Gene-hormone therapy interaction and fracture risk in postmenopausal women

Youjin Wang, Jean Wactawski-Wende, Lara E. Sucheston-Campbell, Leah Preus, Kathleen M. Hovey, Jing Nie, Rebecca D. Jackson, Samuel K. Handelman, Rami Nassir, Carolyn J. Crandall, and Heather M. Ochs-Balcom

The Journal of Clinical Endocrinology & Metabolism
Endocrine Society

Submitted: August 09, 2016
Accepted: February 28, 2017
First Online: March 06, 2017

The Journal of Clinical Endocrinology & Metabolism; Copyright 2017 DOI: 10.1210/jc.2016-2936

Gene-hormone therapy interaction and fracture risk in postmenopausal women

Youjin Wang[1], Jean Wactawski-Wende[1], Lara E. Sucheston-Campbell[2,3], Leah Preus[1, 4], Kathleen M. Hovey[1], Jing Nie[1], Rebecca D. Jackson[5], Samuel K. Handelman[6], Rami Nassir[7], Carolyn J. Crandall[8], and Heather M. Ochs-Balcom[1]

[1] *Department of Epidemiology and Environmental Health, School of Public Health and Health Professions, University at Buffalo, The State University of New York, Buffalo, NY*

[2] *College of Pharmacy, The Ohio State University, Columbus, OH*

[3] *Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH*

[4] *Department of Cancer Prevention and Control, Division of Cancer Prevention and Population Sciences, Roswell Park Cancer Institute, Buffalo, NY*

[5] *Department of Internal Medicine, Division of Endocrinology, Diabetes and Metabolism, The Ohio State University, Columbus, OH*

[6] *Center for Pharmacogenomics, Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Columbus, OH*

[7] *Department of Biochemistry and Molecular Medicine, University of California Davis, Davis, CA*

[8] *Division of General Internal Medicine and Health Sciences Research, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA*

Received 09 August 2016. Accepted 28 February 2017.

**This manuscript is part of Youjin Wang's doctoral dissertation which was submitted and published on ProQuest/UMI. This manuscript is original and is not being considered for publication in any other peer-reviewed journal, but the results were presented as a poster at the 2016 Women's Health Initiative Investigator Meeting, 2016 School of Public Health and Health Professions J. Warren Perry Poster Day at the State University of New York at Buffalo, and 2015 American Society of Human Genetics Annual Meeting.

Context: Evidence supports a protective effect of menopausal hormone therapy (HT) on bone. However, whether genetic susceptibility modifies the association of HT and fracture risk is not sufficiently explored.

Objective: The objective was to test an interaction between genetic susceptibility and HT on fracture risk.

Design: We constructed two weighted genetic risk scores (GRSs) based on 16 fracture-associated variants (Fx-GRS) and 50 bone mineral density (BMD) variants (BMD-GRS). We used Cox regression to estimate the main effects of GRSs and their interactions with HT on fracture risk. We estimated the relative excess risk due to interaction (RERI) as a measure of additive interaction. We also utilized the case-only approach to test for a multiplicative interaction.

Setting: 40 US clinical centers

Participants: 9,922 genotyped white postmenopausal women (age 50-79) from the Women's Health Initiative HT randomized trials

Main outcome: Adjudicated fracture incidence

Results: Both GRSs were associated with fracture risk (hazard ratio (HR) (95% CI) per one-unit increment in GRS, 1.04 (1.02-1.06) for Fx-GRS and 1.03 (1.02-1.04) for BMD-GRS). We found no evidence for multiplicative interaction for either of the GRS. However, we observed a significant additive interaction, where the highest quartile of both GRSs and randomization to placebo have excess fracture risk: Fx-GRS p-for-RERI=0.047, BMD-GRS p-for-RERI=0.046.
Conclusions: These results suggest that HT reduces fracture risk in postmenopausal women especially in those at highest genetic risk of fracture and low BMD.

We evaluated gene-menopausal hormone therapy (HT) interaction on fracture. We found that HT reduces fracture risk especially in postmenopausal women at highest genetic risk of fracture and low BMD.

INTRODUCTION

Menopausal hormone therapy (HT) prevents bone loss and reduces fracture risk in postmenopausal women. The Women's Health Initiative (WHI) clinical trials demonstrated that estrogen alone (E) or estrogen plus progestin (E+P) therapy reduced the risk of hip fracture by 33-35% and total fracture by 24-29[1,2].

Evidence indicates that contributors to fracture risk are multifactorial and include both genetic and environmental factors[3-12]. The majority of previous gene-environment interaction studies published on bone-related phenotypes have relied on candidate gene approach which have seldom been replicated due to small effect size and small sample size. Previous studies have demonstrated that an aggregate genetic risk score (GRS) based on common genetic variants identified from genome-wide association studies (GWAS) can be a useful measure of genetic susceptibility to fracture[6,7]. In a meta-GWAS including 17 studies, a GRS with 16 single nucleotide polymorphisms (SNPs) that were associated with both bone mineral density (BMD) and fracture was associated with any type of fracture[6]. Another study showed that the GRS with 16 SNPs, and the GRS based on 63 BMD SNPs identified from the meta-GWAS were associated with any type of fracture and hip fractures risk[7]. In a recent study by Ho-Le et al, a BMD GRS showed an increased fracture risk independent of age, prior fracture, and falls[13].

Given the strength of the evidence supporting a protective effect of HT on fracture risk, the existing meta-GWAS, and availability of the HT clinical trial data, we performed a gene-environment interaction study to untangle the complex interplay of genetic susceptibility and HT on risk of fracture. We hypothesized that genetic susceptibility modifies the association of HT and fracture risk.

MATERIALS AND METHODS

Women's Health Initiative hormone therapy clinical trials

Between 1993 and 1998, 27,347 women participated in one of two WHI HT trials[14]. If a participant had a hysterectomy (N=10,739), she was randomized into the estrogen alone study (conjugated equine estrogen 0.625 mg/day or matching placebo)[15] whereas if she had an intact uterus (N=16,608), she was randomized to the estrogen+progestin study (conjugated equine estrogen 0.625 mg plus medroxyprogesterone acetate 2.5 mg/day or matching placebo). Due to the increased risk of breast cancer and inconsistent risk-benefit profiles, the estrogen plus progestin trial was ended in 2002 (median follow-up 5.6 years)[16,17]. The estrogen alone trial was also terminated in 2004 (median follow-up 7.2 years) due to the increased stroke risk with no evidence of coronary heart disease or global benefit[17,18]. However, the women were followed until the planned stop date (March 31, 2005).

Study participants

Among 22,030 self-identified European American (EA) HT trial participants, 10,634 women were genotyped as part of one of two sub-studies: the Genomics and Randomized Trials Network (GARNET; N=4,894) and the Women's Health Initiative Memory Study + (WHIMS+; N=5,740). The WHI GARNET was designed to identify genetic factors that impact treatment response to HT, utilizing a nested case-control study design. The WHIMS+ sub-study includes WHIMS EA participants who are not in GARNET, plus additionally selected HT trial participants who are neither in WHIMS nor GARNET[19]. We oversampled for women age>65 since all WHIMS participants are aged 65 and older[20]. All participants provided informed consent and the study was approved by the University at Buffalo Health Sciences IRB.

Among the 10,634 genotyped individuals, 701 were excluded prior to imputation in GARNET if sample-chromosome combination with chromosomal abnormalities and/or missingness>5%, or overall missingness>2%, and were excluded in WHIMS+ if missingness>3% or were one of a related pair, leaving a total of 9,933 and 9,932 respective self-reported white individuals with complete imputed SNP data available for Fx-GRS and BMD-GRS. Data from an additional 11 individuals were excluded due to a lack of follow-up data. The current study includes data from the remaining 9,922 (9,921 for BMD-GRS analyses) women (**Supplemental Figure 1**).

HT intervention

In order to classify women according to HT use, we utilized the assignment of HT at randomization. We combined the two hormone arms (E alone and E+P) and classified the participants into two groups (randomized to HT intervention or placebo) as the findings of the two WHI HT trials on fracture prevention were similar[1,2].

Ascertainment of fracture

The primary outcome of interest is incident fracture of any type. Fracture incidence was initially self-reported via semi-annual questionnaires and adjudicated in a blind manner using radiology reports[1,2]. Total fracture was defined as adjudicated fractures excluding those of the ribs, sternum, skull or face, fingers, toes, and cervical vertebrae[1,2,21]. For exploratory fracture subtype analyses, we considered the following three types: central body (hip, spine, and pelvis), lower limb (ankle, patella, shaft of femur, tarsal/metatarsal, tibia/fibula, and tibial plateau) and upper limb (carpal, elbow, metacarpal, radius/ulna, upper radius/ulna, lower humerus, humerus, and upper humerus)[22].

SNP genotyping, quality control (QC) and imputation

Samples were genotyped using the Illumina HumanOmni1-Quad v1-0 B SNP array (GARNET) or the Illumina HumanOmniExpressExome-8v1_B array (WHIMS+). In GARNET, SNPs were removed if they failed the recommended quality control (QC) procedure; intensity only, technical failure by the genotyping center, 100% missing, minor allele frequency (MAF)=0 for unrelated study participants, call rate<98%, >0 discordant calls in 35 duplicate pairs, Hardy-Weinberg equilibrium (HWE) p-value<1.0×10^{-4}. The genetic QC for WHIMS+ data was conducted following the Gene, Environment Association Studies Consortium (GENEVA) protocol [23,24]; missing call rate≥2%, HWE p-value<1.0×10^{-4}, and MAF<0.01. Genotype imputation was conducted with BEAGLE[25] using the 1000 Genomes reference panel (20100804 sequence and alignment release) (GARNET) and Minimac using 1000 Genomes reference panel v3.20101123 (WHIMS+). Detailed quality control is described elsewhere[26]. Allelic dosage was calculated for the imputed genotype, ranging from 0 to 2.

Genetic risk scores (GRS)

We constructed two genetic risk scores, one for fracture-associated SNPs (Fx-GRS) and one for BMD-associated SNPs (BMD-GRS), where each GRS was weighted by allele effect size using SNPs that were identified in the GEnetic Factors for Osteoporosis consortium (GEFOS) meta-GWAS[6]. The WHI Genetic Components of HIP Fracture Consortium GWAS (GeCHIP) sample was included in the GEFOS meta-GWAS as a replication sample. The fracture GRS (Fx-GRS) was based on SNPs associated with femoral neck (FN) or lumbar spine (LS) BMD that were also associated with any type of fracture at Bonferroni-corrected significance level ($P<5\times10^{-4}$) (**Supplemental Table 1**). In the Fx-GRS, 4 (2) SNPs were typed and 11 (13) were imputed. A typed proxy ($r^2=1$) for rs1373004 (1), rs7898709, was used as the former SNP was not available in GARNET (WHIMS+) data (http://analysistools.nci.nih.gov/LDlink/)[27]. Weights were calculated as a log-transformation of the reported odds ratios (OR) for fracture divided by the mean of log-transformed ORs across all GRS SNPs.

We constructed the BMD-GRS using 50 SNPs from an established set of 63 BMD associated SNPs from the GEFOS meta-GWAS (**Supplemental Table 2**). In the score, a total of 13 (11) SNPs were typed, 36 (38) were imputed and 1 (1) was a typed proxy in GARNET (WHIMS+). The typed proxy rs7898709 was again used for rs1373004. Out of 63 SNPs, 13 variants were excluded for following reasons: failed QC (rs12821008, rs1566045, rs17040773), showed secondary signals only after conditional analysis but were not themselves associated with BMD (rs10226308, rs13245690, rs1564981, rs17482952, rs4792909, rs736825, rs7521902, rs7751941), or did not show an association with FN-BMD among females (beta-coefficient for FN-BMD=0) (rs1878526, rs7071206). Weights for BMD-GRS were based on the stage 1+2 meta-analysis female only effects sizes (beta-coefficients) for FN-BMD, which were standardized by dividing each beta-coefficient by the mean of the effects.

Statistical analyses

We compared the baseline characteristics between randomization arms using Chi-square tests for categorical variables and t-test for continuous variables. We used Cox proportional-hazards regression to test the association of GRS with fracture and the interactions between HT and GRS on the risk of fracture. The analyses were conducted with both continuous and categorical parameterization of GRS. For the categorical analyses, participants were categorized into 3 groups based on GRS quartiles; quartile 1 (Q1), quartile 2-3 (Q2-3), and quartile 4 (Q4). The time-to-event was the number of days since enrollment to the first fracture. The proportional hazards assumptions were evaluated by examining Schoenfeld residuals and no significant deviations were found. The multivariable models were adjusted for age and WHI GWAS (WHIMS+ or GARNET).

The joint effects of both GRS and HT on fracture risk were tested on both additive and multiplicative scales. Multiplicative interactions were examined by testing the statistical significance of the regression coefficient of the cross-product term (GRS*HT) while including both main effects in the model. Further, we assessed multiplicative interaction by evaluating the GRS-HT association in logistic regression models using a case-only approach with 1,608 total fracture cases. The case-only study design increases power to detect gene-environment interaction because the need to estimate the association between gene and environment in non-cases is alleviated under the assumption of gene-environment independence in the source population[28]. The gene-environment independence assumption was tested in logistic regression models with 8,314 non-cases and was satisfied.

To assess GRS-HT additive interactions, we constructed a composite variable of aforementioned GRS categories and HT arm and estimated the relative excess risk due to interaction (RERI) using the suggested methods by Li and Chambless[29]. We calculated strata-specific HRs and 95% CIs using those in the lowest GRS quartile who were assigned to HT intervention as the reference group. Variances of the RERI were calculated using the Hosmer and Lemeshow's delta method[30].

We further evaluated the HRs for HT intervention within the strata of GRSs to examine the effect modification of genetic susceptibility on the association between HT and fracture risk. Heterogeneity of the HT effect was tested using Cochran's Q test for meta-analysis.

All statistical analyses were conducted using SAS version 9.4 (SAS institute, Cary, NC). All p-values reported were two-sided, and statistical significance was defined as $p<0.05$.

RESULTS

The baseline characteristics according to HT arm are summarized in **Table 1**. As expected because of randomization, the intervention and placebo arms did not differ according to previous personal HT use, previous fracture or in terms of physical measurements. Participants had a mean age of 67 years (SD=6.5). Approximately 66% had never used HT and 9% were current smokers at randomization. The mean (SD) GRSs were 14.7 (2.5) and 50.6 (4.8) for Fx-GRS and BMD-GRS, respectively, for participants without any fracture, and 14.9 (2.5) and 51.3 (4.7) for those with fracture incidence.

Both the Fx-GRS and BMD-GRS were significantly associated with total fracture incidence; confirming genetic associations with the meta-GWAS hits. Each unit increment in Fx-GRS from 16 SNPs resulted in a 4% increase hazards for total fracture (multivariate adjusted HR: 1.04, 95% CI: 1.02-1.06). For BMD-GRS, the HR was 1.03 (95% CI: 1.02-1.04). (**Supplemental Table 3**). In the analysis of categorical GRS, the annualized incidence rate of total fracture was 1.96%, 2.31% and 2.49% in the Fx-GRS Q1, Q2-3 and Q4, respectively. Similar results were observed for BMD-GRS. The annualized incidence rate of total fracture was 1.85%, 2.33% and 2.59% in the Q1, Q2-3 and Q4, respectively. Similar to total fracture, higher GRSs were associated with increased risk of all fracture subgroups. We did not observe any interaction between GRSs and age on fracture risk (**Supplemental Table 4)**. We examined previous fracture, number of falls, and smoking variables in the regression models, however no factors significantly changed the effect estimate of GRS.

During an average follow-up of 7.1 (±2.3) years, 698 women assigned to HT intervention and 910 women assigned to placebo experienced a fracture. The incidence rate of any fracture was 19 per 1,000 person-years in HT intervention group and 26 per 1,000 person-years in placebo group. **Figures 1A** and **1B** show that women assigned to placebo experienced higher fracture incidence compared to women randomized to HT intervention. In women assigned to placebo, we observed significant linear trends of higher fracture risk as genetic susceptibility increased. For Fx-GRS, these higher fracture risks ranged from HR=1.26, 95% CI: 1.02-1.55 for the lowest genetic susceptibility group to HR=1.75, 95% CI: 1.44-2.14 for the most genetically susceptible (p-for-trend<0.001). The pattern was similar for the BMD-GRS with p-for-trend<0.0001.

We observed a significant additive interaction between the GRSs and HT. The joint effect of randomization to placebo and high genetic susceptibility on total fracture risk was larger than expected from the sum of the individual effects. The multivariable-adjusted RERI values (95% CI, p-value) were 0.35 (0.01-0.69; p=0.047) for the Q4 of Fx-GRS, and 0.38 (0.01-0.75;

p=0.046) for Q4 for the BMD-GRS, indicating that those who were assigned to placebo are at higher risk of fracture if their genetic predisposition is high.

We found no evidence for statistically significant multiplicative interaction for either GRSs on total fracture. In Cox proportional hazard regression, the multivariable adjusted p-value for multiplicative interaction was 0.49 for Fx-GRS and 0.63 for BMD-GRS. The results from case-only analyses similarly showed no multiplicative interaction between GRS and HT (Fx-GRS p-for-interaction=0.53, BMD-GRS p-for-interaction=0.84).

When we compared the effect of HT intervention within GRS strata, somewhat greater fracture risk reduction was observed in women with high genetic predisposition for fracture or low BMD (**Figure 2A and 2B**). Multivariable-adjusted relative risk reductions with HT intervention were 21% for Fx-GRS Q1, 26% for Q2-3, and 34% for Q4 (**Figure 2A**). However, the difference among GRS strata was not statistically significant (p-for-heterogeneity=0.40). We observed similar risk reductions in BMD-GRS quartile strata (24%, 24%, and 35% for BMD-GRS Q1, Q2-3 and Q4, respectively), however, the heterogeneity was statistically insignificant (p=0.40) (**Figure 2B**). **Supplemental Figure 2** shows the Kaplan Meier plots for the fracture and BMD GRSs, respectively. These plots show the highest cumulative hazards for women who were randomized to placebo, where women with the highest genetic susceptibility are at highest fracture risk.

In exploratory analyses using fracture subgroups, we found no evidence for statistically significant multiplicative interaction for either score or any of fracture sites (p-for-interaction>0.05) (**Supplemental Figure 3**). However, we again noted a significant additive interaction between GRS and HT on the risk of central body and upper limb fracture in the highest GRS quartile (Fx-GRS Q4 p-for-RERI=0.04 for upper limb fracture, BMD-GRS Q4 p-for-RERI=0.01 for central body fracture) (data not shown). Relative risk reduction with HT use was stronger in women with high genetic predisposition in central and upper limb fracture, but the heterogeneity was not statistically significant.

Supplemental Table 5 shows the evidence for interaction of HT with single SNPs that make up the GRS. Three SNPs were nominally significant (rs7812088 in *ABCF2*, rs1346004 in *GALNT3*, rs3801387 in *WNT16*) but not significant after adjusting for multiple testing.

DISCUSSION

We examined cumulative effects of previously reported 16 SNPs associated with both BMD and fracture, and 50 BMD associated SNPs on fracture risk. We observed that higher fracture and BMD GRS are significantly associated with increased fracture risk and that there is a significant additive interaction for both GRSs and HT use on risk of total fracture. Although statistically insignificant, we observed a trend towards greater total fracture risk reduction with HT use among the most genetically susceptible women. In exploratory analyses, we also found evidence of additive interaction for central body and upper limb fractures.

Our study confirms previous findings of cumulative main effects of the GEFOS meta-GWAS variants with fracture in our cohort of 9,922 European American women. Estrada et al. showed that a GRS based on 16 SNPs associated with both BMD and fracture had a significant association with any fracture among elderly women[6]. Furthermore, Erikson et al. demonstrated that a GRS based on 63 BMD SNPs were also associated with the incidence of any type of fracture and hip fractures in older participants[7]. In our analyses, we constructed the BMD-GRS with 50 QC passed SNPs that showed an association with FN-BMD among females while excluding variants that showed secondary conditional signals. Although we constructed BMD-

GRS with a subset of 63 SNPs, our reported findings are in agreement with the previous studies. The results provide solid impetus for our gene-HT interaction study, as these variants are meta-GWAS significant and replicated in our sample.

Within the context of a randomized placebo controlled trial, we were able to examine and formally test for the presence of interaction of HT and GRS on fracture on both additive and multiplicative scales. Our results suggest that the interaction between HT and genetic susceptibility is additive rather than multiplicative. Additive interaction is of a greater public health and clinical importance because it allows us to identify groups that may get more benefit from intervention through risk stratification.

Herein we found that women assigned to placebo had a higher risk of fracture, and these risks were even more elevated in the presence of high genetic susceptibility. Our results point to the potential future benefit of genetic risk assessment in clinical decision making for a targeted intervention, should our results be replicated in other studies. If replicated, these study findings may increase our evidence for the utility of genetic information as an additional factor to consider in evaluating the benefit-risk profile of osteoporosis treatment, and further contribute to our knowledge of personalized recommendations for fracture prevention. Benefits associated with other therapies such as bisphosphonates for osteoporosis prevention and treatment may also be greater in these high-risk women. In an observational study of 1,023 postmenopausal Korean women, Lee et al analyzed GRS based on SNPs from GEFOS meta-analysis (GRS63) for their predictability of fracture among women taking HT or bisphosphonates[31]. Among women who were not on either treatment, those in the highest tertile had almost 4 times increased risk of any incidental fracture compared to those in the medium and lowest tertiles, while there was no statistically significant increase in fracture risk with increase in genetic risk among women taking HT or bisphosphonates. Although further evaluation in other race/ethnic groups is required, findings from the study suggest that bisphosphonates as well as HT reduce the risk of fracture among women with at high genetic risk.

Estrogen deficiency causes imbalances in bone homeostasis, favoring bone resorption over formation, which leads to bone loss and susceptibility to fractures. Estrogen exerts effects on bone cells and the immune system[32-36]. Although some of the genes in the GRSs in our analyses are observed to be clustered within several biological bone-related pathways[6], the roles of most of these genes on bone response to HT use are largely unknown. Future large-scale studies are needed elucidate the biologic mechanism of interactions of genes with HT on fracture risk.

We observed stronger relative risk reduction with HT intervention by increasing GRS quartile level in total, central and upper limb fracture, while the opposite was noted in lower limb fracture. Although we do not know the exact reason for this inconsistency, findings from the multicenter Study of Osteoporotic Fractures (SOF) suggest that ankle fractures were not associated with total hip, total spine and FN-BMD[37]. In our sample, ankle fracture constituted approximately 35% of lower limb fracture. Major risk factors for ankle fractures include body weight and falls. Because both GRSs are derived from SNPs identified in the GEFOS for BMD, it is possible that some variants that account for fracture risk other than BMD such as falls are unrepresented. Therefore, analyses of a GRS that includes variants identified from future GWAS for fracture risk are necessary to improve fracture risk stratification at each anatomic site.

To our knowledge, this study is the first to investigate gene-HT interaction on fracture in EA women using multiple genetic variants from a meta-GWAS with the utilization of the powerful randomized clinical trial design. The random assignment of HT ensures that both known and unknown characteristics that can impact the outcome are uniformly distributed across

intervention arms, thereby minimizing biases and confounding. In addition, the well-defined cohort, comprehensive and uniform data collection on participants' characteristics, prospective follow-up and adjudicated fracture outcomes are also strengths of our study.

Our study also has several limitations. First, both Fx-GRS and BMD-GRS in our analyses were derived from common variants identified in a meta-GWAS, which account for only a small portion of genetic variance in BMD[6,7]. In addition, GRSs in our analyses only account for BMD-dependent SNPs. Although BMD is strongest predictor of fracture, fracture risk is multifactorial and it is possible that some genetic variants that account for fracture risk are unrepresented. Another limitation of our study is our inability to determine the influence of BMD on the association of GRS and fracture risk in our sample because not all of the participants had their BMD measured. A previous study reported that although the associations between GRS and risk of total fracture remained statistically significant after adjusting for clinical risk factors, the association were attenuated after being adjusted for FN-BMD[13]. However, because assessment of a fracture risk prediction model for its clinical utility was not the purpose of our study, the BMD variable is not relevant in the current analyses. Further, we had limited power to investigate interactions in the fracture subtype analyses due to sample size. Lastly, we examined only EA postmenopausal women who enrolled in WHI HT trials, therefore our findings may not be generalizable to other race/ethnic groups. We observed that the additive interaction was largely driven by the WHIMS+ sample who are slightly older than GARNET sample. However, we have lower power to detect the interaction when our sample is stratified, so we are cautious about drawing strong conclusions in this case.

Our results support further consideration of GRS as another factor in determining potential risk and benefit ratio of osteoporosis prevention and treatment. Future randomized trials of new osteoporosis treatment can benefit from the GRS-treatment interaction analysis to determine whether the additive interactions between genetic susceptibility and anti-osteoporotic therapy exist, and to assess the benefits of targeted treatment to women at genetically highest fracture risk.

ACKNOWLEDGMENTS

Corresponding author and person to whom reprint request should be addressed: Heather M. Ochs-Balcom, Ph.D., University at Buffalo, The State University of New York, 270 Farber Hall, Buffalo, NY 14214-8001, Phone: 716-829-5338, Fax: 716-829-2979, Email: hmochs2@buffalo.edu

Funding / support:

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201600003C, HHSN268201600002C, HHSN268201600004C, HHSN268201600001C, and HHSN271201100004C. The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf.

Funding support for WHI GARNET was provided through the National Human Genome Research Institute (NHGRI) Genomics and Randomized Trials Network (GARNET) (Grant Number U01 HG005152). Assistance with phenotype harmonization and genotype cleaning, as

well as with general study coordination, was provided by the GARNET Coordinating Center (U01 HG005157). Assistance with data cleaning was provided by the National Center for Biotechnology Information. Funding support for genotyping, which was performed at the Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, was provided by the NIH Genes, Environment and Health Initiative (U01 HG004424).

The datasets used for the analyses described in this manuscript were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap through dbGaP accession phs000675.v2.p3 (WHIMS+) and phs000315.v6.p3 (GARNET).

Grants or fellowships supporting the writing of the paper

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201600003C, HHSN268201600002C, HHSN268201600004C, HHSN268201600001C, and HHSN271201100004C. The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf.

Funding support for WHI GARNET was provided through the National Human Genome Research Institute (NHGRI) Genomics and Randomized Trials Network (GARNET) (Grant Number U01 HG005152). Assistance with phenotype harmonization and genotype cleaning, as well as with general study coordination, was provided by the GARNET Coordinating Center (U01 HG005157). Assistance with data cleaning was provided by the National Center for Biotechnology Information. Funding support for genotyping, which was performed at the Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, was provided by the NIH Genes, Environment and Health Initiative (U01 HG004424).

The datasets used for the analyses described in this manuscript were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap through dbGaP accession phs000675.v2.p3 (WHIMS+) and phs000315.v6.p3 (GARNET).

Author contributions:

Study conception and design: YW, JWW, HMO; Collection and assembly of data: JWW, LP, JN, HMO; Data analysis: YW, LS, HMO; Data interpretation: YW, JWW, LS, HMO; Drafting of the manuscript: YW, HMO; Critical revision of the manuscript for content: YW, JWW, LS, LP, KMH, JN, RDJ, SKH, RN, CJC, HMO; Approving final version of manuscript: YW, JWW, LS, LP, KMH, JN, RDJ, SKH, RN, CJC, HMO; Obtained funding: JWW, HMO

Disclosures

The authors have nothing to disclose.

REFERENCES

1. Jackson RD, Wactawski-Wende J, LaCroix AZ, et al. Effects of conjugated equine estrogen on risk of fractures and BMD in postmenopausal women with hysterectomy: results from the women's health initiative randomized trial. *J Bone Miner Res.* 2006;21(6):817-828.
2. Cauley JA, Robbins J, Chen Z, et al. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. *Jama.* 2003;290(13):1729-1738.
3. Cauley JA, Wu L, Wampler NS, et al. Clinical risk factors for fractures in multi-ethnic women: the Women's Health Initiative. *J Bone Miner Res.* 2007;22(11):1816-1826.
4. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporos Int.* 2008;19(4):385-397.
5. Woolf AD, Akesson K. Preventing fractures in elderly people. *BMJ.* 2003;327(7406):89-95.
6. Estrada K, Styrkarsdottir U, Evangelou E, et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat Genet.* 2012;44(5):491-501.
7. Eriksson J, Evans DS, Nielson CM, et al. Limited clinical utility of a genetic risk score for the prediction of fracture risk in elderly subjects. *J Bone Miner Res.* 2015;30(1):184-194.
8. Michaelsson K, Melhus H, Ferm H, Ahlbom A, Pedersen NL. Genetic liability to fractures in the elderly. *Arch Intern Med.* 2005;165(16):1825-1830.
9. Deng HW, Chen WM, Recker S, et al. Genetic determination of Colles' fracture and differential bone mass in women with and without Colles' fracture. *J Bone Miner Res.* 2000;15(7):1243-1252.
10. Andrew T, Antioniades L, Scurrah KJ, Macgregor AJ, Spector TD. Risk of wrist fracture in women is heritable and is influenced by genes that are largely independent of those influencing BMD. *J Bone Miner Res.* 2005;20(1):67-74.
11. Liu C-T, Karasik D, Zhou Y, et al. Heritability of prevalent vertebral fracture and volumetric bone mineral density and geometry at the lumbar spine in three generations of the framingham study. *Journal of Bone and Mineral Research.* 2012;27(4):954-958.
12. Karasik D, Rivadeneira F, Johnson ML. The genetics of bone mass and susceptibility to bone diseases. *Nat Rev Rheumatol.* 2016;12(6):323-334.
13. Ho-Le TP, Center JR, Eisman JA, Nguyen HT, Nguyen TV. Prediction of Bone Mineral Density and Fragility Fracture by Genetic Profiling. *J Bone Miner Res.* 2016.
14. The Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials.* 1998;19(1):61-109.
15. Stefanick ML, Cochrane BB, Hsia J, Barad DH, Liu JH, Johnson SR. The Women's Health Initiative postmenopausal hormone trials: overview and baseline characteristics of participants. *Ann Epidemiol.* 2003;13(9 Suppl):S78-86.
16. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *Jama.* 2002;288(3):321-333.
17. Manson JE, Chlebowski RT, Stefanick ML, et al. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. *Jama.* 2013;310(13):1353-1368.

18. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. *Jama.* 2004;291(14):1701-1712.
19. NCBI Database of Genotypes and Phenotypes (dbGaP). WHIMS+ GWAS: GWAS on Selected Women's Health Initiative (WHI) Hormone Trial European Americans http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000675.v2.p3&phv=196087&phd=&pha=&pht=3567&phvf=&phdf=&phaf=&phtf=&phs_code=&dssp=1&consent=&temp=1. Accessed 01/16, 2016.
20. Shumaker SA, Reboussin BA, Espeland MA, et al. The Women's Health Initiative Memory Study (WHIMS): a trial of the effect of estrogen therapy in preventing and slowing the progression of dementia. *Control Clin Trials.* 1998;19(6):604-621.
21. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354(7):669-683.
22. Crandall CJ, Yildiz VO, Wactawski-Wende J, et al. Postmenopausal weight change and incidence of fracture: post hoc findings from Women's Health Initiative Observational Study and Clinical Trials. *BMJ.* 2015;350:h25.
23. Laurie CC, Doheny KF, Mirel DB, et al. Quality control and quality assurance in genotypic data for genome-wide association studies. *Genet Epidemiol.* 2010;34(6):591-602.
24. Cornelis MC, Agrawal A, Cole JW, et al. The Gene, Environment Association Studies consortium (GENEVA): maximizing the knowledge obtained from GWAS by collaboration across studies of multiple conditions. *Genet Epidemiol.* 2010;34(4):364-372.
25. Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet.* 2009;84(2):210-223.
26. NCBI Database of Genotypes and Phenotypes (dbGaP). WHI Harmonized and Imputed GWAS Data. http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000746.v1.p3. Accessed 01/16, 2016.
27. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics.* 2015;31(21):3555-3557.
28. Thomas D. Gene--environment-wide association studies: emerging approaches. *Nat Rev Genet.* 2010;11(4):259-272.
29. Li R, Chambless L. Test for additive interaction in proportional hazards models. *Ann Epidemiol.* 2007;17(3):227-236.
30. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. *Epidemiology.* 1992;3(5):452-456.
31. Lee SH, Cho EH, Ahn SH, et al. Prediction of Future Osteoporotic Fracture Occurrence by Genetic Profiling: A 6-year Follow-up Observational Study. *J Clin Endocrinol Metab.* 2016:jc20153972.
32. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. *J Clin Invest.* 2006;116(5):1186-1194.
33. Manolagas SC, O'Brien CA, Almeida M. The role of estrogen and androgen receptors in bone health and disease. *Nat Rev Endocrinol.* 2013;9(12):699-712.
34. Nakamura T, Imai Y, Matsumoto T, et al. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell.* 2007;130(5):811-823.

35. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev.* 2000;21(2):115-137.
36. Marcus R, Feldman D, Dempster DW, Luckey M, Cauley JA. Osteoporosis. Fourth edition. ed. Amsterdam: Elsevier/Academic Press,; 2013: ClinicalKey https://www.clinicalkey.com/dura/browse/bookChapter/3-s2.0-C20111045666.
37. Stone KL, Seeley DG, Lui LY, et al. BMD at multiple sites and risk of fracture of multiple types: long-term results from the Study of Osteoporotic Fractures. *J Bone Miner Res.* 2003;18(11):1947-1954.

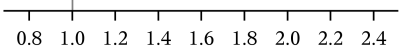
Table 1. Baseline characteristics of participants according to hormone therapy randomization.

	HT Intervention (N=5,023)	Placebo (N=4,899)	
Characteristics	**N (%), Means(SD)**	**N (%), Means(SD)**	**P**
WHI GWAS			0.02
WHIMS+[1]	2818 (56.1%)	2858 (58.3%)	
GARNET[2]	2205 (43.9%)	2041 (41.7%)	
Age at screening, y	66.9 (6.5)	67.1 (6.4)	0.13
Years since menopause, y			0.06
< 10	697 (15.4%)	625 (14.0%)	
≥ 10, <20	1703 (37.6%)	1768 (39.7%)	
≥ 20	2125 (47.0%)	2066 (46.3%)	
BMI (kg/m^2)	29.0 (5.7)	28.8 (5.8)	0.10
Self-reported general health			0.85
Excellent/Very good/Good	4631 (92.8%)	4529 (92.9%)	
Fair/Poor	358 (7.2%)	345 (7.1%)	
Recent personal HT use at baseline			0.77
Never used	3309 (65.9%)	3260 (66.6%)	
Past user	1354 (27.0%)	1294 (26.4%)	
Recent user	359 (7.1%)	343 (7.0%)	
Recent personal HT duration, y			0.16
None	3309 (65.9%)	3260 (66.5%)	
< 5	1003 (20.0%)	987 (20.1%)	
≥ 5, < 10	312 (6.2%)	253 (5.2%)	
≥ 10	399 (7.9%)	399 (8.1%)	
Smoking status			0.93
Never	2551 (51.3%)	2466 (51.0%)	
Former	1976 (39.8%)	1939 (40.1%)	
Current	444 (8.9%)	428 (8.9%)	
Falls in last 12 months			0.89
0	3101 (65.5%)	3063 (65.5%)	
1	992 (20.9%)	990 (21.2%)	
≥2	644 (13.6%)	621 (13.3%)	
Fracture at Age 55+	884 (21.9%)	918 (22.7%)	0.35
Total MET-hours/week[3]	11.1 (13.2)	11.0 (12.8)	0.87
Height (cm)	161.3 (6.0)	161.4 (6.0)	0.34
Weight (kg)	75.5 (15.7)	75.1 (15.7)	0.20

[1] WHIMS+: The Women's Health Initiative Memory Study +
[2] GARNET: The Genomics and Randomized Trials Network
[3] MET: Metabolic Equivalent of Task

A. Fx-GRS

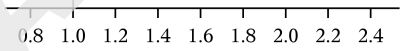
GRS quartile	N of event (censored)	HR for Fracture	HR (95% CI)	RERI (95% CI)
HT intervention				
Q1	159 (1093)		1.00 (Reference)	
Q2-3	358 (2145)		1.14 (0.94, 1.37)	
Q4	181 (1087)		1.15 (0.93, 1.42)	
p for trend			0.21	
HT placebo				
Q1	193 (1035)		1.26 (1.02, 1.55)	
Q2-3	462 (1997)		1.53 (1.28, 1.83)	0.13 (-0.15, 0.42), p=0.359
Q4	255 (957)		1.75 (1.44, 2.14)	0.35 (0.01, 0.69), p=0.047
p for trend			<0.001	



p for multiplicative interaction
= 0.49 (Cox regression)
0.53 (Case-only analysis)

B. BMD-GRS

GRS quartile	N of event (censored)	HR for Fracture	HR (95% CI)	RERI (95% CI)
HT intervention				
Q1	145 (1112)		1.00 (Reference)	
Q2-3	368 (2150)		1.27 (1.05, 1.54)	
Q4	185 (1062)		1.28 (1.03, 1.59)	
p for trend			0.03	
HT placebo				
Q1	187 (1036)		1.32 (1.06, 1.64)	
Q2-3	456 (1987)		1.66 (1.38, 2.00)	0.07 (-0.24, 0.39), p=0.640
Q4	267 (966)		1.98 (1.62, 2.42)	0.38 (0.01, 0.75), p=0.046
p for trend			<0.0001	

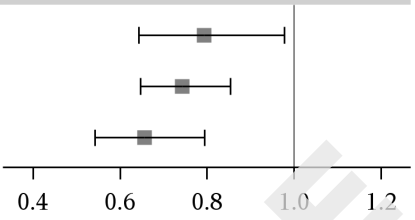


p for multiplicative interaction
= 0.63 (Cox regression)
0.84 (Case-only analysis)

A. Fx-GRS

GRS quartile	HT intervention N of cases (annualized %)	Placebo N of cases (annualized %)	HR (95% CI)
Q1 (Lowest)	159 (1.74)	193 (2.20)	0.79 (0.64, 0.98)
Q2-3	358 (1.98)	462 (2.66)	0.74 (0.65, 0.85)
Q4(Highest)	181 (1.99)	255 (3.05)	0.66 (0.54, 0.79)

P for Heterogeneity = 0.40



B. BMD-GRS

GRS quartile	HT intervention N of cases (annualized %)	Placebo N of cases (annualized %)	HR (95% CI)
Q1 (Lowest)	145 (1.59)	187 (2.12)	0.76 (0.61, 0.94)
Q2-3	368 (2.01)	456 (2.66)	0.76 (0.66, 0.87)
Q4(Highest)	185 (2.07)	267 (3.14)	0.65 (0.54, 0.79)

P for Heterogeneity = 0.40