Published in final edited form as:

Breast Cancer Res Treat. 2015 December ; 154(3): 543–555. doi:10.1007/s10549-015-3634-6.

ESR1 and ESR2 polymorphisms in the BIG 1-98 trial comparing adjuvant letrozole versus tamoxifen or their sequence for early breast cancer

Brian Leyland-Jones, MB BS PhD.,

Avera Cancer Institute, Sioux Falls, SD

Kathryn P. Gray, PhD.,

International Breast Cancer Study Group Statistical Center, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Harvard T. H. Chen School of Public Health, 450 Brookline Ave, Boston, MA 02215

Mark Abramovitz, PhD.,

VM Institute of Research, 6100 Av Royalmount, Montréal H4P 2R2, QC

Mark Bouzyk, PhD.,

AKESOGen, Inc., 3155 Northwoods PI NW, Norcross, GA 30071

Brandon Young, BS.,

Avera Cancer Institute, Sioux Falls, SD 11099 North Torrey Pines, Suite 160, La Jolla, CA 92037 USA

Bradley Long, MS.,

Genomic and Molecular Pathology, University of Chicago, 900 E 57th St. Room 1260D, Chicago, IL 60637

Roswitha Kammler, BA.,

International Breast Cancer Study Group Coordinating Center and Central Pathology Office, Effingerstrasse 40 3008 Bern, Switzerland

Patrizia Dell'Orto, DSc.,

International Breast Cancer Study Group Central Pathology Office, Division of Pathology and Laboratory Medicine, European Institute of Oncology, Via Ripamonti 435, 20146 Milan, Italy

Maria Olivia Biasi, ScD.,

Division of Pathology and Laboratory Medicine, European Institute of Oncology, Via Ripamonti 435, 20146 Milan, Italy

Co-Corresponding Authors: Brian Leyland-Jones, M.B. B.S., Ph.D. Vice President of Molecular and Experimental Medicine; Avera Cancer Institute; 1000 E 23rd Street, Sioux Falls, SD 57105 Telephone: (605) 322-6900, Fax: (605) 322-6901, bleylandj@aol.com. Karen N Price, International Breast Cancer Study Group Statistical Center, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston MA, 02215. Tel: 617-632-2459; Fax: 617-632-2444. price@jimmy.harvard.edu.

Members of the BIG 1-98 Collaborative Group who submitted tumor blocks are listed in Supplementary Appendix, available with the full text of this article at [journal website].

The BIG 1-98 trial registration ID: NCT00004205

Conflicts of Interest

None of the authors have a conflict of interest.

Beat Thürlimann, MD.,

Breast Center, Kantonsspital St. Gallen, Rorschacher Strasse 95 CH- 9007 St. Gallen, Switzerland, Swiss Group for Clinical Cancer Research (SAKK) and International Breast Cancer Study Group Bern, Switzerland

Vernon Harvey, MD.,

Auckland City Hospital, PO Box 26498, Epsom, Auckland 1344, New Zealand and Australia and New Zealand Clinical Trials Group, Newcastle, Australia and International Breast Cancer Study Group, Bern, Switzerland

Patrick Neven, MD.,

KULeuven (University of Leuven), Department of Oncology, Multidisciplinary Breast Center, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium

Laurent Arnould, MD.,

Institute Georges François Leclerc, 1 rue Professeur Marion BP 77 980 21079 Dijon cedex - France

Rudolf Maibach, PhD.,

International Breast Cancer Study Group Coordinating Center, Effingerstrasse 40 3008 Bern, Switzerland

Karen N. Price, BS.,

International Breast Cancer Study Group Statistical Center, Frontier Science and Technology Research Foundation, 450 Brookline Ave, Boston, MA 02215, USA

Alan S. Coates, MD.,

International Breast Cancer Study Group, Bern, Switzerland and University of Sydney, Sydney, Australia

Aron Goldhirsch, MD, PhD.,

Program for Breast Health (Senology), European Institute of Oncology, Via Ripamonti 435, 20146 Milan, Italy, International Breast Cancer Study Group, Bern, Switzerland

Richard D. Gelber, PhD.,

International Breast Cancer Study Group Statistical Center, Dana-Farber Cancer Institute, Harvard Medical School, Harvard T.H. Chan School of Public Health, Frontier Science and Technology Research Foundation, 450 Brookline Ave, Boston, MA 02215 USA

Olivia Pagani, MD.,

Institute of Oncology of Southern Switzerland (IOSI), Ospedale Italiano, Via Capelli, 6962 Viganello, Switzerland. Swiss Group for Clinical Cancer Research (SAKK) and International Breast Cancer Study Group, Bern, Switzerland

Giuseppe Viale, MD.,

International Breast Cancer Study Group Central Pathology Office, Division of Pathology and Laboratory Medicine, European Institute of Oncology, University of Milan, Via Ripamonti 435, 20146 Milan, Italy

James M. Rae, PhD., and

Division of Hematology/Oncology, University of Michigan Comprehensive Cancer Center, 1500 E Medical Cntr Drive/SPC 5946, Ann Arbor, MI 48109

Meredith M. Regan, ScD.

International Breast Cancer Study Group Statistical Center, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, and Harvard Medical School, 450 Brookline Ave, Boston, MA 02215, USA

on behalf of the BIG 1-98 Collaborative Group

Brian Leyland-Jones: bleylandj@aol.com; Kathryn P. Gray: pkruan@jimmy.harvard.edu; Mark Abramovitz: mark.abramovitz@gmail.com; Mark Bouzyk: mbouzyk@akesogen.com; Brandon Young: Brandon.Young@avera.org; Bradley Long: Bradley.Long@uchospitals.edu; Roswitha Kammler: Rosita.Kammler@ibcsg.org; Patrizia Dell'Orto: patrizia.dellorto@ieo.it; Maria Olivia Biasi: olivia.biasi@ieo.it; Beat Thürlimann: beat.thuerlimann@kssg.ch; Vernon Harvey: vernonh@adhb.govt.nz; Patrick Neven: patrick.neven@uzleuven.be; Laurent Arnould: larnould@dijon.fnclcc.fr; Rudolf Maibach: rudolf.maibach@ibcsg.org; Karen N. Price: price@jimmy.harvard.edu; Alan S. Coates: alan.coates@ibcsg.org; Aron Goldhirsch: aron.goldhirsch@ibcsg.org; Richard D. Gelber: gelber@jimmy.harvard.edu; Olivia Pagani: olivia.pagani@ibcsg.org; Giuseppe Viale: giuseppe.viale@ieo.it; James M. Rae: jimmyrae@med.umich.edu; Meredith M. Regan: mregan@jimmy.harvard.edu

Abstract

Purpose—Estrogen receptor 1 (*ESR1*) and *ESR2* gene polymorphisms have been associated with endocrine-mediated physiological mechanisms, and inconsistently with breast cancer risk and outcomes, bone mineral density changes and hot flushes/night sweats (HF/NS).

Methods—DNA was isolated and genotyped for six *ESR1* and two *ESR2* single nucleotide polymorphisms (SNPs) from tumor specimens from 3691 postmenopausal women with hormone receptor-positive breast cancer enrolled in the BIG 1-98 trial to receive tamoxifen and/or letrozole for five years. Associations with recurrence and adverse events (AE) were assessed using Cox proportional hazards models.

Results—3401 samples were successfully genotyped for five SNPs. *ESR1* rs9340799(XbaI) (T>C) variants CC or TC were associated with reduced breast cancer risk (HR=0.82,95%CI=0.67–1.0), *ESR1* rs2077647(T>C) variants CC or TC was associated with reduced distant recurrence risk (HR=0.69,95%CI=0.53–0.90), both regardless of the treatments. No differential treatment effects (letrozole vs. tamoxifen) were observed for the association of outcome with any of the SNPs. Letrozole-treated patients with rs2077647(T>C) variants CC,TC had a reduced risk of bone AE (HR=0.75,95%CI=0.58–0.98, $P_{\text{interaction}}=0.08$), whereas patients with rs4986938(G>A) genotype variants AA and AG had an increased risk of bone AE (HR=1.37,95%CI=1.01–1.84, $P_{\text{interaction}}=0.07$).

Conclusions—We paradoxically observed 1) rare *ESR1* homozygous polymorphisms were associated with lower recurrence, and 2) *ESR1* and *ESR2* SNPs were associated with bone AEs in letrozole-treated patients. Genes that are involved in estrogen signaling and synthesis have the potential to affect both breast cancer recurrence and side effects, suggesting that individual treatment strategies can incorporate not only oncogenic drivers but also SNPs related to estrogen activity.

Keywords

ESR1; ESR2; letrozole; tamoxifen; polymorphism

Introduction

The relationship between estrogen receptors (ER) and their ligand, the hormone estrogen (17 β -estradiol), and the enzymes that synthesize it are not well understood. The *ESR1* and *ESR2* genes encode for ER α and ER β , respectively, which are responsible for the effects of estrogens and are therapeutic targets of selective estrogen receptor modulators (SERMs) including the first breast cancer targeting drug tamoxifen. The ERs are complex genes with many polymorphic and splice variants. Genetically determined variants in sex steroid hormone pathways have been related to several measures of health status (i.e. circulating hormone concentrations, menstrual cycle profiles, lipids, diabetes mellitus, depressive symptoms, measures of cognition, bone mineral density (BMD) and vasomotor symptoms) in a community-based population of premenopausal women[1]. Polymorphisms in the *ESR1* and *ESR2* genes have been reported to be associated with multiple endocrine-mediated physiological mechanisms including lipid profile[2–6], mammographic density[7], venous thromboembolism[8, 9], cognition[10]; and inconsistently associated with breast cancer risk and outcomes and BMD changes[11–17].

The role that *ESR1* and *ESR2* polymorphisms play in breast cancer outcomes and the effects that they exert on BMD in postmenopausal women requires further studies to confirm any effect they may have in other populations. No pharmacogenetic studies have been published on *ESR1* and *ESR2* polymorphisms and the differential effectiveness or side effect profile of SERMs or aromatase inhibitors (AIs) in postmenopausal women with breast cancer.

We investigated the clinical relevance of *ESR1* and *ESR2* SNPs in postmenopausal women treated with tamoxifen and/or letrozole in the Breast International Group (BIG) 1-98 trial. We hypothesized that *ESR1* and/or *ESR2* phenotypes that potentially result in receptor activity changes could affect disease outcomes that varies by type of endocrine therapy. We also investigated potential associations between *ESR1* and *ESR2* SNPs and hot flashes or night sweats (HF/NS) during the first two years of treatment, and bone adverse events (AEs) during and subsequent to treatment.

Materials and Methods

Patients

The BIG 1-98 trial is a randomized, phase III, double blind study that recruited postmenopausal women with estrogen receptor (ER) and/or progesterone receptor (PgR)-positive early breast cancer. From 1998 to 2000, women were randomly assigned to receive monotherapy with letrozole (Femara®, Novartis) 2.5mg daily or tamoxifen 20 mg daily for five years, and from 1999 to 2003, to one of four arms: tamoxifen or letrozole for five years or sequential therapy comprising letrozole for two years followed by tamoxifen for three years, or tamoxifen for two years followed by letrozole for three years[18–22].

A total of 8010 women were enrolled between March 1998 and May 2003. After the initial trial results were released in 2005, patients assigned to tamoxifen monotherapy were offered the chance to switch to letrozole for the remainder of their adjuvant therapy, and 619 (25.2%) of them did so.

All participants provided written informed consent. Ethics committees and relevant health authorities approved the protocol. Trial participants were followed clinically at baseline, every 6 months for the first 5 years during blinded study drug dispensing, and yearly thereafter. AEs were recorded at study visits and graded according to the Common Toxicity Criteria (CTC) v2.0.

Tissue Collection, DNA Extraction, and ESR1 and ESR2 Genotyping

The International Breast Cancer Study Group (IBCSG), between 1998 and 2010 in accordance with institutional guidelines and national laws, carried out retrospective tissue collection. Novartis, the BIG 1-98 pharmaceutical partner, provided funding to partially cover associated institution costs. The IBCSG Biological Protocols Working Group approved this project. All material processing and genotyping were done without the knowledge of patients' treatment assignments or outcomes. Formalin-fixed, paraffin-embedded (FFPE) primary breast cancer tissue blocks for 5786 of 8010 enrolled in BIG 1-98 patients were assessed for availability of material for translational research. An area that was representative of the invasive tumor component was identified, and one or two 1-mm cores were punched in this area. DNA was isolated for potential ESR1 and ESR2 genotyping for 3691 of 8010 trial patients, and 3401 were included in the analytic cohort (Figure 1).

Genomic DNA was extracted using the QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA) according to manufacturer's instructions. DNA was eluted with 60 µL of sterile distilled water, quantified and quality controlled according to the 260/280 nm ratio using the Infinite M 200 NanoQuant (Tecan, Mannedorf, Switzerland) and aliquoted at a concentration of 10 ng/µL.

DNA samples were genotyped for six ESR1 (rs9340799(XbaI), rs2234693(PvuII), rs11963577, rs2077647, rs9341070, rs746432) and two ESR2 (rs4986938, rs1256049) SNPs, selected based on location and/or whether they had been evaluated in prior studies. Five of eight SNPs (4 in ESR1, 1 in ESR2) passed genotype quality assurance and were included in our statistical analyses. These SNPs included three *ESR1* intronic SNPs, rs9340799 T>C(XbaI), rs2234693 T>C(PvuII) and rs11963577 C>T; and one *ESR1* synonymous SNP, rs2077647 T>C; and one *ESR2* SNP in the 3'UTR, rs4986938 G>A.

All samples were processed using polymerase chain reaction (PCR)-based methods and were genotyped on the GenomeLab SNPstream Genotyping System (Beckman Coulter, Brea, CA) in a Clinical Laboratory Improvement Amendments (CLIA)-compliant laboratory. All reagents were preformulated and included in the GenomeLab SNPware Reagent kit. The GenomeLab SNPstream Genotyping System Software Suite v2.3 and SNP genotyping software (ABI) were used for array imaging and genotype calling. One SNP rs2234693 was re-run on the 7900HT Fast Real-Time 200 PCR System (Applied Biosystems, Foster City, CA) using a commercially-available predesigned TaqMan assay. The optimization employed Applied Biosystems' TaqMan probes, in conjunction with the KlearKall Mastermix from KBioscience (Beverly, MA).

The *ESR1* and *ESR2* genotypes were tested for deviations from Hardy-Weinberg Equilibrium (HWE). Significant violations were not observed across most sites, with some minor exceptions (Supplementary Table S1). As an additional control, we compared genotypes derived from tumor versus whole blood DNA in 120 matched samples as an extension of our recently published report[23]. All five of the *ESR* genotypes tested (*ESR1*:rs9340799, rs2234693, rs11963577 and rs2077647; *ESR2*:rs4986938) showed very high to complete concordance between tumor versus whole blood (97.3% to 100%) suggesting that tumor-derived genotypes accurately represented germline genotypes.

Statistical Analyses

Disease endpoints were: 1) breast cancer-free interval (BCFI): time from randomization to first occurrence of invasive breast cancer recurrence at a local, regional, or distant site, or a new invasive cancer in the contralateral breast; and 2) distant recurrence-free interval (DRFI): time from randomization to recurrence at a distant site. In the absence of an event, the endpoints were censored at last follow-up time, or in the analyses focused on 5-year monotherapy, at time of selective crossover from tamoxifen to letrozole after dissemination of the primary trial results in 2005[18]. Cox proportional hazards models were stratified by randomization option (2 or 4-arm), and prior chemotherapy (and by treatment assignment for analyses that included all patients). The models adjusted for these characteristics at randomization: local therapy (mastectomy vs. breast-conserving surgery), tumor grade (2/3 vs. 1/unknown), tumor size (>2cm vs. ≤2cm/unknown), nodal status (positive vs. negative/Nx), peritumoral vascular invasion (present vs. absent/unknown), HER2 status (positive vs. negative/unknown) and Ki-67 labeling index (≥14% vs. <14%/unknown). The proportional hazards assumption was assessed by testing for genotype-by-time interaction and using Schoenfeld residuals.

To investigate the association of *ESR1* and *ESR2* genotypes with early onset of HF/NS, all treatment groups were included (N=3401) (Figure 1), and the endpoint was defined as the time from randomization to the first report of new or worsening HF/NS of any grade within 23 months of randomization (prior to the treatment switch in the sequential arms). The Cox proportional hazards model was adjusted for race (white vs. other), age (years, in quartiles), body mass index (<18.5, 18.5–29.9, ≥30 kg/m²), history of HRT use (never, within last 3 months, ≥3 months ago), and baseline HF/NS (present vs. absent).

For the association of bone AEs, analyses were limited to patients assigned to five years of letrozole or tamoxifen monotherapy (N=1940) (Figure 1). Time to bone AE was defined as time from randomization to first report of grade 3–4 osteoporosis or any bone fracture during or subsequent to protocol treatment. Cox regression models were adjusted for age, BMI, prior HRT use, smoking history (yes vs. no/unknown), history of osteoporosis, bone fracture or bisphosphonate use (yes vs. no/unknown), and bisphosphonate use since randomization as a time-varying covariate.

We first assessed SNP variant effects in a genotype model using the logrank test and an additive model (df=1) that compared 0 vs. 1 vs. 2 (the number indicates minor alleles in the three variant categories: 0=common allele, 1=minor allele) using a trend test. If appropriate, dominant and recessive effects of the variant (rare) allele were also estimated, where the

dominant model combined variant (rare) homozygote and heterozygote compared with the wild-type homozygote (reference), or recessive model combined variant (rare) homozygote was compared versus the combined heterozygote and wild-type homozygote (reference).

With a range of 616–3288 assessable patients, 10–36% event rates, and 13–69% prevalence of variant genotypes (assumed for calculations to be associated with increased hazard of event), there was 80% power (two-sided type I error of 0.05) to detect hazard ratios ranging from 1.2 (for $n=3067$, 36% event rate, 69% prevalence of variant genotype scenario) to 2.9 (for $n=613$, 10% event rate, 13% prevalence of variant genotype scenario) comparing variant groups versus wild-type.

Results are presented in accordance with REMARK criteria[24]. All statistical tests were two-sided. No multiple comparisons adjustments were implemented, and P -values less than 0.05 in the overall cohort, or interaction P -values less than 0.10, were considered statistically significant.

Results

Patients with DNA available for genotyping (Figure 1) were representative of the BIG 1-98 population characteristics and clinical outcomes (Table 1). Most (97%) patients were reported to be white/Caucasian, 36% had lymph node-positive disease and 70% had no previous chemotherapy.

Association between *ESR1* and *ESR2* genotype variants and disease outcomes

For the overall analytic cohort, 14% of patients treated with letrozole or tamoxifen, alone or in sequence, had breast cancer events. There were statistically significant associations between either BCFI or DRFI and two *ESR1* SNPs. The presence of rs9340799(XbaI) variants CC or TC was associated with a reduced hazard of a breast cancer event ($HR=0.82$, 95% $CI=0.67-1.0$). A similar effect was observed for a distant recurrence ($HR=0.79$, 95% $CI=0.63-1.0$) (Table 2 and Figures 2A,B). Patients with *ESR1* rs2077647 variants CC or TC had a reduced hazard of a distant recurrence ($HR=0.69$, 95% $CI=0.53-0.90$) (Figure 2C), consistent with BCFI, though not statistically significant.

There were no statistically significant differential treatment effects (letrozole vs. tamoxifen) for the association of either BCFI or DRFI with any of the five SNPs in the subset of 1,940 patients assigned to a 5-year monotherapy regimen (Table 2).

Association between *ESR1* and *ESR2* genotype variants and bone adverse events and early onset hot flushes or night sweats

Over the 5-year treatment period and subsequent follow-up through 2010, 34% of patients reported grade 3–4 osteoporosis or any grade bone fracture. In the overall cohort, there was no prognostic association of bone AEs with any of the genotypes. In the subset of 1,940 patients assigned to a monotherapy regimen, there were statistically significant differential treatment effects (letrozole vs. tamoxifen) for the association of bone AEs with *ESR1* rs2077647 (T>C) genotype variants (treatment-by-genotype interaction $P=0.08$; Table 2 and Figure 3A,B). For patients treated with letrozole, having the rare allele (C), either

homozygous or heterozygous, was associated with a 25% reduced risk of a bone AE (HR=0.75, 95% CI=0.58–0.98), whereas for patients treated with tamoxifen, the hazard of a bone AE was not reduced (HR=1.04, 95% CI=0.77–1.40). Similarly, a statistically significant differential treatment effect was observed for bone AEs with *ESR2* rs4986938(G>A) genotype variants (interaction P=0.07, Figure 3C,D). For patients treated with letrozole, having the rare allele of A, either homozygous or heterozygous, was associated with a 37% increased risk of a bone AE (HR=1.37, 95% CI=1.01–1.84), whereas for patients treated with tamoxifen, the hazard of a bone AE was not increased (HR=0.93, 95% CI=0.67–1.30).

Over the first two years of protocol treatment with letrozole or tamoxifen monotherapy, 35% of patients reported HF/NS. Patients with *ESR2* rs4986938 homozygous (AA) variant had a 29% increased risk of early onset of HF/NS events (HR=1.29, 95% CI=1.04–1.61; Table 2, Figure 4) compared to patients who had heterozygous (AG) or wild-type (GG) variants.

Discussion

There have been no studies evaluating the association between *ESR* polymorphisms in postmenopausal ER-positive breast cancer patients treated with SERMs or AIs and breast cancer outcomes. In this study, we found a statistically significant association between two *ESR1* SNPs and BCFI or DRFI among postmenopausal endocrine therapy-treated patients in the BIG 1-98 trial[18]. Both rs9340799(XbaI) (variants CC or TC) and rs2077647 (variants CC or TC) were associated with a reduced hazard of a breast cancer event with the pattern of association more strongly observed for distant recurrence. With regard to HF/NS, patients with the *ESR2* SNP rs4986938 variant minor allele AA reported an increased number of early onset HF/NS. In distinct contrast, there was no predictive (differential effect by treatment) association of breast cancer outcomes or early onset of HF/NS with any of the five SNPs. Of note, in the same patient population, we found that patients who had both *CYP2D6* poor metabolizer and intermediate metabolizer phenotypes had an increased risk of tamoxifen-induced hot flushes compared to those with the extensive metabolizer phenotype, although no association with breast cancer outcome was found[25].

With regard to bone AEs known to occur in postmenopausal women receiving adjuvant AI therapy, we found that patients with the *ESR1* SNP rs2077647 variants CC or TC had a reduced risk of a bone AE, while those with the *ESR2* SNP rs4986938 rare allele (A) had an increased hazard of a bone AE when treated with letrozole and not tamoxifen.

A number of published studies have investigated the association of *ESR1* and *ESR2* polymorphisms and breast cancer risk with varied and equivocal results. There has been only one study published on the association of *ESR1* polymorphisms and breast cancer survival[11], and although no overall association was observed between *ESR1* gene polymorphisms and breast cancer, there were interactive effects of *ESR1* gene polymorphisms and ER status on outcome.

The two most commonly studied *ESR1* polymorphisms, rs2234693 and rs9340799 (PvuII and XbaI restriction fragment length polymorphisms (RFLPs), respectively), are found in

intron 1, 50 bp apart, and have been investigated with regard to breast cancer risk with equivocal results overall being reported depending on patient ethnicity and age. In an early large-scale population-based case-control study, *ESR1* PvuII and XbaI RFLPs were investigated in 1,069 Chinese breast cancer cases (~64% ER+) and 1,166 age-matched controls. The PvuII polymorphism was associated with an increased risk of breast cancer while the XbaI polymorphism was associated with a non-significantly elevated risk, mainly confined to postmenopausal women[26]. This increased risk was not observed in another study in a similar Chinese population of 614 women with breast cancer[27].

In a smaller study on 205 Korean women with breast cancer, the *ESR1* rs2234693(PvuII) RFLP did not show any difference between cases and controls, however rs9340799(XbaI) RFLP, moreso in postmenopausal than premenopausal women, was found to modify individual breast cancer risk[28]. Genotyping of four *ESR1* SNPs, rs746432, rs2234693(PvuII), rs9340799(XbaI) and rs1801132, was performed on 1,183 Caucasian postmenopausal women (393 breast cancer cases and 790 controls) from the Study of Osteoporotic Fractures[29]. A protective effect of SNP rs9340799(XbaI) was observed, while no statistically significant association was found for any of the other three SNPs and breast cancer risk in this postmenopausal population. In a case-control study conducted with a total of 846 pairs (388 Japanese, 79 Japanese Brazilians and 379 non-Japanese Brazilians) in pre and postmenopausal breast cancer women, none of the five *ESR1* SNPs (rs2234693, rs9340799, rs1801132, rs3798577 and rs2228480) or two *ESR2* SNPs (rs4986938 and rs1256049) were associated with breast cancer risk[30]. In a large meta-analysis of 1,678 breast cancer cases and 1,678 general population controls from Asian populations, the association between *ESR1* XbaI and PvuII SNPs and breast cancer risk was evaluated[31]. The risk of breast cancer was not observed in premenopausal and postmenopausal women with the rs9340799(XbaI) polymorphism, however, premenopausal women with breast cancer with the rs2234693(PvuII) variant had a significantly elevated breast cancer risk, while postmenopausal women showed a non-significant increased risk.

There have also been several studies focused on *ESR2* SNPs and their association with breast cancer risk. In one study, three common *ESR2* polymorphisms, rs1256049 (G1082A), rs4986938 (G1730A) and rs928554 (Cx+56 A-->G), were not found to be significantly associated with breast cancer risk in 723 breast cancer cases (323 sporadic and 400 familial cases)[32]. In a meta-analysis of the two most commonly studied *ESR2* polymorphisms, rs4986938 and rs2987983 (nine studies of 10,837 cases and 16,021 controls for rs4986938; 8 studies of 11,652 cases and 15,726 controls for rs1256049), rs4986938 AA/AG vs. GG was associated with a significant but small decreased breast cancer risk, while rs2987983 was not[33]. In our study, the *ESR2* SNP rs4986938 was not prognostic or predictive of outcome in tamoxifen or letrozole-treated postmenopausal ER+ women.

Given the differing results in our European population, taken together, studies suggest that ethnicity may influence how *ESR1* and *ESR2* SNPs affect breast cancer risk and that no general conclusion can be drawn regarding the role they might play in breast cancer outcomes in either pre or postmenopausal women.

There have been relatively few studies that have investigated the role of *ESR* SNPs as they relate to endocrine treatment AEs. AIs profoundly reduce already low circulating estrogen levels in postmenopausal women by a further 80–90% compared with tamoxifen, which is associated with a modest increase of deleterious effects on the musculoskeletal system, a reason for treatment discontinuation[34]. In a recent study, the *ESR1* SNP rs2234693(PvuII) variants TT and TC were associated with a lower risk of musculoskeletal AEs, while SNP rs9340799(XbaI) variant TT was associated with a higher risk of musculoskeletal AEs in 436 postmenopausal Chinese Han women receiving adjuvant AI[35]. However, we found no association of *ESR1* SNPs rs2234693(PvuII) and rs9340799(XbaI) with bone AEs in a predominantly European population. Although the observed differences may be related to ethnicity, other factors may be at play, including perhaps the smaller number in the Chinese study.

Both *ESR1* rs2234693(PvuII) and *ESR2* rs4986938 influence the prevalence of hot flushes in tamoxifen-treated postmenopausal women[36]. In 297 participants after four months of tamoxifen treatment, postmenopausal women with *ESR2-02* (rs4986938 in our study) GG genotypes had a 4.6-fold increase in the number of hot flush scores compared to other postmenopausal women. On the other hand, postmenopausal women with the *ESR2-02* AA genotype were significantly less likely to experience tamoxifen treatment-induced hot flushes than women who carried at least one *ESR2-02* G allele[36]. These results are contrary to our findings that *ESR1* rs2234693(PvuII) had no effect while patients with the *ESR2* rs4986938 variant minor homozygous allele (AA) had an increased risk of early onset HF/NS overall, although not significantly associated with tamoxifen treatment.

BMD has also been a concern for women on hormone replacement therapy (HRT). A few studies show that *ESR* SNPs can affect bone loss[17, 37, 38]. Women with the *ESR1* PvuII genotypes PP and Pp have a greater risk of relatively fast bone loss after menopause and therefore, may derive more benefit from HRT[38]. A later study also found similar results and plus found *ESR1* XX genotype was associated with lower rates of bone loss and hence would benefit more from HRT[17]. In a more recent study[37] women carrying the C allele of *ESR1* rs2234693(PvuII) had a decreased risk of all-cause mortality with HRT. They also showed in distinct contrast that women who were homozygous for the T allele had a significantly increased risk of cancer-related mortality. The findings were similar for *ESR1* rs9340799(XbaI) and *ESR2* rs1271572.

Our study has limitations. We tested four *ESR1* SNPs and one *ESR2* SNP, but testing more *ESR1* and *ESR2* SNPs might have provided additional informative variants. There may be potential sources of false-positive results, including sample size or imbalance between patient groups. Patients were from heterogeneous ethnic groups, though in general, genotype frequencies corresponded well with those reported in the literature for a predominantly European Caucasian population. Nonetheless, we cannot rule out the possibility that the observed association with *ESR* genotypes were due to linkage disequilibrium with another causative gene and/or were the result of background genetic factors confined to an ethnically-defined subgroup.

Pharmacogenomics of breast cancer-related studies can provide information that can provide patients with the optimal therapy that will result in the best outcome, while limiting potential AEs. Our findings, together with those from previous studies, have implicated polymorphisms in ESR genes affecting outcomes and AEs. We found that two *ESR1* SNPs were predictive of disease outcome, one of which was also associated with a decreased risk of a bone AE but only in letrozole-treated patients, and one *ESR2* SNP was associated with an increased risk of early onset of HF/NS in postmenopausal endocrine-responsive breast cancer patients. These results provide increasing evidence that genes involved in estrogen signaling and synthesis affect outcomes and AEs, which if incorporated into a therapeutic strategy will increase both quality-of-life and survival in women with breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are indebted to the women, physicians, nurses, and data managers who participated in this clinical trial; to the many pathologists who submitted tumor blocks; to the BIG 1-98 Steering Committee; to Novartis for funding of the clinical trial and of the collection of tumor blocks; to the IBCSG for the design of the trial, coordination, data management, medical review, statistical support, and to the IBCSG Central Pathology Office for collection and processing of tumor blocks; to the BIG-198 Collaborative Group (members who submitted tumor blocks are listed in Supplementary Appendix, available online); to Susan G. Komen for the Cure Promise Grant.

Financial support including source and number of grants for each author

The BIG 1-98 trial sponsor, Novartis, contracted with the IBCSG to design the BIG 1-98 trial; collect, analyze, and interpret the data; and report the results. This report presents a study based on collected tumor material (collection partially funded by Novartis) and genotyping (funded by a Promise Grant from the Susan G. Komen for the Cure). The translational research, including DNA extraction and genotyping, was funded by Susan G. Komen for the Cure Promise Grant (KG080081 to GV, OP, MMR) and by The Breast Cancer Research Foundation (BCRF) (N003173 to JMR), the National Institutes of Health (1RO1GM099143 to JMR). The Breast International Group (BIG) 1-98 trial was funded by Novartis and coordinated by the International Breast Cancer Study Group (IBCSG). Other support for the IBCSG: United States National Cancer Institute (CA75362 to MMR). The writing of this manuscript did not include representatives from Novartis or Susan G. Komen for the Cure. The BIG 1-98 Steering Committee reviewed the article, suggested changes and is responsible for the decision to publish.

References

1. Sowers MR, Wilson AL, Karvonen-Gutierrez CA, Kardia SR. Sex steroid hormone pathway genes and health-related measures in women of 4 races/ethnicities: the Study of Women's Health Across the Nation (SWAN). *The American journal of medicine*. 2006; 119(9 Suppl 1):S103–110.10.1016/j.amjmed.2006.07.012 [PubMed: 16949383]
2. Almeida S, Hutz MH. Estrogen receptor 1 gene polymorphisms in premenopausal women: interaction between genotype and smoking on lipid levels. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica* [et al]. 2008; 41(10):872–876.
3. Hayes DF, Skaar TC, Rae JM, Henry NL, Nguyen AT, Stearns V, Li L, Philips S, Desta Z, Flockhart DA. Consortium on Breast Cancer P. Estrogen receptor genotypes, menopausal status, and the effects of tamoxifen on lipid levels: revised and updated results. *Clinical pharmacology and therapeutics*. 2010; 88(5):626–629.10.1038/clpt.2010.143 [PubMed: 20827267]
4. Molvarec A, Nagy B, Kovacs M, Walentin S, Imreh E, Rigo J Jr, Szalay J, Fust G, Prohaszka Z, Karadi I. Lipid, haemostatic and inflammatory variables in relation to the estrogen receptor alpha (*ESR1*) PvuII and XbaI gene polymorphisms. *Clinica chimica acta; international journal of clinical chemistry*. 2007; 380(1–2):157–164.10.1016/j.cca.2007.02.005

5. Ntukidem NI, Nguyen AT, Stearns V, Rehman M, Schott A, Skaar T, Jin Y, Blanche P, Li L, Lemler S, Hayden J, Krauss RM, Desta Z, Flockhart DA, Hayes DF. Consortium on Breast Cancer P. Estrogen receptor genotypes, menopausal status, and the lipid effects of tamoxifen. *Clinical pharmacology and therapeutics*. 2008; 83(5):702–710.10.1038/sj.clpt.6100343 [PubMed: 17713466]
6. Sowers MR, Symons JP, Jannausch ML, Chu J, Kardia SR. Sex steroid hormone polymorphisms, high-density lipoprotein cholesterol, and apolipoprotein A-1 from the Study of Women's Health Across the Nation (SWAN). *The American journal of medicine*. 2006; 119(9 Suppl 1):S61–68.10.1016/j.amjmed.2006.07.008 [PubMed: 16949390]
7. Crandall CJ, Sehl ME, Crawford SL, Gold EB, Habel LA, Butler LM, Sowers MR, Greendale GA, Sinsheimer JS. Sex steroid metabolism polymorphisms and mammographic density in pre- and early perimenopausal women. *Breast cancer research: BCR*. 2009; 11(4):R51.10.1186/bcr2340 [PubMed: 19630952]
8. Onitilo AA, McCarty CA, Wilke RA, Glurich I, Engel JM, Flockhart DA, Nguyen A, Li L, Mi D, Skaar TC, Jin Y. Estrogen receptor genotype is associated with risk of venous thromboembolism during tamoxifen therapy. *Breast cancer research and treatment*. 2009; 115(3):643–650.10.1007/s10549-008-0264-2 [PubMed: 19082882]
9. Oger E, Lacut K, Mercier B, Le Gal G, Leroyer C, Pasquier E, Ferec C, Mottier D. Estrogen receptor alpha polymorphism and venous thromboembolism in male and female: data from the EDITH study. *Thrombosis research*. 2007; 119(4):433–439.10.1016/j.thromres.2006.05.002 [PubMed: 16762402]
10. Yaffe K, Lindquist K, Sen S, Cauley J, Ferrell R, Penninx B, Harris T, Li R, Cummings SR. Estrogen receptor genotype and risk of cognitive impairment in elders: findings from the Health ABC study. *Neurobiology of aging*. 2009; 30(4):607–614.10.1016/j.neurobiolaging.2007.08.003 [PubMed: 17889406]
11. Boyapati SM, Shu XO, Ruan ZX, Cai Q, Smith JR, Wen W, Gao YT, Zheng W. Polymorphisms in ER-alpha gene interact with estrogen receptor status in breast cancer survival. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 2005; 11(3):1093–1098. [PubMed: 15709176]
12. Dunning AM, Healey CS, Baynes C, Maia AT, Scollen S, Vega A, Rodriguez R, Barbosa-Morais NL, Ponder BA, Low YL, Bingham S, Haiman CA, Le Marchand L, Broeks A, Schmidt MK, Hopper J, Southey M, Beckmann MW, Fasching PA, Peto J, Johnson N, Bojesen SE, Nordestgaard B, Milne RL, Benitez J, Hamann U, Ko Y, Schmutzler RK, Burwinkel B, Schurmann P, Dork T, Heikkinen T, Nevanlinna H, Lindblom A, Margolin S, Mannermaa A, Kosma VM, Chen X, Spurdle A, Change-Claude J, Flesch-Janys D, Couch FJ, Olson JE, Severi G, Baglietto L, Borresen-Dale AL, Kristensen V, Hunter DJ, Hankinson SE, Devilee P, Vreeswijk M, Lissowska J, Brinton L, Liu J, Hall P, Kang D, Yoo KY, Shen CY, Yu JC, Anton-Culver H, Ziogas A, Sigurdson A, Struwing J, Easton DF, Garcia-Closas M, Humphreys MK, Morrison J, Pharoah PD, Pooley KA, Chenevix-Trench G. Search, Epic, Mec, Abcs, Abcfs, Bbcc, Bbcs, Cggs, Cnio BCS, Genica, Gc H, Habcs, Hebcs, Karbac, Kbcs, kConFab the AMG, for M, Marie, Mccs, Nbcs, Nhs, Origo, Pbcs, Sasbac, Sebcs, Twbcs, Ucibcs, Usrts, Bcac. Association of ESR1 gene tagging SNPs with breast cancer risk. *Human molecular genetics*. 2009; 18(6):1131–1139.10.1093/hmg/ddn429 [PubMed: 19126777]
13. Gennari L, Merlotti D, De Paola V, Calabro A, Becherini L, Martini G, Nuti R. Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. *American journal of epidemiology*. 2005; 161(4):307–320.10.1093/aje/kwi055 [PubMed: 15692074]
14. Heilberg IP, Hernandez E, Alonzo E, Valera R, Ferreira LG, Gomes SA, Bellorin-Font E, Weisinger JR. Estrogen receptor (ER) gene polymorphism may predict the bone mineral density response to raloxifene in postmenopausal women on chronic hemodialysis. *Renal failure*. 2005; 27(2):155–161. [PubMed: 15807179]
15. Henry NL, Nguyen A, Azzouz F, Li L, Robarge J, Philips S, Cao D, Skaar TC, Rae JM, Storniolo AM, Flockhart DA, Hayes DF, Stearns V. Lack of association between oestrogen receptor polymorphisms and change in bone mineral density with tamoxifen therapy. *British journal of cancer*. 2010; 102(2):294–300.10.1038/sj.bjc.6605460 [PubMed: 19953095]

16. Li N, Dong J, Hu Z, Shen H, Dai M. Potentially functional polymorphisms in ESR1 and breast cancer risk: a meta-analysis. *Breast cancer research and treatment*. 2010; 121(1):177–184.10.1007/s10549-009-0532-9 [PubMed: 19760036]
17. Rapuri PB, Gallagher JC, Knezetic JA, Haynatzka V. Estrogen receptor alpha gene polymorphisms are associated with changes in bone remodeling markers and treatment response to estrogen. *Maturitas*. 2006; 53(4):371–379.10.1016/j.maturitas.2005.07.007 [PubMed: 16139450]
18. Thurlimann B, Keshaviah A, Coates AS, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Castiglione-Gertsch M, Gelber RD, Rabaglio M, Smith I, Wardley A, Price KN, Goldhirsch A. Breast International Group 1-98 Collaborative G. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *The New England journal of medicine*. 2005; 353(26):2747–2757.10.1056/NEJMoa052258 [PubMed: 16382061]
19. Coates AS, Keshaviah A, Thurlimann B, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Castiglione-Gertsch M, Gelber RD, Colleoni M, Lang I, Del Mastro L, Smith I, Chirgwin J, Nogaret JM, Pienkowski T, Wardley A, Jakobsen EH, Price KN, Goldhirsch A. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2007; 25(5):486–492.10.1200/JCO.2006.08.8617 [PubMed: 17200148]
20. Colleoni M, Giobbie-Hurder A, Regan MM, Thurlimann B, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Lang I, Smith I, Chirgwin J, Pienkowski T, Wardley A, Price KN, Gelber RD, Coates AS, Goldhirsch A. Analyses adjusting for selective crossover show improved overall survival with adjuvant letrozole compared with tamoxifen in the BIG 1-98 study. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2011; 29(9):1117–1124.10.1200/JCO.2010.31.6455 [PubMed: 21321298]
21. Group BIGC, Mouridsen H, Giobbie-Hurder A, Goldhirsch A, Thurlimann B, Paridaens R, Smith I, Mauriac L, Forbes J, Price KN, Regan MM, Gelber RD, Coates AS. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. *The New England journal of medicine*. 2009; 361(8):766–776.10.1056/NEJMoa0810818 [PubMed: 19692688]
22. Rae JM, Regan MM, Thibert JN, Gersch C, Thomas D, Leyland-Jones B, Viale G, Pusztai L, Hayes DF, Skaar T, Van Poznak C. Concordance between CYP2D6 genotypes obtained from tumor-derived and germline DNA. *J Natl Cancer Inst*. 2013; 105(17):1332–1334.10.1093/jnci/djt204 [PubMed: 23958736]
23. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Statistics Subcommittee of the NCIEWGoCD. Reporting recommendations for tumor marker prognostic studies. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2005; 23(36):9067–9072.10.1200/JCO.2004.01.0454 [PubMed: 16172462]
24. Regan MM, Leyland-Jones B, Bouzyk M, Pagani O, Tang W, Kammler R, Dell'orto P, Biasi MO, Thurlimann B, Lyng MB, Ditzel HJ, Neven P, Debled M, Maibach R, Price KN, Gelber RD, Coates AS, Goldhirsch A, Rae JM, Viale G. Breast International Group 1-98 Collaborative G. CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. *Journal of the National Cancer Institute*. 2012; 104(6):441–451.10.1093/jnci/djs125 [PubMed: 22395644]
25. Cai Q, Shu XO, Jin F, Dai Q, Wen W, Cheng JR, Gao YT, Zheng W. Genetic polymorphisms in the estrogen receptor alpha gene and risk of breast cancer: results from the Shanghai Breast Cancer Study. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2003; 12(9):853–859.
26. Sakoda LC, Blackston CR, Doherty JA, Ray RM, Lin MG, Gao DL, Stalsberg H, Feng Z, Thomas DB, Chen C. Selected estrogen receptor 1 and androgen receptor gene polymorphisms in relation to risk of breast cancer and fibrocystic breast conditions among Chinese women. *Cancer epidemiology*. 2011; 35(1):48–55.10.1016/j.canep.2010.08.005 [PubMed: 20846920]
27. Shin A, Kang D, Nishio H, Lee MJ, Park SK, Kim SU, Noh DY, Choe KJ, Ahn SH, Hirvonen A, Kim JH, Yoo KY. Estrogen receptor alpha gene polymorphisms and breast cancer risk. *Breast cancer research and treatment*. 2003; 80(1):127–131. [PubMed: 12889606]

28. Wang J, Higuchi R, Modugno F, Li J, Umblas N, Lee J, Lui LY, Ziv E, Tice JA, Cummings SR, Rhee B. Estrogen receptor alpha haplotypes and breast cancer risk in older Caucasian women. *Breast cancer research and treatment*. 2007; 106(2):273–280.10.1007/s10549-007-9497-8 [PubMed: 17268813]
29. Iwasaki M, Hamada GS, Nishimoto IN, Netto MM, Motola J Jr, Laginha FM, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Kobayashi M, Ishihara J, Yamamoto S, Hanaoka T, Tsugane S. Isoflavone, polymorphisms in estrogen receptor genes and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians. *Cancer science*. 2009; 100(5):927–933.10.1111/j.1349-7006.2009.01118.x [PubMed: 19298602]
30. Li LW, Xu L. Menopausal status modifies breast cancer risk associated with ESR1 PvuII and XbaI polymorphisms in Asian women: a HuGE review and meta-analysis. *Asian Pacific journal of cancer prevention: APJCP*. 2012; 13(10):5105–5111. [PubMed: 23244119]
31. Maguire P, Margolin S, Skoglund J, Sun XF, Gustafsson JA, Borresen-Dale AL, Lindblom A. Estrogen receptor beta (ESR2) polymorphisms in familial and sporadic breast cancer. *Breast cancer research and treatment*. 2005; 94(2):145–152.10.1007/s10549-005-7697-7 [PubMed: 16261413]
32. Yu KD, Rao NY, Chen AX, Fan L, Yang C, Shao ZM. A systematic review of the relationship between polymorphic sites in the estrogen receptor-beta (ESR2) gene and breast cancer risk. *Breast cancer research and treatment*. 2011; 126(1):37–45.10.1007/s10549-010-0891-2 [PubMed: 20390341]
33. Henry NL, Giles JT, Ang D, Mohan M, Dadabhoy D, Robarge J, Hayden J, Lemler S, Shahverdi K, Powers P, Li L, Flockhart D, Stearns V, Hayes DF, Storniolo AM, Clauw DJ. Prospective characterization of musculoskeletal symptoms in early stage breast cancer patients treated with aromatase inhibitors. *Breast cancer research and treatment*. 2008; 111(2):365–372.10.1007/s10549-007-9774-6 [PubMed: 17922185]
34. Wang J, Lu K, Song Y, Xie L, Zhao S, Wang Y, Sun W, Liu L, Zhao H, Tang D, Ma W, Pan B, Xuan Q, Liu H, Zhang Q. Indications of clinical and genetic predictors for aromatase inhibitors related musculoskeletal adverse events in Chinese Han women with breast cancer. *PloS one*. 2013; 8(7):e68798.10.1371/journal.pone.0068798 [PubMed: 23894347]
35. Jin Y, Hayes DF, Li L, Robarge JD, Skaar TC, Philips S, Nguyen A, Schott A, Hayden J, Lemler S, Storniolo AM, Flockhart DA, Stearns V. Estrogen receptor genotypes influence hot flash prevalence and composite score before and after tamoxifen therapy. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2008; 26(36):5849–5854.10.1200/JCO.2008.16.8377 [PubMed: 19018086]
36. Ryan J, Canonico M, Carcaillon L, Carriere I, Scali J, Dartigues JF, Dufouil C, Ritchie K, Scarabin PY, Ancelin ML. Hormone treatment, estrogen receptor polymorphisms and mortality: a prospective cohort study. *PloS one*. 2012; 7(3):e34112.10.1371/journal.pone.0034112 [PubMed: 22457817]
37. Salmen T, Heikkinen AM, Mahonen A, Kroger H, Komulainen M, Saarikoski S, Honkanen R, Maenpaa PH. Early postmenopausal bone loss is associated with PvuII estrogen receptor gene polymorphism in Finnish women: effect of hormone replacement therapy. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 2000; 15(2):315–321.10.1359/jbmr.2000.15.2.315

Figure 1.

Flow diagram showing the derivation of the analytic cohort of 3401 patients.

Figure 2(A, B, C). Kaplan-Meier estimates of breast cancer-free interval (BCFI) (A) and distant recurrence-free interval (DRFI) according to *ESR1* SNP rs9340799(XbaI)(T>C) variants (B) and DRFI according to *ESR1* SNP rs2077647(T>C) variants (C) for patients in the BIG 1-98 trial. Hazard ratios (HR) and confidence intervals (CI) were estimated using the adjusted Cox proportional hazards model.

Figure 3 (A, B, C, D).

Kaplan-Meier estimates of freedom from adverse bone events according to *ESR1* SNP rs2077647(T>C) variants (**A and B**); and according to *ESR2* SNP rs4986938(G>A) variants (**C and D**) for patients in the BIG 1-98 trial assigned to treatment 5-years letrozole (**A and C**) or 5-years tamoxifen (**B and D**). Hazard ratios (HR) and confidence intervals (CI) were estimated using the adjusted Cox proportional hazards model in which treatment-by-genotype interaction for rs2077647 was $P=0.08$ and for rs4986938 was $P=0.07$.

Figure 4. Kaplan-Meier estimates of hot flushes or night sweats according to *ESR2* SNP rs4986938(G>A) variants for patients in the BIG 1-98 trial. Hazard ratios (HR) and confidence intervals (CI) were estimated using the adjusted Cox proportional hazards model.

Table 1

Characteristics and clinical outcomes of patients in the BIG 1-98 trial according to availability of DNA for genotyping^a

Characteristic	DNA for <i>ESR1</i> , <i>ESR2</i> Genotyping ^b	
	No (n=4319)	Yes (n=3691)
Four-arm randomization option, %	70	86
Median follow-up, months	100	97
Postmenopausal, %	98	99
White race, %	98	97
Age, median (IQR), years	61 (56,67)	61 (56,68)
BMI, median (IQR), kg/m ²	26 (23,29)	26 (23,30)
Mastectomy, %	52	33
Previous (neo)adjuvant chemotherapy, %	21	30
Lymph node-positive, %	46	36
Tumor size >2 cm, %	40	35
Tumor grade 2 or 3, %	79	79
Peritumoral vascular invasion present, %	19	19
Centrally-assessed tumor features ^c		
ER-absent, %	2	1
HER2-positive, %	8	6
Ki-67 LI of immunostained cells, median (IQR), %	13 (7,18)	12 (6,19)
Clinical outcomes		
Breast cancer events, %	19	14
Distant recurrences, %	15	10
Early onset of adverse hot flushes or night sweats events ^d , %	39	35
Bone adverse events ^e , %	27	34

^a Eligible for enrollment in the randomized, phase III double-blind Breast International Group (BIG) 1-98 trial for postmenopausal women with hormone receptor-positive operable invasive breast cancer.

IQR = interquartile range; BMI = body mass index; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; LI = labeling index.

^b Genomic DNA was isolated from formalin-fixed paraffin-embedded primary breast cancer tissue blocks from 3,691 BIG 1-98 trial patients. Genotyping for four *ESR1* and one *ESR2* single nucleotide polymorphisms (SNPs) was done using polymerase chain reaction-based methods.

^c Centrally-assessed tumor features were available for a subset of the trial patients (2,654 of 4319 patients with no DNA for genotyping; 3,625 of 3691 patients with DNA available for genotyping).

^d New or worsening hot flushes or night sweats of any grade adverse events reported during the first 23 months protocol treatment period.

^e Bone adverse events include grade 3–4 osteoporosis or any grade bone fracture reported during or subsequent to the 5-year protocol treatment period.

Table 2

Associations between *ESR1* and *ESR2* genotype variants and breast cancer-free interval, distance recurrence, bone adverse events and early onset hot flushes or night sweats in the overall cohort and in the cohorts according to treatment regimen in the BIG 1-98 trial

		Overall Cohort			Monotherapy cohort 5-year Letrozole		Monotherapy cohort 5-year Tamoxifen			
SNP	Genetic model	N Patients (N Events)	HR (95%CI)	P	N Patients (N Events)	HR (95%CI)	N Patients (N Events)	HR (95%CI)	Interaction <i>p</i> ^a	
Breast Cancer-Free Interval (BCFI)										
rs9340799(XbaI)	0 vs. 1 vs. 2(C)	2902(400)	0.90(0.78,1.03)	0.14	845(106)	1.10(0.84,1.42)	812(131)	0.96(0.76,1.21)	0.52	
	CC,TC vs. TT (ref)		0.82(0.67,1.00)	0.05		1.03(0.70,1.51)		0.85(0.60,1.20)	0.45	
rs2234693(PvuII)	0 vs. 1 vs. 2(C)	3288(467)	1.06(0.93,1.20)	0.38	960(121)	1.11(0.86,1.44)	914(153)	1.00(0.80,1.25)	0.46	
rs11963577	TT,TC vs. CC (ref)	2729(390)	1.09(0.81,1.47)	0.57	794(104)	1.04(0.59,1.85)	761(122)	1.49(0.86,2.57)	0.44	
rs2077647	0 vs. 1 vs. 2(C)	2314(317)	0.88(0.75,1.03)	0.11	664(75)	0.92(0.67,1.26)	650(108)	0.93(0.71,1.23)	0.80	
	CC,TC vs. TT (ref)		0.85(0.68,1.07)	0.17		0.76(0.48,1.21)		0.94(0.63,1.40)	0.43	
rs4986938	0 vs. 1 vs. 2(A)	2138(311)	0.91(0.76,1.09)	0.32	616(81)	0.86(0.59,1.25)	616(103)	0.94(0.69,1.29)	0.46	
Distant Recurrence-Free Interval (DRFI)										
rs9340799(XbaI)	0 vs. 1 vs. 2(C)	2902(289)	0.86(0.73,1.01)	0.06	845(75)	1.03(0.76,1.41)	812(93)	0.90(0.68,1.19)	0.73	
	CC,TC vs. TT (ref)		0.79(0.63,1.00)	0.05		0.94(0.60,1.49)		0.81(0.53,1.23)	0.81	
rs2234693(PvuII)	0 vs. 1 vs. 2(C)	3288(337)	1.04(0.90,1.21)	0.58	960(86)	1.09(0.80,1.48)	914(109)	0.97(0.74,1.27)	0.57	
rs11963577	TT,TC vs. CC (ref)	2729(289)	1.16(0.82,1.62)	0.40	794(73)	1.03(0.52,2.04)	761(91)	1.72(0.92,3.23)	0.44	
rs2077647	0 vs. 1 vs. 2(C)	2314(233)	0.73(0.60,0.88)	<0.001	664(51)	0.70(0.47,1.04)	650(79)	0.83(0.60,1.16)	0.43	
	CC,TC vs. TT (ref)		0.69(0.53,0.90)	0.01		0.58(0.33,1.01)		0.84(0.53,1.35)	0.22	
rs4986938	0 vs. 1 vs. 2(A)	2138(225)	0.96(0.78,1.19)	0.72	616(54)	0.95(0.61,1.48)	616(77)	1.07(0.74,1.53)	0.56	
Bone Adverse Events										
rs9340799(XbaI)	0 vs. 1 vs. 2(C)	2902(999)	0.96(0.88,1.04)	0.34	845(285)	0.86(0.73,1.01)	812(246)	0.98(0.83,1.17)	0.15	
rs2234693(PvuII)	0 vs. 1 vs. 2(C)	3288(1123)	1.00(0.92,1.08)	0.93	960(321)	0.91(0.78,1.07)	914(277)	1.00(0.85,1.18)	0.21	
rs11963577	TT,TC vs. CC (ref)	2729(941)	0.90(0.74,1.10)	0.31	794(267)	0.84(0.57,1.25)	761(230)	1.01(0.66,1.55)	0.27	
rs2077647	0 vs. 1 vs. 2(C)	2314(790)	0.96(0.87,1.06)	0.47	664(227)	0.82(0.68,0.99)	650(196)	1.04(0.84,1.28)	0.06	
	CC,TC vs. TT (ref)		0.95(0.83,1.10)	0.53		0.75(0.58,0.98)		1.04(0.77,1.40)	0.08	
rs4986938	0 vs. 1 vs. 2(A)	2138(729)	1.05(0.93,1.18)	0.43	616(215)	1.19(0.95,1.48)	616(183)	0.88(0.69,1.13)	0.07	
	AA,AG vs. GG (ref)		1.11(0.95,1.30)	0.21		1.37(1.01,1.84)		0.93(0.67,1.30)	0.07	

		Overall Cohort			Monotherapy cohort 5-year Letrozole			Monotherapy cohort 5-year Tamoxifen			
SNP	Genetic model	N Patients (N Events)	HR (95%CI)	P	N Patients (N Events)	HR (95%CI)	N Patients (N Events)	HR (95%CI)	N Patients (N Events)	HR (95%CI)	Interaction p^a
Early Onset Hot Flashes or Night Sweats											
		Overall Cohort			2-year Letrozole			2-year Tamoxifen			Interaction p^b
rs9340799(XbaI)	0 vs.1 vs.2(C)	2902(1040)	1.04(0.96,1.13)	0.35	1459(491)	1.03(0.91,1.16)			1443(549)	1.06(0.94,1.19)	0.72
	CC vs. TC,TT(ref)		1.16(0.99,1.37)	0.07		1.06(0.84,1.35)				1.28(1.03,1.59)	0.27
rs2234693(PvuII)	0 vs.1 vs.2(C)	3288(1176)	0.98(0.90,1.06)	0.55	1653(553)	0.96(0.86,1.08)			1635(623)	0.99(0.88,1.10)	0.66
rs11963577	TT,TC vs. CC(ref)	2729(992)	0.97(0.80,1.17)	0.73	1375(471)	0.97(0.74,1.27)			1354(521)	0.98(0.75,1.29)	0.87
rs2077647	0 vs.1 vs.2(C)	2314(840)	1.04(0.95,1.15)	0.38	1156(386)	1.01(0.89,1.16)			1158(454)	1.07(0.93,1.22)	0.47
	CC,TC vs. TT(ref)		1.09(0.95,1.26)	0.23		1.12(0.91,1.38)				1.06(0.87,1.29)	0.86
rs4986938	0 vs.1 vs.2(A)	2138(765)	1.07(0.95,1.21)	0.25	1061(359)	1.12(0.94,1.32)			1077(406)	1.01(0.86,1.19)	0.61
	AA vs. AG,GG (ref)		1.29(1.04,1.61)	0.02		1.40(1.02,1.93)				1.16(0.86,1.58)	0.47

Abbreviations: BIG = Breast International Group; SNP = single nucleotide polymorphism; HR = hazard ratio; CI = confidence interval. HRs, CIs and P -values estimated using adjusted Cox proportional hazards models.

^a Interaction P -value: Wald test of treatment assignment (5-year letrozole vs. 5-year tamoxifen) by genotype interaction.

^b Interaction P -value: Wald test of treatment assignment (2-year letrozole vs. 2-year tamoxifen) by genotype interaction.