Published in final edited form as:

Breast Cancer Res Treat. 2020 October ; 183(3): 705–716. doi:10.1007/s10549-020-05812-1.

Variation in the *UGT2B17* genotype, exemestane metabolism and menopause-related toxicities in the CCTG MAP.3 trial

Vikki Ho^{1,2,*}, Romain Pasquet^{1,*}, Shaman Luo³, Gang Chen³, Paul Goss⁴, Dongsheng Tu⁵, Philip Lazarus³, Harriet Richardson⁵ on behalf of MAP3 Investigators

¹University of Montreal, Department of Social and Preventive Medicine, Montreal, Quebec, Canada

²University of Montreal Hospital Research Centre (CRCHUM), Health Innovation and Evaluation Hub, Montreal, Quebec, Canada

Correspondence to: Dr. Harriet Richardson, Queen's University, Cancer Research Institute, Divisions of Canadian Cancer Trials Group and Cancer Care & Epidemiology, 10 Stuart Street, Room 220, K7L 3N6, Kingston, Ontario, Canada, Telephone: 613-533-6430, Fax: 613-533-2941, hrichardson@ctg.queensu.ca.

*Shared first authorship. These authors contributed equally to this work.

Addresses

Vikki Ho, University of Montreal Hospital Research Centre (CRCHUM), 850 rue Saint-Denis, Tour Saint-Antoine, 3rd Floor, S03.424, H2X0A9, Montréal, Quebec, Canada

Romain Pasquet, University of Montreal Hospital Research Centre (CRCHUM), 850 rue Saint-Denis, Tour Saint-Antoine, 3rd Floor, S03.412, H2X0A9, Montréal, Quebec, Canada

Shaman Luo, Washington State University, 412 E. Spokane Falls Blvd, PBS 431, Spokane WA 99202-2131

Gang Chen, Washington State University, 412 E. Spokane Falls Blvd, PBS 431, Spokane WA 99202-2131

Paul Goss, Harvard Medical School, MGH Cancer Center, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114, United States

Dongsheng Tu, Queen's University, Cancer Research Institute, Division of Canadian Cancer Trials Group, 10 Stuart Street, K7L 3N6, Kingston, Ontario, Canada

Philip Lazarus, Washington State University, 412 E. Spokane Falls Blvd, PBS 431, Spokane WA 99202-2131

Harriet Richardson, Queen's University, Cancer Research Institute, Divisions of Canadian Cancer Trials Group and Cancer Care & Epidemiology, 10 Stuart Street, Room 220, K7L 3N6, Kingston, Ontario, Canada

Contributor Statements

PG, HR and the MAP3 Investigators directed the MAP3 trial. Each author has directly participated in the planning, execution, or analysis of the current study. VH, RP, PL and HR designed the study's analytic strategy and interpreted the results. DT, and HR provided advice on study design or data analysis. VH, RP and HR drafted the manuscript. All authors critically revised and commented on the manuscript.

Conflicts of interest: There are no competing financial interests or conflicts of interest for any of the authors of this manuscript.

Disclosure of potential conflicts of interest: This study was funded by a grant (RO1-CA164366) from the National Cancer Institute at the National Institutes of Health to P. Lazarus (PI) and H. Richardson (Sub-award PI). Employment or Leadership Position: None; Consultant or Advisory Role: None; Stock Ownership: None; Honoraria: None; Expert Testimony: None; Patents, Royalties, and Licenses: None; Other Remuneration: None. Research Funding from company: Harriet Richardson and the CCTG held an IIR grant from Pfizer for the MAP3 trial (2004–2016); Vikki Ho holds a Sex and Gender Science Chair in Cancer Research from the Canadian Institutes for Health Research and is currently supported by the Cancer Research Society, Fonds de recherche du Québec – Santé (FRQS) and Ministère de l'Économie, de la Science et de l'Innovation du Québec (MESI). Shaman Luo received a China Scholarship Council grant (File No. 201406600026). Romain Pasquet declares that he has no conflict of interest. Gang Chen declares that he has no conflict of interest. Paul Goss declares that he has no conflict of interest. Dongsheng Tu declares that he has no conflict of interest. Philip Lazarus declares that he has no conflict of interest.

Compliance with Ethical Standards:

Research involving human participants: All procedures performed in this study were in accordance with the ethical standards of the Queen's Health Sciences Research Ethics Board and with the 1964 Helsinki declaration on medical research in human subjects and the Canadian Tri-Council Policy Statement on the Ethical Conduct for Research Involving Humans (TCPS).

Informed Consent: Informed consent was obtained from all individual participants included in the study and only those that also consented to genetic testing were included in the analyses presented in this paper.

Publisher's Disclaimer: This Author Accepted Manuscript is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication but has not been copyedited or corrected. The official version of record that is published in the journal is kept up to date and so may therefore differ from this version.

³Washington State University, College of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutical Sciences, Spokane, WA, USA

⁴Massachusetts General Hospital Cancer Center, Boston, MA, USA

⁵Queen's University, Cancer Research Institute, Division Canadian Cancer Trials Group, and Department of Public Health Sciences, Kingston, Ontario, Canada

Abstract

Purpose—To examine associations between the *UGT2B17* gene deletion and exemestane metabolites, and commonly reported side-effects (fatigue, hot flashes, and joint pain) among postmenopausal women participating in the MAP.3 chemoprevention trial.

Methods—The analytical samples for the *UGT2B17* analysis comprised 1,752 women on exemestane and 1,721 women on placebo; the exemestane metabolite analysis included 1,360 women on exemestane with one-year serum samples. Both the *UGT2B17* gene deletion and metabolites were measured in blood. The metabolites were conceptualized as a ratio (17-DHE-Gluc:17-DHE). Symptoms were assessed using the CTCAE v4.0 at approximately 1-year intervals. Log-binomial regression was used to examine the associations between *UGT2B17* deletion, exemestane metabolites and each side effect at 1 and up to 5 year follow-up, adjusting for potential confounders.

Results—Among individuals on exemestane with the *UGT2B17* gene deletion (i.e. lower detoxification), a higher risk of severe fatigue (RR=2.59 95% CI: 1.14–5.89) was observed at up to 5-year follow-up. Among individuals on placebo, those with the *UGT2B17* gene deletion had a higher risk of any fatigue (RR=1.39, 95% CI: 1.02–1.89) at year 1. A lower metabolite ratio (poor detoxification) was associated with a higher risk of any fatigue, hot flashes and joint pain at year 1 (fatigue: RR=1.89, 95% CI: 1.16–3.09; hot flashes: RR=1.77, 95% CI: 1.40–2.24; joint pain: RR=2.05, 95% CI: 1.35–3.12); similar associations were observed at 5-year follow-up.

Conclusion—Variation in the metabolism of exemestane through the *UGT2B17*-mediated pathway is associated with subsequent risk of commonly reported symptoms in MAP.3.

Keywords

Exemestane; chemoprevention; randomized clinical trial; menopausal symptoms; metabolism; pharmacogenetics

INTRODUCTION

Exemestane is an aromatase inhibitor (AI) with demonstrated efficacy in reducing the incidence or recurrence of breast cancer in postmenopausal women [1–3]. However, menopause-related side-effects due to estrogen suppression from exemestane can limit uptake and adherence. Variation in exemestane metabolism may provide valuable insights about treatment efficacy, side effects and symptoms of exemestane therapy.

The UDP-glucuronosyltransferase 2B17 is an enzyme that is encoded by the *UGT2B17* gene and can play an important role in xenobiotic and sex steroid metabolism in humans [4–5]. Exemestane is extensively metabolized through the activity of several CYP450s and aldo-

keto reductases. The major metabolite is 17-dihydroexemestane (17-DHE), which is also a potent aromatase inhibitor [6–7]. 17-DHE is primarily metabolized by UGT2B17; this phase II metabolism reaction facilitates the excretion of 17-DHE in urine via glucuronidation.

A homozygous (double) deletion in the *UGT2B17* gene has been associated with a decreased ability to metabolize 17-DHE [4–8], with individuals with the homozygous deletion exhibiting higher levels of 17-DHE and lower levels of 17-DHE-Gluc in the plasma of subjects taking exemestane [7, 9]. As 17-DHE is a major active metabolite of exemestane, lower glucuronidation capacity, as represented by lower concentrations of 17-DHE relative to 17-DHE-Gluc, may lead to higher estrogen depletion and higher risk of exemestane-induced toxicities.

Paradoxically, in the absence of aromatase inhibitors, the *UGT2B17* homozygous gene deletion is associated with altered sex steroid metabolism [4, 8, 10]. Emerging evidence in postmenopausal women has suggested that increasing androgen concentrations may also result in increased estrogen production through aromatase activity, which is of particular importance due to already-low estrogen levels in these women [11–13].

This study was nested within the Mammary Prevention 3 (MAP.3) trial, led by the Canadian Cancer Trials Group (CCTG). The main objectives of this current study were to examine associations between adverse symptomology and: 1) variation in the *UGT2B17* genotype in MAP.3 participants (stratified by exemestane and placebo treatment) and; 2) exemestane metabolites among women randomized to exemestane.

MATERIALS AND METHODS

Study Population

The MAP.3 chemoprevention trial was conducted by the Canadian Cancer Trials Group [1]. MAP.3 was an international, multicentre, double-blind, placebo-controlled, phase III randomized trial that aimed to assess the use of exemestane for breast cancer prevention purposes in 4,560 postmenopausal women [1]. MAP.3 participants consisted of postmenopausal women 35 years of age or older with at least one of the following risk factors: 60 years of age or older; Gail 5-year risk score greater than 1.66% or prior benign breast disease [1]. All eligible and consenting women were randomized to either exemestane or placebo for up to 5 years and serum samples were collected at baseline and year 1. Figure 1 presents a flowchart describing the analytical sample for Objective 1 and 2. Briefly, Objective 1 was examined separately among the exemestane treatment versus placebo group and consisted of those who consented to undergo genetic testing. Further, given that the genotype and metabolism of exemestane has been shown to vary by race and more than 90% of the MAP.3 cohort was Caucasian, this analysis was restricted to Caucasian women only including 1,752 women on exemestane and 1,721 women on placebo. For Objective 2, we further excluded 392 participants who did not have serum collected at year 1; thus, resulting in a final analytical sample of 1,360 women in the exemestane group only.

UGT2B17 gene deletion

The *UGT2B17* double gene deletion was measured from baseline blood samples as previously described [10, 11]. *UGT2B17* genotype was categorized into two groups for the primary analysis: wild type (+/+) plus heterozygous (+/0), versus homozygous/null (0/0). Sensitivity analysis also explored associations among the three groups [i.e. (+/+), (+/0) and (0/0)].

Exemestane Metabolites

Exemestane metabolites, 17-DHE-Gluc and 17-DHE, were quantified from serum collected 1 year after randomization, from participants on the exemestane arm, using ultra-performance liquid-chromatography tandem mass spectrometry (UPLC-MS/MS) as described previously [7]. There were 174 participants, with concentrations of either 17-DHE-Gluc or 17-DHE below the limit of detection (0.0005 ug/ml and 0.0001 ug/ml, respectively); for these individuals, values of half the minimum value observed for 17-DHE-Gluc or 17-DHE in the study population was assigned. The metabolite concentrations were conceptualized both as a ratio (i.e. 17-DHE-Gluc/17-DHE) and a percentage (i.e. 17-DHE / (17-DHE-Gluc+17-DHE)*100). For the ratio of 17-DHE-Gluc:17-DHE, a higher ratio would indicate greater metabolism and excretion of 17-DHE; we parametrized the ratio measure as a 3-level categorical variable. The categories of the 3-level categorical variable were selected *a priori* in order to broadly differentiate between “poor” detoxifiers (ratio < 0.8), “intermediate” detoxifiers (0.8 ratio 1.2), and “good” detoxifiers (ratio > 1.2); where, the “good” detoxifiers were used as the referent category.

Assessment of Toxicities

Within MAP.3, symptoms were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 at baseline, 6 months, 12 months and annually thereafter. The most common exemestane-related side effects reported previously, and in the MAP.3 trial, included hot flashes, joint pain and fatigue. These were also the most common reasons cited for early discontinuation and were thus, the outcomes of interest in this study. The CTCAE uses ‘grade’ to refer to the severity of each toxicity. Grade 1 and 2 refers to asymptomatic or mild symptoms, respectively while grade 3 describes symptoms as severe or medically significant but not immediately life-threatening. Grade 4 symptoms refer to those of life-threatening consequences needing urgent intervention while grade 5 (where applicable) indicates death. For each toxicity, two contrasts were considered: 1) severe toxicity (i.e. toxicity at grade 3 or higher) versus less than severe (i.e. less than grade 3) or no toxicity and; 2) any toxicity (i.e. at any grade) versus no toxicity. Further, these contrasts considered toxicities occurring within the first year from randomization and occurring up to 5 years from randomization (median duration follow-up was 3 years).

Covariates

Potential covariates of interest, collected at baseline, included age, education level, income, smoking status, BMI, family history of breast cancer, concomitant use of nonsteroidal anti-inflammatory drugs (NSAIDs) or bisphosphonates and ever use of hormone replacement therapy. For twenty-three participants with missing values for any one of these covariates,

we imputed the mode. A women's lifetime number of cumulative menstrual cycles (LCMC) was also considered as a covariate; LCMC is a composite measure that takes into account age at menarche, number of full term births, breastfeeding duration, oral contraceptive use, number of non-full-term pregnancies and age at menopause [14]. Due to insufficient detail for some variables, adjustments to this method were needed. Assumptions made included: regularization of cycles started at menarche, each menstrual cycle was of the same length, non-full-term pregnancies resulted in a 17-week absence of cycles and each pregnancy was an average of 40-weeks. The LCMC was parametrized into five categories defined by equidistant cutpoints and included a missing category for those with missing data on any of the variables used to derive the LCMC.

Statistical Analysis

For Objective 1, the associations between the *UGT2B17* gene deletion and each toxicity at 1-year and up to 5-years after randomization were examined using log-binomial regression in separate models for the exemestane and placebo groups, adjusted for potential confounders. Log-binomial regression was also used to examine the associations between metabolite levels and each toxicity at 1-year and up to 5-years after randomization, in the exemestane group only (Objective 2), adjusted for potential confounders. A backward deletion procedure ($p = 0.20$) was used to determine the covariates to include in each of the models; only hormone therapy (HT) remained in the final models. All analyses were performed using SAS version 9.4. (SAS Institute Inc, Cary, North Carolina, USA).

RESULTS

The descriptive characteristics of our study populations are shown in Table 1. Focusing on the placebo group included in objective 1, the mean age of our participants was 62 (S.D.=7.0); about 62% had at least some university education and almost 81% had an income greater than \$40,000. Approximately half the women had never smoked (52%). Of the ever smokers, only 13% were current smokers and 62% had used hormone therapy (HT) in the past. The distributions were similar in the exemestane arms for Objective 1 and 2. The median ratio of the exemestane metabolites, 17-DHE-Gluc:17-DHE, was 2.03 in women with the *UGT2B17* homozygous/null (0/0) genotype and 37.73 in women with the wild type (+/+) or heterozygous (+/0) genotype.

At year-1 and year-5 follow-up, hot flashes were the most commonly reported toxicity in both the exemestane and placebo group (Tables 2 and 3), although the large majority of hot flashes occurred within the first year of the study. There were approximately twice as many reports for fatigue and joint pain up to 5-years compared to year 1. Table 2 presents the results comparing the *UGT2B17* homozygous/null (0/0) versus wild type (+/+) and heterozygous (+/0) in relation to each toxicity at 1- year and up to 5-years, among those on the exemestane treatment arm. While no associations were observed between the *UGT2B17* gene deletion and joint pain, significant positive associations were observed between the *UGT2B17* gene deletion and severe toxicity of fatigue at up to 5 years after randomization (RR=2.59, 95% CI: 1.14–5.89). Similar associations were also observed between the *UGT2B17* gene deletion and severe hot flashes at both 1 and 5 years after randomization,

although not quite statistically significant (RR=2.06, 95% CI: 0.98–4.34 and 1.85 RR=1.85, 95% CI: 0.96–3.56, respectively). When considering the *UGT2B17* deletion polymorphism in three categories [i.e., (+/+), (0/+), (0/0)], results were similar to those previously discussed (Supplementary Table S1).

Table 3 presents the associations between the *UGT2B17* gene deletion and each toxicity at 1- and up to 5-years follow-up among those on the placebo arm. A weak relationship was only observed between the *UGT2B17* gene deletion and any fatigue at 1-year (RR=1.39, 95% CI: 1.02–1.89). No association between the genotype and severe fatigue, hot flashes or joint pain was observed in the placebo group. Similar null results were observed when considering the *UGT2B17* deletion polymorphism in three categories among those on placebo (Supplementary Table S2).

Table 4 presents the distribution of the toxicities versus the 3-level categorical representation of the ratio of 17-DHE-Gluc:17-DHE at both 1-year and up to 5-years, among the exemestane group. The proportion of events was higher among those with a metabolite ratio < 0.8 (poor detoxifiers) for all toxicity contrasts at 5-years. The association between metabolite levels of 17-DHE-Gluc and 17-DHE in relation to toxicities was examined while considering the metabolite levels as a ratio (17-DHE-Gluc:17-DHE). Table 5 presents the results of the ratio measure parameterized as a 3-level categorical variable, with the “good” detoxifier (ratio > 1.2) as the referent category, in relation to reported toxicities at 1- and up to 5-year follow-up, respectively. Due to the low reporting of severe fatigue at 1-year, we only considered comparing any fatigue versus no fatigue. Overall, a lower ratio of 17-DHE-Gluc:17-DHE, hypothesized to represent poor detoxification, was associated with a statistically higher risk of any fatigue, hot flashes or joint pain at year-1 (RRs between 1.89 and 2.05; p-values<0.05) when comparing ratios of <0.8 versus >1.2. Similar significant associations were observed with any fatigue, hot flashes and joint pain up to 5-years although effect estimates were weaker (RRs between 1.32 and 1.56; p-values<0.05). A similar trend was also observed for severe hot flashes, with a lower ratio of 17-DHE-Gluc:17-DHE associated with a statistically higher risk of hot flashes at year-1 (RR=4.32, 95% CI: 1.04–17.89) and a non-significant association at year-5 (RR=3.30, 95% CI: 0.81–13.51) when comparing the <0.8 versus >1.2 17-DHE-Gluc:17-DHE groups. When examining the metabolite levels of 17-DHE and 17-DHE-Gluc as a percentage (i.e. 17-DHE / (17-DHE-Gluc + 17-DHE * 100) in relation to toxicities, a similar positive association was observed, with an increasing percentage of plasma 17-DHE, hypothesized to represent more circulating active metabolite of exemestane, and increasing toxicities (Supplementary Table S3).

DISCUSSION

The NCCN guidelines for Breast Cancer Risk Reduction include AIs as chemopreventive agents for postmenopausal women at elevated risk (category 1), [3] and the elucidation of biomarkers of AI toxicity could potentially enhance uptake and adherence. Studies of adjuvant breast cancer therapy have reported that non-compliance is largely attributable to the presence of adverse side effects [15–19] and when questioned directly, patients name treatment-emergent symptoms and side-effects as the most common reasons for

discontinuing therapy [20–23]. Low treatment adherence (<80%) and decreased duration of therapy in turn have been shown to negatively affect survival [24–25]. Within the MAP.3 trial, 32% of women on the exemestane arm discontinued treatment protocol early (compared to 28% on placebo). Perceived treatment side-effects were the most common reason for early discontinuation (~50%) [26]. Knowing what predicts symptoms might enhance treatment monitoring and adherence in prevention.

We examined the associations between the *UGT2B17* gene deletion, and the three most commonly reported symptoms of hot flashes, joint pain and fatigue in the MAP.3 trial, separately among patients on the exemestane and the placebo arm. The hypothesis for this study posited that a loss of the *UGT2B17* function via a double gene deletion would increase the incidence of menopause-specific symptoms, in postmenopausal women randomized to exemestane through increased 17-DHE levels. Conversely, those randomized to placebo were hypothesized to experience fewer menopause-specific symptoms, due to the effects of *UGT2B17* on endogenous hormone concentrations.

Consistent with our first hypothesis, the *UGT2B17* gene deletion trended towards an association with higher risk of fatigue and hot flashes at 1- and 5-year follow-up among patients on exemestane; effects were stronger for severe symptoms but was only statistically significant for severe fatigue at 5-years. We further considered the role of exemestane metabolites in association with increased symptom burden, among patients continuing on exemestane treatment at year-1 and with a one-year serum sample. Exemestane metabolites were considered as a ratio of 17-DHE-Gluc:17-DHE and parameterized as a 3-level variable; consistent and statistically significant associations were observed between decreasing levels of detoxification mediated by *UGT2B17* (i.e. lower concentrations of 17-DHE-Gluc relative to 17-DHE) and higher risks of any fatigue, hot flashes and joint pain at 1-year and up to 5-years post-randomization. While the associations were more consistent for the comparisons of any toxicity versus no toxicity, the ratio of 17-DHE-Gluc:17-DHE was most strongly associated with severe hot flashes at 1-year and up to 5-years. In contrast, we did not observe a consistent protective effect of the *UGT2B17* gene deletion on menopausal symptoms among women in the placebo group, as originally posited in our second hypothesis.

Previous Literature

Previous studies suggest that determinants of response to AIs may depend on variability in exemestane metabolism, as significant inter-individual variability in formation of the important exemestane metabolite 17-dihydro-exemestane (17-DHE) has been observed [32]. However, the mechanism by which putative genetic polymorphisms affect exemestane metabolism has not been previously investigated in relation to toxicities.

A deletion in the *UGT2B17* has been associated with a decreased ability to metabolize exemestane metabolites [6]. In a recent study of 96 patients with breast cancer, those who took exemestane and had the *UGT2B17* gene deletion had a significant 30–40-fold decrease in 17-DHE-glucuronide levels in both plasma and urine, with a significant 30% increase in plasma DHE levels instead [7]. Another study observed that those with the gene deletion (~10%) had a 36-fold lower intrinsic clearance of 17-DHE compared to those who possessed

the gene, and that there was a significant correlation between 17-DHE glucuronide formation and liver UGT2B17 mRNA expression [6]. Our results are in line with the experimental evidence whereby a lower detoxification capacity (as represented by having a lower ratio of 17-DHE-Gluc:17-DHE) is associated with a risk of treatment-related side-effects. The metabolite ratio was consistently and significantly associated with any fatigue, hot flashes and joint pain at 1-year and up to 5- years. Furthermore, there were suggestive elevated risks for severe fatigue, hot flashes and joint pain at 1-year among women with the UGT2B17 gene deletion on exemestane. Combined, these results suggest that the *UGT2B17* gene deletion can affect exemestane metabolism and may in part explain the variability in toxicity that is observed with exemestane's clinical use, especially for fatigue and hot flashes.

In the absence of an AI, such as exemestane, the homozygous *UGT2B17* gene deletion may confer some protection for postmenopausal women. The *UGT2B17* metabolic pathway is involved in the local inactivation, glucuronidation and excretion of androgens [4]. The *UGT2B17* gene deletion can lead to increased circulating androgen concentrations that in turn can lead to increased estrogen production through aromatase activity. Several studies have observed higher bone mineral density (marker of increased estrogen exposure) in postmenopausal women with the homozygous *UGT2B17* gene deletion compared to women with one or both alleles of the *UGT2B17* gene [12–13], and when restricted to women not taking HT [11]. However, to our knowledge, the impact of the *UGT2B17* gene deletion on non-bone related menopause symptoms has not been previously described. Our results suggest that altered *UGT2B17* activity had conflicting influence on menopause-related symptoms in women who were not on exemestane, at least when based on toxicities reported on the CTCAE by a study nurse or physician. While there were suggestive protective effects for hot flashes, we observed a significant increased risk for fatigue at 1-year among women on placebo with the *UGT2B17* gene deletion.

Methodological Limitations

The CTCAE is a validated tool developed by the National Cancer Institute to systematically collect information on adverse events from patients participating in clinical trials. However, the most commonly reported symptoms of hot flashes, joint pain and fatigue are subjective in nature. While the healthcare team may be blinded to the treatment assignment of the participants, physician-graded toxicity may still not adequately capture presence and severity of subjective toxicities or symptoms, which in turn can lead to attenuated effect estimates towards the null. In contrast, patient reported outcomes (PROs), such as the menopause-specific quality of life (QOL) questionnaire (MENQOL) [27] based on participant responses, and take into account the level of discomfort or dysfunction associated with each reported symptom or toxicity. A change of 5–10% of the scale breadth of a QOL instrument, like the MENQOL, is thought to be potentially clinically meaningful to patients, according to experts in the literature [28–29]. Future studies may want to assess the utility of using PROs compared to CTCAE toxicity scores to describe menopause-specific outcomes, which may result in better classification of symptom severity and may lead to more accurate estimates.

Several other study limitations should be addressed. The timing of the blood draw was not standardized and because the half-life of exemestane is quite short (approximately 24 hours), a degree of misclassification could have been introduced to the level of metabolite concentrations. However, the parametrization of the metabolites as a ratio is thought to help mitigate some of the influence of this misclassification.

While the distribution of the exemestane metabolites (ratio) differed significantly by the *UGT2B17* genotype (p-value<0.001), with 15% of those women with the gene deletion having a low (<0.8) ratio of 17 DHE-GLUC:17 DHE compared to only 1.5% in the non-deleted group. The signal between the *UGT2B17* genotype and toxicities was weak in comparison to the results for the exemestane metabolites. One limitation was that the analysis for the *UGT2B17* deletion and menopause-related toxicities was generally underpowered, especially when considering severe toxicities. This was a function of the low prevalence of the double gene deletion (~10%) and the small number of severe toxicity events (defined as CTCAE grade 3 or greater). In addition, the metabolic pathway for exemestane is likely more complex than originally hypothesized. The *UGT2B17*-mediated conjugation is not the only mechanism in which 17-DHE is inactivated, but such alternate mechanisms were not considered in our analysis. Metabolites, such as 6-HME (6-(hydroxymethyl)androsta-1,4,6-triene-3,17-dione), that are active may also contribute to worsened symptoms. Consequently, the more robust associations observed between exemestane metabolite levels and menopause-related toxicities may be because they are downstream of the *UGT2B17* gene deletion, and represent a more precise measurement of activity.

In the MAP.3 trial 15% of women on exemestane and 11% of women on placebo discontinued therapy early, because of toxicity [26]. The most commonly cited toxicities associated with therapy discontinuation included hot flashes, joint pain and/or fatigue. [26]. For the exemestane metabolite analyses, women were excluded if they had discontinued therapy in the first year because serum samples were not collected at time of study discontinuation. Approximately 50% of participants cited toxicities as their primary reason for early discontinuation [26] and it is quite possible that the more severe cases were excluded from the second objective, which could have biased the results, likely attenuating the RR towards the null. Furthermore, participants were only counted as having an event (symptom) if their symptom severity had increased by at least one grade from baseline. Consequently, many of the analyses in the second objective were underpowered, given the small number of new and severe symptoms.

Finally, the prevalence of *UGT2B17* is known to vary by race, but since 93% of the MAP.3 study participants were Caucasian, it was not possible to assess any race-specific effects. Therefore, the results of this study can only be generalized to white women. While the homozygous gene deletion is relatively rare in Caucasian populations (~10%) [30–31], it has been observed to be much more common in Asian populations (>40%) [8, 32] and therefore, it would be important to replicate this work in studies with larger Asian populations, given the potential relevance of this gene-drug interaction in populations with higher frequencies of the gene polymorphism.

CONCLUSION

To date, this is the largest study to contribute novel information on the role of the *UGT2B17* gene deletion on menopause-related toxicities in postmenopausal women participating on the International MAP.3 trial of exemestane versus placebo for breast cancer prevention. The reduced detoxification of exemestane metabolites as a consequence of the *UGT2B17* gene deletion may help to explain the subsequent risk of commonly reported symptoms among postmenopausal women randomized to exemestane and could have important clinical relevance if confirmed in future studies. Measurement of the *UGT2B17* gene deletion in blood prior to the start of AI therapy or measurement of metabolite ratios from serum collected within the first year of starting therapy could have the potential to predict exemestane toxicity, and possibly efficacy, which in turn could help contribute to the discussion of the risk-benefit trade-off when considering therapy and /or improve treatment compliance. However, confirmatory studies are urgently needed, especially in the adjuvant setting, in order to clarify the role of the *UGT2B17* gene pathway and menopausal-related toxicities, given that the vast majority of exemestane is prescribed to women with breast cancer. Furthermore, data from adjuvant studies of exemestane would have the study power to evaluate the influence of the *UGT2B17* gene pathway on treatment efficacy; an endpoint that is rare in cancer prevention trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the 4,560 women who agreed to participate in this study; the trial committee; the many investigators, pharmacists, and clinical research associates involved in the trial; the Central Office staff of the Canadian Clinical Trials Group who contributed to the conduct of the main trial and Pfizer Pharmaceuticals for support and for providing exemestane and placebo. We also thank the entire Mammary Prevention 3 (MAP.3) investigator group. Finally, we thank the Mass Spectrometry Core facility and the Genomic Core facility at Washington State University -Spokane for their help with UPLC-MS and genotyping, respectively. We are also very grateful to Zuping Xia in the Department of Pharmaceutical Sciences at Washington State University for providing 17 β -DHE as a standard for these studies.

Financial Support: The current work was supported by a grant (RO1-CA164366) from the National Cancer Institute at the National Institutes of Health to P. Lazarus (PI) and H. Richardson (Sub-award PI) and a China Scholarship Council grant (File No. 201406600026) for Shaman Luo. Dr. Ho holds a Sex and Gender Science Chair in Cancer Research from the Canadian Institutes for Health Research and is currently supported by the Cancer Research Society, Fonds de recherche du Québec – Santé (FRQS) and Ministère de l'Économie, de la Science et de l'Innovation du Québec (MESI).

REFERENCES

1. Goss PE, Ingle JN, Ales-Martinez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, McTiernan A, Robbins J, Johnson KC, Martin LW et al.: Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med* 2011, 364(25):2381–2391. [PubMed: 21639806]
2. Gatti-Mays ME, Venzon D, Galbo CE, Singer A, Reynolds J, Makariou E, Kallakury B, Heckman-Stoddard BM, Korde L, Isaacs C et al.: Exemestane Use in Postmenopausal Women at High Risk for Invasive Breast Cancer: Evaluating Biomarkers of Efficacy and Safety. *CancerPrevRes(Phila)* 2016, 9(3):225–233.
3. Breast Cancer Risk Reduction [https://www2.tri-kobe.org/nccn/guideline/breast/english/breast_risk.pdf]

4. Chouinard S, Pelletier G, Bélanger A, Barbier O. "Cellular specific expression of the androgen-conjugating enzymes UGT2B15 and UGT2B17 in the human prostate epithelium". *Endocr. Res.* 2005, 30 (4):717–25..
5. Lazarus P, Zheng Y, Aaron Runkle E, et al. "Genotype-phenotype correlation between the polymorphic UGT2B17 gene deletion and NNAL glucuronidation activities in human liver microsomes". *Pharmacogenet. Genomics.* 2006, 15 (11): 769–78.
6. Sun D, Chen G, Dellinger RW, Sharma AK, Lazarus P: Characterization of 17-dihydroexemestane glucuronidation: potential role of the UGT2B17 deletion in exemestane pharmacogenetics. *Pharmacogenet Genomics* 2010, 20(10):575–585. [PubMed: 20697310]
7. Luo S, Chen G, Truica C, Baird CC, Leitzel K, Lazarus P: Role of the UGT2B17 deletion in exemestane pharmacogenetics. *Pharmacogenomics J* 2018, 18(2):295–300. [PubMed: 28534527]
8. McCarroll SA, Hadnott TN, Perry GH, Sabeti PC, Zody MC, Barrett JC, Dallaire S, Gabriel SB, Lee C, Daly MJ et al.: Common deletion polymorphisms in the human genome. *Nat Genet* 2006, 38(1):86–92. [PubMed: 16468122]
9. Lazarus P, Sun D: Potential role of UGT pharmacogenetics in cancer treatment and prevention: focus on tamoxifen and aromatase inhibitors. *Drug Metab Rev* 2010, 42(1):182–194. [PubMed: 19821643]
10. Gauthier-Landry L, Belanger A, Barbier O: Multiple roles for UDP-glucuronosyltransferase (UGT)2B15 and UGT2B17 enzymes in androgen metabolism and prostate cancer evolution. *J Steroid Biochem Mol Biol* 2015, 145:187–192. [PubMed: 24861263]
11. Giroux S, Bussières J, Bureau A, Rousseau F. UGT2B17 gene deletion associated with an increase in bone mineral density similar to the effect of hormone replacement in postmenopausal women. *Osteoporos Int.* 2012;23:1163–1170. [PubMed: 21614655]
12. Yang TL, Chen XD, Guo Y, Lei SF, Wang JT, Zhou Q, Pan F, Chen Y, Zhang ZX, Dong SS, et al. Genome-wide copy-number-variation study identified a susceptibility gene, UGT2B17, for osteoporosis. *Am J Hum Genet* 2008 83:663–674. [PubMed: 18992858]
13. Kamdem LK, Xi J, Clark BL et al. Exemestane may be less detrimental than letrozole to bone health in women homozygous for the *UGT2B17*2* gene deletion. *Breast Cancer Res Treat* 175, 297–303 (2019). [PubMed: 30747308]
14. Chavez-MacGregor M, Elias SG, Onland-Moret NC, vanderSchouw YT, VanGils CH, Monninkhof E, Grobbee DE, Peeters PHM: Postmenopausal breast cancer risk and cumulative number of menstrual cycles. *Cancer Epidemiol Biomarkers Prev* 2005, 14(4):799–804. [PubMed: 15824146]
15. Barron TI, Connolly R, Bennett K, Feely J, Kennedy MJ: Early discontinuation of tamoxifen: a lesson for oncologists. *Cancer* 2007, 109(5):832–839. [PubMed: 17243168]
16. Chlebowski RT, Geller ML: Adherence to endocrine therapy for breast cancer. *Oncology* 2006, 71(1–2):1–9. [PubMed: 17344666]
17. Fontein DB, Nortier JW, Liefers GJ, Putter H, Meershoek-Klein Kranenbarg E, van den Bosch J, Maartense E, Rutgers EJ, van de Velde CJ: High non-compliance in the use of letrozole after 2.5 years of extended adjuvant endocrine therapy. Results from the IDEAL randomized trial. *Eur J Surg Oncol* 2012, 38(2):110–117. [PubMed: 22172646]
18. Hadji P: Improving compliance and persistence to adjuvant tamoxifen and aromatase inhibitor therapy. *Crit Rev Oncol Hematol* 2010, 73(2):156–166. [PubMed: 19299162]
19. Partridge AH, LaFountain A, Mayer E, Taylor BS, Winer E, Asnis -Alibozek A: Adherence to initial adjuvant anastrozole therapy among women with early-stage breast cancer. *J Clin Oncol* 2008, 26(4):556–562. [PubMed: 18180462]
20. Aiello Bowles EJ, Boudreau DM, Chubak J, Yu O, Fujii M, Chestnut J, Buist DS: Patient-reported discontinuation of endocrine therapy and related adverse effects among women with early-stage breast cancer. *J Oncol Pract* 2012, 8(6):e149–157. [PubMed: 23598850]
21. Fallowfield L: Quality of life issues in relation to the aromatase inhibitor. *J Steroid Biochem Mol Biol* 2007, 106(1–5):168–172. [PubMed: 17587567]
22. Henry NL, Azzouz F, Desta Z, Li L, Nguyen AT, Lemler S, Hayden J, Tarpinian K, Yakim E, Flockhart DA et al.: Predictors of aromatase inhibitor discontinuation as a result of treatment-emergent symptoms in early-stage breast cancer. *J Clin Oncol* 2012, 30(9):936–942. [PubMed: 22331951]

23. Kyvernitis I, Ziller V, Hars O, Bauer M, Kalder M, Hadji P: Prevalence of menopausal symptoms and their influence on adherence in women with breast cancer. *Climacteric* 2014, 17(3):252–259. [PubMed: 23805799]
24. Hershman DL, Shao T, Kushi LH, Buono D, Tsai WY, Fehrenbacher L, Kwan M, Gomez SL, Neugut AI: Early discontinuation and non-adherence to adjuvant hormonal therapy are associated with increased mortality in women with breast cancer. *Breast Cancer Res Treat* 2011, 126(2):529–537. [PubMed: 20803066]
25. Makubate B, Donnan PT, Dewar JA, Thompson AM, McCowan C: Cohort study of adherence to adjuvant endocrine therapy, breast cancer recurrence and mortality. *Br J Cancer* 2013, 108(7):1515–1524. [PubMed: 23519057]
26. Maunsell E, Goss PE, Chlebowski RT, Ingle JN, Ales-Martinez JE, Sarto GE, Fabian CJ, Pujol P, Ruiz A, Cooke AL et al.: Quality of life in MAP.3 (Mammary Prevention 3): a randomized, placebo-controlled trial evaluating exemestane for prevention of breast cancer. *J Clin Oncol* 2014, 32(14):1427–1436. [PubMed: 24711552]
27. Hilditch JR, Lewis J, Peter A, van Maris B, Ross A, Franssen E, Guyatt GH, Norton PG, Dunn EA: A menopause-specific quality of life questionnaire: development and psychometric properties. *Maturitas*. 1996 7;24(3):161–75. [PubMed: 8844630]
28. Cella D, Hahn EA, Dineen K: Meaningful change in cancer-specific quality of life scores: Differences between improvement and worsening. *Qual Life Res*. 2002;11:207–221. [PubMed: 12074259]
29. Osoba D, Bezjak A, Brundage M, Zee B, Tu D, Pater J: Analysis and interpretation of health-related quality-of-life data from clinical trials: Basic approach of the National Cancer Institute of Canada Clinical Trials Group. *Eur J Cancer*. 2005;41:280–287. [PubMed: 15661554]
30. Wilson W, Pardo-Manuel De Villena F, Lyn-Cook BD, et al. Characterization of a common deletion polymorphism of the UGT2B17 gene linked to UGT2B15. *Genomics*. 2004;84:707–714. [PubMed: 15475248]
31. Lazarus P, Zheng Y, Aaron Runkle E, Muscat JE, Wiener D: Genotype-phenotype correlation between the polymorphic UGT2B17 gene deletion and NNAL glucuronidation activities in human liver microsomes. *Pharmacogenet Genomics*. 2005;15:769–778. [PubMed: 16220109]
32. Wang LZ, Goh SH, Wong ALA, et al. Validation of a rapid and sensitive LC-MS/MS method for determination of exemestane and its metabolites, 17 β -hydroxyexemestane and 17 β -hydroxyexemestane-17-O- β -D-glucuronide: Application to human pharmacokinetics study. *PLoS One*. 2015;10:e0118553. doi:10.1371/journal.pone.0118553. [PubMed: 25793887]

Fig. 1.
Flowchart of study participants by objectives

Table 1.

Descriptive characteristics of study population

Variable		Objective 1 UGT-Toxicities (Exemestane) N = 1,752 n (%)	Objective 1 UGT-Toxicities (Placebo) N = 1,721 n (%)	Objective 2 Metabolites-Toxicities (Exemestane) N = 1,360 n (%)
Age (year)				
	Mean (std)	62.5 (7.1)	62 (7.0)	63.3 (7.1)
Education level				
	Elementary school	47 (2.7)	38 (2.2)	33 (2.4)
	Some high school	51 (2.9)	39 (2.3)	37 (2.7)
	High school diploma	287 (16.4)	278 (16.1)	223 (16.4)
	Technical/Community college or CEGEP	315 (18.0)	297 (17.3)	256 (18.8)
	Some university	251 (14.3)	268 (15.6)	183 (13.5)
	Bachelor's Degree at University (BA, BSc, LLB)	378 (21.6)	400 (23.2)	296 (21.8)
	University Degree above a Bachelor's Degree	420 (23.9)	398 (23.1)	329 (24.2)
	Missing	3 (0.2)	3 (0.2)	3 (0.2)
Income				
	< 20,000\$	101 (5.7)	77 (4.5)	75 (5.5)
	20,000\$ to < 40,000\$	273 (15.6)	248 (14.4)	214 (15.7)
	40,000\$ to < 60,000\$	329 (18.8)	290 (16.9)	260 (19.1)
	60,000\$ to < 80,000\$	257 (14.6)	271 (15.7)	197 (14.5)
	80,000\$	591 (33.8)	622 (36.1)	463 (34.1)
	Don't know/refuse to answer	201 (11.5)	213 (12.4)	151 (11.1)
Smoking				
	< 100 cigarettes	906 (51.7)	905 (52.6)	710 (52.2)
	100 cigarettes	839 (47.9)	811 (47.1)	645 (47.4)
	Missing	7 (0.4)	5 (0.3)	5 (0.4)
BMI				
	Normal	507 (28.9)	497 (28.9)	387 (28.5)
	Overweight	648 (37.0)	636 (36.9)	464 (34.1)
	Obese	590 (33.7)	585 (34.0)	505 (37.1)
	Missing	7 (0.4)	3 (0.2)	4 (0.3)
Family history of Breast cancer				
	No	701 (40.0)	707 (41.1)	550 (40.5)
	Yes	1,046 (59.7)	1,011 (58.7)	807 (59.3)
	Missing	5 (0.3)	3 (0.2)	3 (0.2)
Concomitant use of NSAIDs or bisphosphonates				
	No	55 (3.1)	46 (2.7)	42 (3.1)
	Yes	1,697 (96.9)	1,675 (97.3)	1,318 (96.9)
Ever had hormone replacement therapy				

Variable		Objective 1 UGT-Toxicities (Exemestane) N = 1,752 n (%)	Objective 1 UGT- Toxicities (Placebo) N = 1,721 n (%)	Objective 2 Metabolites- Toxicities (Exemestane) N = 1,360 n (%)
LCMC	No	678 (38.7)	654 (38.0)	535 (39.3)
	Yes	1,074 (61.3)	1,067 (62.0)	825 (60.7)
	< 350	368 (21.0%)	354 (20.6%)	276 (20.3)
	350 to < 400	258 (14.7%)	252 (14.6%)	197 (14.5)
	400 to < 450	373 (21.3%)	368 (21.4%)	283 (20.8)
	450 to < 500	359 (20.5%)	360 (20.8%)	274 (20.1)
	500	345 (19.7%)	336 (19.5%)	270 (19.9)
	Missing	49 (2.8%)	51 (3.0%)	60 (4.4)
	17-DHE-Gluc:17-DHE ratio (Median[IQR])	N/A	N/A	
UGT2B17 deletion (0,0), n=146				2.03 [1.15–4.51]
UGT2B17 Wild type/heterozygous (+/+, +/-), n=1214				37.73 [12.42–86.91]

Table 2

Adjusted RRs (95% CIs) for the association between the *UGT2B17* gene deletion and toxicities in the exemestane group

Contrasts	UGT2B17 deletion (0/0) (N = 184) n events (%)	No UGT2B17 deletion (+/+, (+/0) (N = 1,568) n events (%)	Adjusted RR (95% CI) ^{a, b}	P-value
Fatigue 1-year				
Severe toxicity vs. less severe/no toxicity	4 (2.2)	15 (0.96)	2.48 (0.83 – 7.45)	0.11
Any toxicity vs. no toxicity	41 (22.3)	277 (17.7)	1.30 (0.97 – 1.74)	0.08
Fatigue 5-year				
Severe toxicity vs. less severe/no toxicity	7 (3.8)	26 (1.66)	2.59 (1.14 – 5.89)	0.02
Any toxicity vs. no toxicity	65 (35.3)	480 (30.6)	1.20 (0.97 – 1.48)	0.09
Hot flashes 1-year				
Severe toxicity vs. less severe/no toxicity	8 (4.4)	38 (2.4)	2.06 (0.98 – 4.34)	0.06
Any toxicity vs. no toxicity	73 (39.7)	570 (36.4)	1.12 (0.93 – 1.35)	0.25
Hot flashes 5-year				
Severe toxicity vs. less severe/no toxicity	10 (5.4)	53 (3.4)	1.85 (0.96 – 3.56)	0.07
Any toxicity vs. no toxicity	91 (49.5)	749 (47.8)	0.98 (0.84 – 1.14)	0.80
Joint pain 1-year				
Severe toxicity vs. less severe/no toxicity	5 (2.7)	26 (1.7)	1.76 (0.68 – 4.54)	0.25
Any toxicity vs. no toxicity	33 (17.9)	310 (19.8)	0.92 (0.67 – 1.28)	0.62
Joint pain 5-year				
Severe toxicity vs. less severe/no toxicity	7 (3.8)	64 (4.1)	1.03 (0.48 – 2.22)	0.93
Any toxicity vs. no toxicity	75 (40.8)	654 (41.7)	1.00 (0.83 – 1.20)	0.99

^aRRs obtained using log binomial regressions.

^bAll models were adjusted for hormonal replacement therapy (yes/no).

Table 3

Adjusted RRs (95% CIs) for the association between the *UGT2B17* gene deletion and toxicities in the placebo group

Contrasts	UGT2B17 deletion (0/0) (N = 177) n events (%)	No UGT2B17 deletion (+/+), (+/0) (N = 1,544) n events (%)	Adjusted RR (95% CI) ^{a, b}	P-value
Fatigue 1-year				
Severe toxicity vs. less severe/no toxicity	1 (0.57)	9 (0.6)	0.95 (0.12 – 7.48)	0.96
Any toxicity vs. no toxicity	37 (20.9)	233 (15.1)	1.39 (1.02 – 1.89)	0.04
Fatigue 5-year				
Severe toxicity vs. less severe/no toxicity	3 (1.7)	21 (1.4)	1.24 (0.37 – 4.11)	0.73
Any toxicity vs. no toxicity	61 (34.5)	455 (29.5)	1.17 (0.94 – 1.45)	0.16
Hot flashes 1-year				
Severe toxicity vs. less severe/no toxicity	2 (1.1)	27 (1.8)	0.66 (0.16 – 2.74)	0.56
Any toxicity vs. no toxicity	52 (29.4)	441 (28.6)	1.03 (0.81 – 1.32)	0.79
Hot flashes 5-year				
Severe toxicity vs. less severe/no toxicity	3 (1.7)	39 (2.5)	0.68 (0.21 – 2.18)	0.52
Any toxicity vs. no toxicity	70 (39.6)	701 (45.4)	0.87 (0.73 – 1.06)	0.17
Joint pain 1-year				
Severe toxicity vs. less severe/no toxicity	0 (0.0)	9 (0.6)	-	-
Any toxicity vs. no toxicity	31 (17.5)	260 (16.8)	1.05 (0.75 – 1.47)	0.80
Joint pain 5-year				
Severe toxicity vs. less severe/no toxicity	3 (1.7)	25 (1.6)	1.06 (0.32 – 3.47)	0.92
Any toxicity vs. no toxicity	67 (37.9)	600 (38.9)	0.98 (0.80 – 1.19)	0.82

^aRRs obtained using log binomial regressions.

^bAll models were adjusted for hormonal replacement therapy (yes/no).

Table 4.Distribution of the 3-category 17-DHE-Gluc:17-DHE ratio variable by toxicity contrast ^a

Toxicities ^a	Total Population (N=1,360) N _{Events} (%) ^b	17-DHE-Gluc:17-DHE ratio		
		< 0.8 (N = 39)	0.8 to 1.2 (N = 37)	> 1.2 (N = 1,284)
		N _{Events} (%) ^b	N _{Events} (%) ^b	N _{Events} (%) ^b
Fatigue 1-year				
Severe toxicity vs. less severe/no toxicity	10 (0.7)	0 (0.0)	0 (0.0)	10 (0.8)
Any toxicity vs. no toxicity	247 (18.2)	12 (30.8)	6 (16.2)	229 (17.8)
Fatigue 5-year				
Severe toxicity vs. less severe/no toxicity	21 (1.5)	1 (2.6)	0 (0.0)	20 (1.6)
Any toxicity vs. no toxicity	424 (31.2)	16 (41.0)	13 (35.1)	395 (30.8)
Hot flashes 1-year				
Severe toxicity vs. less severe/no toxicity	21 (1.5)	2 (5.1)	0 (0.0)	19 (1.5)
Any toxicity vs. no toxicity	525 (38.6)	25 (64.1)	17 (45.9)	483 (37.6)
Hot flashes 5-year				
Severe toxicity vs. less severe/no toxicity	27 (2.0)	2 (5.1)	1 (2.7)	24 (1.9)
Any toxicity vs. no toxicity	711 (52.3)	26 (66.7)	20 (54.1)	665 (51.8)
Joint pain 1-year				
Severe toxicity vs. less severe/no toxicity	20 (1.5)	1 (2.6)	0 (0.0)	19 (1.5)
Any toxicity vs. no toxicity	267 (19.6)	15 (38.5)	3 (8.1)	249 (19.4)
Joint pain 5-year				
Severe toxicity vs. less severe/no toxicity	51 (3.8)	1 (2.6)	1 (2.7)	49 (3.8)
Any toxicity vs. no toxicity	573 (42.1)	24 (61.5)	9 (24.3)	540 (3.9)

^aContrasts included: 1) severe toxicity (i.e. toxicity at grade 3 or higher) versus less than severe (i.e. less than grade 3) or no toxicity and; 2) any toxicity (i.e. at any grade) versus no toxicity.

^bColumn percentages.

Table 5.

Adjusted RRs (95% CIs) for the association between a 3-category 17-DHE-Gluc:17-DHE ratio variables and toxicities.

Contrasts	Fatigue Adjusted RR (95% CI) ^{a,b}	Hot Flashes Adjusted RR (95% CI) ^{a,b}	Joint Pain Adjusted RR (95% CI) ^{a,b}
1-year			
Severe toxicity vs. less severe/no toxicity			
> 1.2	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.8 to 1.2	-	-	-
< 0.8		4.32 (1.04 – 17.89)	1.90 (0.26 – 13.95)
Any toxicity vs. no toxicity			
> 1.2	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.8 to 1.2	0.91 (0.43 – 1.90)	1.26 (0.88 – 1.79)	0.42 (0.14 – 1.25)
< 0.8	1.89 (1.16 – 3.09)	1.77 (1.40 – 2.24)	2.05 (1.35 – 3.12)
5-year			
Severe toxicity vs. less severe/no toxicity			
> 1.2	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.8 to 1.2	-	1.51 (0.21 – 10.80)	0.74 (0.10 – 5.18)
< 0.8	2.01 (0.28 – 14.64)	3.30 (0.81 – 13.51)	0.80 (0.11 – 5.62)
Any toxicity vs. no toxicity			
> 1.2	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.8 to 1.2	1.11 (0.71 – 1.72)	1.06 (0.79 – 1.43)	0.59 (0.33 – 1.04)
< 0.8	1.51 (1.03 – 2.21)	1.32 (1.06 – 1.64)	1.56 (1.22 – 2.00)

^aRRs obtained using log binomial regressions.

^bAll models were adjusted for hormonal replacement therapy (yes/no).