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


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 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 aromatase inhibitor letrozole or exemestane. We performed genetic association analysis and multivariable linear regressions using dominant, recessive, and additive models.

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...
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 Qiagen 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), ER, and 2, progestin receptor (PGR)] coregulation of the estrogen receptor, [ER, E1a binding protein p300 (EP300)], enhancer of zeste 2 polyclamp repressive complex 2 (EZH2), nuclear receptor coactivator 1 (NCOA1), 2 (NCOA2), 3 (NCOA3), nuclear receptor corepressor 1 (NCoR1), 2 (NCoR2), nuclear receptor interaction protein (NRP1), proline-glutamic acid-leucine-rich protein 1 (PELP1), 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
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 Bonferonni-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%)
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 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 < 0.0007). However, in participants taking lipid-altering medications, total cholesterol decreased and HDL-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
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 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...
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 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, antineoplastic therapies in some women may place them at higher risk for cardiovascular disease.
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 holds 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.

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