concentrations at month 3 were below the lower limit of quantification ($n = 278$ of the analyzed cohort), these concentrations were set to 0 for data analysis.

Sample collection and processing of candidate genes. Whole blood was collected at enrollment of each study participant. DNA was extracted from whole blood using Qiafilter Blood DNA Maxi kits (Qiagen, Inc.).

Candidate genes were selected during protocol development based on their known roles in aromatase inhibitor drug metabolism [cytochrome P450 2A6 (*CYP2A6*), and 3A5 (*CYP3A5*)], estrogen metabolism [Armadillo Repeat gene deleted in Velo-Cardio-Facial syndrome (*ARVCF*)], catechol-O-methyltransferase (*COMT*), cytochrome P450 19A1 (*CYP19A1*), *ESR1* and 2, progesterone receptor (*PGR*)], coregulation of the estrogen receptor, [ER, E1a binding protein p300 (*EP300*), enhancer of zeste 2 polycomb repressive complex 2 (*EZH2*), nuclear receptor coactivator 1 (*NCOA1*), 2 (*NCOA2*), 3 (*NCOA3*), nuclear receptor corepressor 1 (*NCOR1*), 2 (*NCOR2*), nuclear receptor interaction protein (*NRIP*), proline-, glutamic acid-, and leucine-rich protein-1 (*PELPI*), and neuropeptide signaling (5-hydroxytryptamine receptor 1A (*HTR1A*), 2A (*HTR2A*), serotonin transporter gene (*SCL6A4*), hypocretin (orexin) neuropeptide precursor (*HCRT*), hypocretin receptor type 1 (*HCRT1*), and 2 (*HCRT2*)]. Genes related to neuropeptide signaling were not included in the analysis as they were not felt to be relevant to lipid metabolism; this determination was made before the statistical analysis. Genotyping for all SNPs was performed using the BioTrove OpenArray platform (Applied Biosystems, Inc.). Genotype quality control was performed before genetic association analysis, by randomly

Figure 1.
Consort diagram.



selecting 10% of the samples and regenotyping them to validate results. *CYP2A6* and *CYP3A5* were genotyped using allelic-discrimination TaqMan SNP genotyping assays (Applied Biosystems) as previously described (21).

The Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria were used for reporting these biomarker results (22).

Statistical analysis

Characteristics of participants at baseline were summarized and described between those on lipid-altering medications and those who were not using Fisher exact test and Wilcoxon rank sum tests. Changes in lipid parameters from baseline to 3 months following treatment initiation were summarized overall and by treatment groups and tested for differences with paired *t* tests. Data analysis was performed using participants who underwent genotyping and follow-up for lipid measurements and who fulfilled criteria for this analysis as described above. For 143/145 SNPs, the minor and major alleles were identified and used to classify participants for each model type (recessive, dominant, additive). *CYP2A6* and *CYP3A5* were genotyped using different assays as described above, therefore these were analyzed separately. SNPs with a homozygous (defined as two copies of the minor allele) genotype frequency of less than 5 participants were excluded from analyses, leaving 92 of 143 SNPs for analysis.

A multivariable linear regression model was run for each combination of the 143 SNPs, SNP genotype (recessive, dominant or additive), 7 treatment subgroups (all patients, all patients on letrozole, patients on letrozole ± lipid-altering medication, all patients on exemestane, patients on exemestane ± lipid-altering medication), and 4 lipid parameters, a multivariable linear regression model was run. There were 12,012 potential models (84

subgroups by 143 SNPs). Eliminating models where there were fewer than 5 participants with the homozygous genotype resulted in 5,796 models across 92 of the 94 SNPs, excluding *CYP2A6* and *CYP3A5*. For each model, the dependent variable was in the difference in the lipid parameter, and independent variables were SNP genotype, participant age, body mass index (BMI), race, change in estradiol levels from baseline to 3 months, and indicators of prior hormonal therapy and tamoxifen use. For each of these models, we specifically tested for Hardy-Weinberg equilibrium, calculated the minor allele frequency, and calculated the additional difference in lipids attributable to the SNP with its corresponding *P* value. The same multivariable linear regression modeling approach as described for the 143 SNPs was used to analyze *CYP2A6* and *CYP3A5*, this resulted in 84 models [3 gene classifications (*CYP3A5* expressed vs. not, *CYP2A6* normal vs. intermediate/slow, *CYP2A6* normal/intermediate versus slow) by 7 patient subgroups by 4 lipid outcomes]. For the models that were run on the entire cohort, we also performed a test for interaction between the SNP and treatment arm on the change in lipid parameter. To adjust for multiple comparisons, models with a resulting Bonferroni-corrected *P* value < 0.00053 (= 0.05/94) were considered significant.

Results

Study population

Of the 503 evaluable participants enrolled in the ELPh trial, 303 met eligibility criteria for this analysis (Fig. 1). Of these, 101 participants were taking lipid-altering medications, and were analyzed separately.

The median age of the overall randomized cohort (*n* = 500) was 59 years (range 35 to 89), which consisted of 441 (88%)

Santa-Maria et al.

Table 1. Characteristics of patients enrolled and randomized in ELPh trial, and subdivided by those included in the analysis

	Entire cohort (<i>n</i> = 500)	Cohort Analyzed (<i>n</i> = 303)	Participants not on LAM (<i>n</i> = 202)	Participants on LAM (<i>n</i> = 101)	<i>P</i> value
Age, median (range)	59 (35, 89)	59 (35, 84)	58 (38, 83)	61 (35, 84)	0.002
BMI at diagnosis, median (range)	29 (17.7, 55.9)	29.3 (18.4, 55.9)	28.6 (18.4, 53.4)	30.5 (20.7, 55.9)	0.02
Race, <i>n</i> (%)					
White	441 (88)	263 (87)	176 (87)	87 (86)	0.81
African American	46 (9)	34 (11)	21 (10)	13 (13)	
Asian	12 (2)	5 (2)	4 (2)	1 (1)	
Other	1 (0)	1 (0)	1 (0)	0 (0)	
Treatment arm					
Exemestane	248 (50)	143 (47)	94 (47)	49 (49)	0.81
Letrozole	252 (50)	160 (53)	108 (53)	52 (51)	
Prior chemotherapy, <i>n</i> (%)	228 (46)	131 (43)	98 (49)	33 (33)	0.01
Prior tamoxifen use, <i>n</i> (%)	184 (37)	100 (33)	70 (35)	30 (30)	0.44
Years on tamoxifen, median (range)	2.3 (0.1, 12.9)	2.1 (0.1, 5.2)	2.1 (0.1, 5.2)	2.1 (0.2, 5)	0.92

NOTE: *P* values for Fisher exact test or Wilcoxon rank sum tests for differences in patients on and not on lipid-altering medication (LAM).Abbreviation: *n*, number.

Caucasians and 46 (9%) African Americans. The median BMI was 29.0 (range 17.7–55.9), over the first 3 months the average change in BMI was 0.15. Of the overall randomized cohort, 228 (46%) women had previously been treated with either adjuvant or neoadjuvant chemotherapy, and 184 (37%) had been treated with adjuvant tamoxifen for a median of 2.3 years duration (range 0.1–12.9). Compliance data was available in 433 patients at 3 months, and 95% of patients (*n* = 412) reported they had not missed a single dose of aromatase inhibitor in the previous week, suggesting a highly compliant cohort. The cohort included in this analysis (*n* = 303) was similar to the overall cohort (Table 1). Patients not taking lipid-altering medications (*n* = 202) were slightly younger (*P* = 0.002), had lower BMI (*P* = 0.02), and were more likely to have received chemotherapy (*P* = 0.01) compared with those taking lipid-altering medication (*n* = 101), but otherwise had similar characteristics. The most common class of lipid-altering medications used by participants was statins.

Changes in lipid profiles in cohort and by aromatase inhibitor

Key changes in lipid profiles are summarized in Table 2. After 3 months of letrozole in participants not taking lipid-altering medications, total cholesterol increased by 5.9 mg/dL (*P* = 0.003) and LDL-C increased by 5.5 mg/dL (*P* < 0.007). However, in participants taking lipid-altering medications, total cholesterol decreased and LDL-C remained unchanged. In participants on exemestane not taking lipid-altering medications, total cholesterol decreased by 5.9 mg/dL (0.02) and HDL-C decreased by 7.8 mg/dL (*P* < 0.001); however, in those taking lipid-altering medications, decreases in total cholesterol were more pronounced, HDL-C remained unchanged, and triglycerides levels were also decreased.

Association of SNPs in candidate genes on lipid profiles

Among participants taking letrozole, variants in the *CYP19A1* gene were associated with significant decreases in triglycerides ranging from 20.2 to 39.3 mg/dL, and decreases in HDL-C (4.2 mg/dL) using additive and recessive models. Among participants taking letrozole on lipid-altering medications, some specific variants of *CYP19A1* were associated with increases of 6.7–9.5 mg/dL in HDL-C and others with decreases of 6.2–6.6 mg/dL in both dominant and additive models. There were no significant associations with SNPs among patients taking letrozole not on lipid-altering medications. Statistically significant changes

(*P* < 0.00053) are summarized in Table 3 and compared with SNPs in other candidate genes not reaching statistical significance in Fig. 2. Mean allele frequency (MAF) ranged from 0.27 to 0.48, and is summarized in Table 3. Triglycerides and HDL-C changes in significant SNPs were further described in recessive, dominant, and additive models (Fig. 3).

We did not observe significant SNP-lipid associations in exemestane-treated participants overall nor by whether they were taking lipid-altering medications or not in these or other variants (Supplementary Table S1). We also did not observe any evidence of interaction between any of the SNPs and treatment arm on the changes in lipid outcomes. We performed a sensitivity analysis with only Caucasian women (Supplementary Table S2) and another analysis without adjusting for changes in plasma estradiol concentrations (Supplementary Table S3), and found similar results. All alleles from significant SNPs were found to be in Hardy–Weinberg Equilibrium.

Discussion

We have prospectively demonstrated that, in women treated with letrozole, variants of *CYP19A1* are associated with mostly favorable effects in triglycerides and HDL-C. These associations were observed despite a general analysis finding no association between letrozole and changes in triglycerides (*P* = 0.44) and HDL-C (*P* = 0.08), suggesting that pharmacogenetic factors play a powerful role in predicting aromatase inhibitor-associated lipid changes. The product of the *CYP19A1* gene is crucial in the conversion of pre-estrogens into estrogens, which can alter lipid concentrations; therefore, a possible mechanism explaining our results may be altered estrogen metabolism in letrozole-treated patients who have specific SNPs in *CYP19A1*. While we observed significant associations in all women treated with letrozole, and in a subset of those taking lipid-altering medications, we did not find an association in the subset of patients not on lipid-altering medications. This result may be due to small numbers in subset analysis, or because the effects lipid-altering medications on lipid profiles may have influenced results.

We did not observe significant associations between SNPs and lipids in exemestane-treated women, although in a general analysis we found that exemestane was associated with decreases in total cholesterol and HDL-C, and that taking

Table 2. Change in lipid parameters after 3 months of aromatase inhibitor therapy in the entire cohort, and in participants treated with letrozole or exemestane whether on lipid-altering medications (LAM) or not

Lipid parameter	N	Baseline, mean (SD)	3 months, mean (SD)	Change, mean (SD)	P	P _{Interaction}
Overall cohort						
Total cholesterol, mg/dL	422	199.7 (37.5)	196.3 (36.1)	-3.4 (27.3)	0.01	
HDL, mg/dL	422	58.6 (17)	54.9 (17.3)	-3.7 (11.4)	<0.001	
LDL, mg/dL	419	117.1 (33.1)	118 (31.3)	1 (26.6)	0.45	
TG, mg/dL	422	121.1 (62.7)	116.9 (62.4)	-4.1 (49.6)	0.09	
Letrozole (not on LAM)						
Total cholesterol, mg/dL	147	201.5 (32.8)	207.4 (33.3)	5.9 (23.6)	0.003	
HDL, mg/dL	147	60.9 (16.7)	59.7 (17.1)	-1.3 (8.5)	0.08	
LDL, mg/dL	147	119.4 (28.6)	124.8 (30.5)	5.5 (24)	0.007	
Triglycerides, mg/dL	147	105.9 (47.1)	108.4 (47.8)	2.4 (38.2)	0.44	
Letrozole (on LAM)						
Total cholesterol, mg/dL	72	186.3 (42.1)	180.1 (38)	-6.2 (25.5)	0.04	0.001
HDL, mg/dL	72	52.9 (15.6)	51.3 (18.1)	-1.6 (13.6)	0.33	0.86
LDL, mg/dL	70	105 (36.9)	100.1 (31)	-4.9 (26.8)	0.13	0.007
Triglycerides, mg/dL	72	138.8 (71.2)	141.4 (74.4)	2.6 (53.3)	0.68	0.98
Exemestane (not on LAM)						
Total cholesterol, mg/dL	132	207.9 (36.4)	201.9 (30.5)	-5.9 (28.6)	0.02	
HDL, mg/dL	132	62.7 (17.8)	54.8 (15.7)	-7.8 (8.2)	<0.001	
LDL, mg/dL	132	122.8 (32.2)	125.6 (26.1)	2.8 (27.2)	0.23	
Triglycerides, mg/dL	132	112 (54.1)	104.6 (53.8)	-7.4 (51.7)	0.1	
Exemestane (on LAM)						
Total cholesterol, mg/dL	71	194.5 (39.9)	179.3 (37.8)	-15.3 (27.7)	<0.001	0.02
HDL, mg/dL	71	51.9 (14)	48.5 (17.1)	-3.5 (16.2)	0.08	0.04
LDL, mg/dL	70	113.4 (36.5)	107.3 (32.5)	-6.1 (28.6)	0.08	0.03
Triglycerides, mg/dL	71	151.3 (80.6)	132.8 (79.5)	-18.5 (59)	0.01	0.19

NOTE: Patients not taking LAM are also further subdivided by treatment arm. *P* values are for changes from baseline to 3 months within patient group. Interaction *P* values test whether changes in lipids after 3 months differ between patients on and not on LAM, separately by treatment arm.

Only patients with data at both time points are included.

Abbreviations: TC, total cholesterol; TG, triglycerides.

lipid-altering medications improved the overall lipid profile. Failure to find any associations in exemestane-treated women in the SNP analysis may have been due to a small sample size being underpowered to detect modest pharmacogenetic effects. Alternatively, nonsteroidal versus steroidal aromatase inhibitors have been shown to have different pharmacodynamic effects, and our results may indeed suggest different biologic effects (15, 23–25).

Our general analysis is similar to results our group has previously reported in the ELPh cohort despite minor differences in cohort derivation (15). Overall, effects of both letrozole and exemestane on lipid profiles are unfavorable. Notably,

while exemestane appears to decrease total cholesterol, this may be due to decreases in HDL-C also observed (total cholesterol = LDL-C + HDL-C + TG/5). While published literature of the effects on aromatase inhibitors has been mixed, many studies have demonstrated decreased in HDL-C with exemestane, and increased LDL-C with letrozole treatment (7–14). However, we report that negative effects on lipid profiles by letrozole and exemestane are eliminated in patients already on lipid-altering medications. To our knowledge, this is the first report finding such results.

Certain strengths and limitations should be considered when interpreting these data. A significant strength of this study

Table 3. Significant findings of multivariable linear regressions analyzing genetic associations between candidate gene SNPs and lipid profiles in aromatase inhibitor-treated participants, adjusted for age, BMI, race, change in plasma estradiol, and prior hormonal therapy and tamoxifen use

Cohort	Lipid parameter	Model	Gene (SNP)	MAF	HWE <i>P</i>	Number of HOM	Minor allele	Mean change, mg/dL (SE)	<i>P</i>
All patients on letrozole (<i>n</i> = 160)	TG	Recessive	<i>CYP19A1</i> (rs10046*)	0.48	0.63	34	A	-36.4 (7.8)	0.000069
	TG	Recessive	<i>CYP19A1</i> (rs2289105*)	0.48	0.87	34	C	-36.45 (7.8)	0.000074
	TG	Recessive	<i>CYP19A1</i> (rs3759811*)	0.43	0.61	25	C	-32.3 (9.0)	0.00045
	TG	Recessive	<i>CYP19A1</i> (rs700518*)	0.44	0.75	29	C	-32.3 (8.4)	0.00019
	TG	Recessive	<i>CYP19A1</i> (rs4775936*)	0.41	1.0	26	C	-33.2 (8.9)	0.00028
	HDL	Additive	<i>CYP19A1</i> (rs4646*)	0.27	1.0	11	A	-4.2 (1.16)	0.00043
	TG	Recessive	<i>CYP19A1</i> (rs749292*)	0.41	0.51	24	A	-39.3 (8.6)	0.000012
Patients on letrozole taking LAM (<i>n</i> = 52)	TG	Additive	<i>CYP19A1</i> (rs749292*)	0.41	0.51	24	A	-20.2 (4.7)	0.000037
	HDL	Additive	<i>CYP19A1</i> (rs749292*)	0.47	0.58	10	A	6.7 (1.4)	0.000037
	HDL	Additive	<i>CYP19A1</i> (rs1062033*)	0.42	0.58	8	G	6.9 (1.5)	0.000043
	HDL	Dominant	<i>CYP19A1</i> (rs1008805*)	0.38	0.24	5	G	-6.6 (1.7)	0.00037
	HDL	Dominant	<i>CYP19A1</i> (rs749292*)	0.47	0.58	10	A	9.5 (2.4)	0.0003
	HDL	Additive	<i>CYP19A1</i> (rs10046*)	0.45	0.57	9	G	-6.2 (1.6)	0.00046
	HDL	Additive	<i>CYP19A1</i> (rs2289105*)	0.45	0.57	9	T	-6.2 (1.6)	0.00046

Abbreviations: A, adenine; C, cytosine; G, guanine; HOM, homozygotes; HWE, Hardy-Weinberg equilibrium; SE, standard error; T, thymine.

Santa-Maria et al.

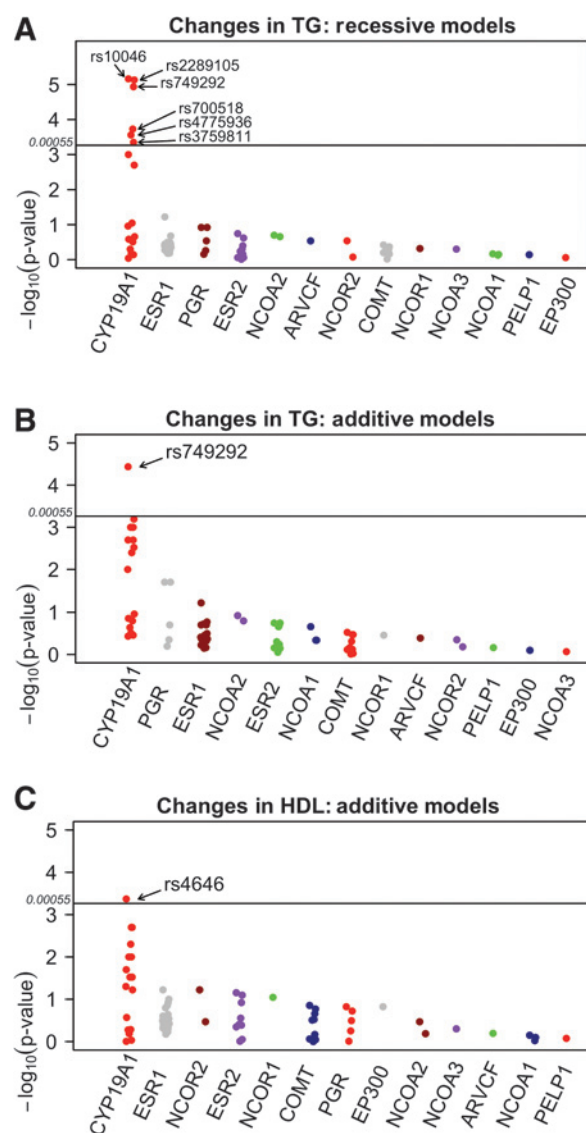


Figure 2. Manhattan plots demonstrating associations between variants in the *CYP19A1* gene and absolute changes in triglycerides (TG) using recessive (A) and additive (B) models, and changes in high density lipoprotein (HDL-C) using additive models (C) in letrozole-treated participants.

is that this is a planned subanalysis of a large prospective study. The subject population is diverse; therefore, results can be extrapolated to similar populations. Correlative study blood samples were collected in the majority of patients, with 79% (393 from 500 evaluable patients) of patients having genotype and lipid data at corresponding time points. A unique strength of our analysis is that changes in estradiol at the same times lipids were collected are adjusted for. The results of this analysis are similar whether estrogen levels are adjusted for or not, suggesting a powerful pharmacodynamic effect.

Limitations to consider when interpreting these data include the fact that allelic frequencies vary among different races and both Caucasian and African American women are included in this analysis. To address this consideration, we statistically adjusted

our analysis for race. Furthermore, we performed a sensitivity analysis in only Caucasian women, and found results to be similar. Another limitation to consider is that our sample was relatively small after excluding participants ineligible for this analysis, which reduces power. However, as mentioned above, this was a planned subanalysis of a prospective randomized cohort, which adds to validity of our findings. Another consideration when interpreting these results is that we investigated only candidate genes relevant to aromatase inhibitor metabolism and estrogen signaling; however, variants in other genes may potentially play a role in determining aromatase inhibitor-mediated effects on lipids. While this may be so, our group has substantial experience with selected candidate genes, and similar panels have been linked to rates of aromatase inhibitor discontinuation and letrozole concentration in aromatase inhibitor-treated women, suggesting that candidate genes selected were appropriate (21, 26, 27). Future large-scale investigation should include genome-wide association studies (GWAS) to validate our findings and to explore new candidate genes. Another consideration when interpreting these results is that while obesity, defined by BMI, is associated with lipid abnormalities, central obesity, estimated by waist circumference, may be a more accurate surrogate of this relationship (28–30). We adjusted our analysis to include the effects of BMI, although a more precise surrogate may have been waist circumference, which may be particularly relevant since up to 96% of women gain weight after breast cancer diagnosis and treatment (31, 32). These results may justify investigating pharmacogenetic strategies to assess cardiovascular risk in breast cancer survivors. While the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial investigators found no difference in non-breast cancer-related deaths between aromatase inhibitors and tamoxifen, a review analyzing 30,023 women found that patients treated with aromatase inhibitors had an increased risk of cardiovascular disease (OR, 1.26; 95% CI, 1.10–1.43, $P < 0.001$) compared with tamoxifen (33, 34). Cardiovascular disease risk is a significant competing comorbidity to breast cancer recurrence, and data from this same cohort of patients used in this study have demonstrated that 43% of women had a predicted 10-year cardiovascular disease risk equivalent to breast cancer recurrence risk and 37% had cardiovascular disease risk higher than breast cancer recurrence risk. Particularly, patients with stage 1 disease had a significantly increased risk of developing heart disease (OR, 6.1; 95% CI, 3.4–11.2, $P < 0.0001$; ref. 18). As dyslipidemias are an important cardiovascular risk factor, identifying those patients at highest risk for dyslipidemias due to aromatase inhibitor treatment is critical. Our data suggest that women taking letrozole with variants in *CYP19A1* may enjoy a favorable modulation in triglycerides, although HDL-C levels may or may not undergo favorable changes.

This study contributes evidence that pharmacogenomic biomarkers play a role as predictors of aromatase inhibitor toxicity (21, 27). Furthermore, pharmacogenomic biomarkers may help explain why different aromatase inhibitors may exert differential effects in target tissues. Additional studies investigating SNP-based models predicting specific aromatase inhibitor toxicity may help identify patients at risk and guide management, particularly in regards to cardiovascular risk factors. This is particularly relevant because many breast cancer survivors are cured of their breast cancer; however, anticancer therapies in some women may place them at higher risk for cardiovascular disease.

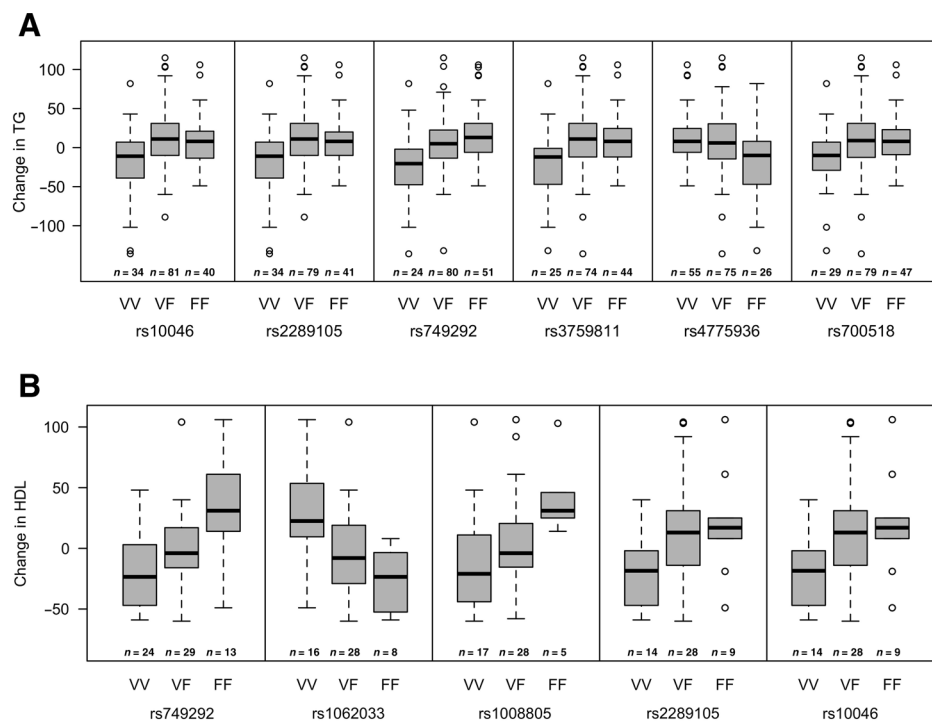


Figure 3. Distribution of changes in triglycerides (TG) in letrozole-treated women not on lipid-altering medications (LAM; A) and HDL-C in letrozole-treated women on lipid-altering medications (B) by *CYP19A1* genotype.

Disclosure of Potential Conflicts of Interest

N.L. Henry reports receiving commercial research grants from AstraZeneca. D.F. Hayes reports receiving commercial research grants from Novartis and Pfizer; other commercial research support from AstraZeneca, Janssen, Lilly, and Puma; and has ownership interest (including patents) in Inbiomotion and OncImmune. V. Stearns reports receiving commercial research grants from Abbvie, Celgene, MedImmune, Merck, Novartis, Pfizer, and Puma. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C.A. Santa-Maria, T.C. Skaar, S. Oesterreich, J.M. Rae, A.M. Storniolo, D.F. Hayes, D.A. Flockhart, V. Stearns
Development of methodology: C.A. Santa-Maria, T.C. Skaar, S. Oesterreich, J.M. Rae, D.F. Hayes, V. Stearns
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.T. Nguyen, T.C. Skaar, S. Philips, J.M. Rae, A.M. Storniolo, D.F. Hayes, V. Stearns
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.A. Santa-Maria, A. Blackford, T.C. Skaar, J.M. Rae, Z. Desta, N.L. Henry, D.F. Hayes, W.S. Post, V. Stearns
Writing, review, and/or revision of the manuscript: C.A. Santa-Maria, A. Blackford, T.C. Skaar, J.M. Rae, Z. Desta, J.D. Robarge, N.L. Henry, A.M. Storniolo, D.F. Hayes, R.S. Blumenthal, P. Ouyang, W.S. Post, V. Stearns

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.T. Nguyen, S. Philips
Study supervision: A.T. Nguyen, A.M. Storniolo, D.F. Hayes, V. Stearns
Other (performed the genotyping of samples and interpreted the final output data to assign them to their respective genotype class): S. Philips

Acknowledgments

The authors thank Dr. Gary Rosner for helpful assistance with the statistical analysis.

Grant Support

This study was funded in part by the NIH/NIGMS Pharmacogenetics Network Grant, P30 CA06973, Pfizer, Novartis, the Breast Cancer Research Foundation (N003173; to J.M. Rae, N.L. Henry), NIH 1R01GM099143 (to J.M. Rae, S. Oesterreich, N.L. Henry), and Fashion Footwear Charitable Foundation of New York/QVC Presents Shoes on Sale (to D.F. Hayes). V. Stearns holds the Breast Cancer Research Chair in Oncology.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 8, 2015; revised August 15, 2015; accepted September 9, 2015; published OnlineFirst October 13, 2015.

References

- Burstein HJ, Prestrud AA, Seidenfeld J, Anderson H, Buchholz TA, Davidson NE, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol* 2010;28:3784-96.
- Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, et al. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. *J Clin Oncol* 2010;28:509-18.
- Higgins MJ, Liedke PE, Goss PE. Extended adjuvant endocrine therapy in hormone dependent breast cancer: the paradigm of the NCIC-CTG MA.17/BIG 1-97 trial. *Crit Rev Oncol Hematol* 2013;86:23-32.
- Goss PE, Ingle JN, Pritchard KI, Ellis MJ, Sledge GW, Budd GT, et al. Exemestane versus anastrozole in postmenopausal women with early breast cancer: NCIC CTG MA.27—a randomized controlled phase III trial. *J Clin Oncol* 2013;31:1398-404.
- Geisler J, Haynes B, Anker G, Dowsett M, Lonning PE. Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, crossover study. *J Clin Oncol* 2002;20:751-7.
- Hayes DF, Skaar TC, Rae JM, Henry NL, Nguyen AT, Stearns V, et al. Estrogen receptor genotypes, menopausal status, and the effects of tamoxifen on lipid levels: revised and updated results. *Clin Pharmacol Ther* 2010;88:626-9.
- Lonning PE, Geisler J, Krag LE, Erikstein B, Bremnes Y, Hagen AI, et al. Effects of exemestane administered for 2 years versus placebo on bone

Santa-Maria et al.

- mineral density, bone biomarkers, and plasma lipids in patients with surgically resected early breast cancer. *J Clin Oncol* 2005;23:5126–37.
8. Francini G, Petrioli R, Montagnani A, Cadirni A, Campagna S, Francini E, et al. Exemestane after tamoxifen as adjuvant hormonal therapy in postmenopausal women with breast cancer: effects on body composition and lipids. *Br J Cancer* 2006;95:153–8.
 9. Montagnani A, Gonnelli S, Cadirni A, Caffarelli C, Del Santo K, Pieropan C, et al. The effects on lipid serum levels of a 2-year adjuvant treatment with exemestane after tamoxifen in postmenopausal women with early breast cancer. *Eur J Intern Med* 2008;19:592–7.
 10. Markopoulos C, Polychronis A, Dafni U, Koukouras D, Zobolas V, Tzorakoleftherakis E, et al. Lipid changes in breast cancer patients on exemestane treatment: final results of the TEAM Greek substudy. *Ann Oncol* 2009;20:49–55.
 11. Markopoulos C, Chrissochou M, Michailidou A, Tzorakoleftherakis E, Xepapadakis G, Papadiamantis J, et al. Effect of exemestane on the lipidemic profile of post-menopausal operable breast cancer patients following 5–7 years of adjuvant tamoxifen: preliminary results of the ATENA substudy. *Anticancer Drugs* 2005;16:879–83.
 12. Markopoulos C, Dafni U, Misitzi J, Zobolas V, Tzorakoleftherakis E, Koukouras D, et al. Extended adjuvant hormonal therapy with exemestane has no detrimental effect on the lipid profile of postmenopausal breast cancer patients: final results of the ATENA lipid substudy. *Breast Cancer Res* 2009;11:R35.
 13. Elisaf MS, Bairaktari ET, Nicolaides C, Kakaidi B, Tzallas CS, Katsaraki A, et al. Effect of letrozole on the lipid profile in postmenopausal women with breast cancer. *Eur J Cancer* 2001;37:1510–3.
 14. Wasan KM, Goss PE, Pritchard PH, Shepherd L, Palmer MJ, Liu S, et al. The influence of letrozole on serum lipid concentrations in postmenopausal women with primary breast cancer who have completed 5 years of adjuvant tamoxifen (NCIC CTG MA.17L). *Ann Oncol* 2005;16:707–15.
 15. Bell LN, Nguyen AT, Li L, Desta Z, Henry NL, Hayes DF, et al. Comparison of changes in the lipid profile of postmenopausal women with early stage breast cancer treated with exemestane or letrozole. *J Clin Pharmacol* 2012;52:1852–60.
 16. Emre A, Sahin S, Erzik C, Nurkalem Z, Oz D, Cirakoglu B, et al. Effect of hormone replacement therapy on plasma lipoproteins and apolipoproteins, endothelial function and myocardial perfusion in postmenopausal women with estrogen receptor-alpha IVS1–397 C/C genotype and established coronary artery disease. *Cardiology* 2006;106:44–50.
 17. Koukouras D, Marioli DJ, Papadopoulos K, Adonakis GL, Armeni AK, Georgopoulos NA, et al. Association of estrogen receptor alpha (ER α) gene polymorphisms with endometrial thickness and lipid profile in women with breast cancer treated with aromatase inhibitors. *Gynecol Endocrinol* 2012;28:859–62.
 18. Bardia A, Arieas ET, Zhang Z, Defilippis A, Tarpinian K, Jeter S, et al. Comparison of breast cancer recurrence risk and cardiovascular disease incidence risk among postmenopausal women with breast cancer. *Breast Cancer Res Treat* 2012;131:907–14.
 19. Henry NL, Giles JT, Ang D, Mohan M, Dadabhoy D, Robarge J, et al. Prospective characterization of musculoskeletal symptoms in early stage breast cancer patients treated with aromatase inhibitors. *Breast Cancer Res Treat* 2008;111:365–72.
 20. Henry NL, Xia R, Banerjee M, Gersch C, McConnell D, Giacherio D, et al. Predictors of recovery of ovarian function during aromatase inhibitor therapy. *Ann Oncol* 2013;24:2011–6.
 21. Desta Z, Kreutz Y, Nguyen AT, Li L, Skaar T, Kamdem LK, et al. Plasma letrozole concentrations in postmenopausal women with breast cancer are associated with CYP2A6 genetic variants, body mass index, and age. *Clin Pharmacol Ther* 2011;90:693–700.
 22. Altman DG, McShane LM, Sauerbrei W, Taube SE. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): explanation and elaboration. *PLoS Med* 2012;9:e1001216.
 23. Goss PE, Hadji P, Subar M, Abreu P, Thomsen T, Banke-Bochita J. Effects of steroidal and nonsteroidal aromatase inhibitors on markers of bone turnover in healthy postmenopausal women. *Breast Cancer Res* 2007;9:R52.
 24. Miller WR, Bartlett J, Brodie AM, Brueggemeier RW, di Salle E, Lonning PE, et al. Aromatase inhibitors: are there differences between steroidal and nonsteroidal aromatase inhibitors and do they matter? *Oncologist* 2008;13:829–37.
 25. Goss PE, Hershman DL, Cheung AM, Ingle JN, Khosla S, Stearns V, et al. Effects of adjuvant exemestane versus anastrozole on bone mineral density for women with early breast cancer (MA.27B): a companion analysis of a randomised controlled trial. *Lancet Oncol* 2014;15:474–82.
 26. Henry NL, Chan HP, Dantzer J, Goswami CP, Li L, Skaar TC, et al. Aromatase inhibitor-induced modulation of breast density: clinical and genetic effects. *Br J Cancer* 2013;109:2331–9.
 27. Henry NL, Skaar TC, Dantzer J, Li L, Kidwell K, Gersch C, et al. Genetic associations with toxicity-related discontinuation of aromatase inhibitor therapy for breast cancer. *Breast Cancer Res Treat* 2013;138:807–16.
 28. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 1983;67:968–77.
 29. Grundy SM, Barnett JP. Metabolic and health complications of obesity. *Dis Mon* 1990;36:641–731.
 30. Pouliot MC, Despres JP, Lemieux S, Moorjani S, Bouchard C, Tremblay A, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol* 1994;73:460–8.
 31. Vance V, Mourtzakis M, McCargar L, Hanning R. Weight gain in breast cancer survivors: prevalence, pattern and health consequences. *Obes Rev* 2011;12:282–94.
 32. Heasman KZ, Sutherland HJ, Campbell JA, Elhakim T, Boyd NF. Weight gain during adjuvant chemotherapy for breast cancer. *Breast Cancer Res Treat* 1985;5:195–200.
 33. Amir E, Seruga B, Niraula S, Carlsson L, Ocana A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. *J Natl Cancer Inst* 2011;103:1299–309.
 34. Cuzick J, Sestak I, Baum M, Buzdar A, Howell A, Dowsett M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol* 2010;11:1135–41.

Clinical Cancer Research

Association of Variants in Candidate Genes with Lipid Profiles in Women with Early Breast Cancer on Adjuvant Aromatase Inhibitor Therapy

Cesar A. Santa-Maria, Amanda Blackford, Anne T. Nguyen, et al.

Clin Cancer Res 2016;22:1395-1402. Published OnlineFirst October 13, 2015.

Updated version Access the most recent version of this article at:
doi:[10.1158/1078-0432.CCR-15-1213](https://doi.org/10.1158/1078-0432.CCR-15-1213)

Supplementary Material Access the most recent supplemental material at:
<http://clincancerres.aacrjournals.org/content/suppl/2015/10/13/1078-0432.CCR-15-1213.DC1>

Cited articles This article cites 34 articles, 6 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/22/6/1395.full#ref-list-1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link <http://clincancerres.aacrjournals.org/content/22/6/1395>. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.