Published in final edited form as:

Breast Cancer Res Treat. 2015 June ; 151(2): 373–384. doi:10.1007/s10549-015-3378-3.

CYP19A1 polymorphisms and clinical outcomes in postmenopausal women with hormone receptor-positive breast cancer in the BIG 1–98 trial

Brian Leyland-Jones¹, Kathryn P. Gray^{2,3}, Mark Abramovitz⁴, Mark Bouzyk⁵, Brandon Young¹, Bradley Long⁶, Roswitha Kammler⁷, Patrizia Dell'Orto⁸, Maria Olivia Biasi⁹, Beat Thürlimann^{10,11}, Maria B. Lyng¹², Henrik J. Ditzel^{12,13}, Vernon J. Harvey¹⁴, Patrick Neven^{15,16}, Isabelle Treilleux¹⁷, Birgitte Bruun Rasmussen¹⁸, Rudolf Maibach¹⁹, Karen N. Price^{2,20}, Alan S. Coates²¹, Aron Goldhirsch²², Olivia Pagani²³, Giuseppe Viale⁸, James M. Rae²⁴, and Meredith M. Regan^{2,25}

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@ibcs.org; Patrizia Dell'Orto: patrizia.dellorto@ieo.it; Maria Olivia Biasi: olivia.biasi@ieo.it; Beat Thürlimann: beat.thuerlimann@kssg.ch; Maria B. Lyng: mlyng@health.sdu.dk; Henrik J. Ditzel: hditzel@health.sdu.dk; Vernon J. Harvey: vernonh@adhb.govt.nz; Patrick Neven: patrick.neven@uzleuven.be; Isabelle Treilleux: isabelle.treilleux@lyon.unicancer.fr; Birgitte Bruun Rasmussen: birgitte.bruun.rasmussen@regionh.dk; Rudolf Maibach: rudolf.maibach@ibcs.org; Karen N. Price: price@jimmy.harvard.edu; Alan S. Coates: alan.coates@ibcs.org; Aron Goldhirsch: aron.goldhirsch@ibcs.org; Olivia Pagani: Olivia.pagani@ibcs.org; Giuseppe Viale: giuseppe.viale@ieo.it; James M. Rae: jimmyrae@med.umich.edu; Meredith M. Regan: mregan@jimmy.harvard.edu

¹Avera Cancer Institute, 1000 E 23rd Street, Sioux Falls, SD 57105, USA ²International Breast Cancer Study Group (IBCSG) Statistical Center, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA, USA ³Harvard School of Public Health, Boston, MA, USA ⁴VM Institute of Research, Montreal, QC, Canada ⁵AKESOG, Inc., Norcross, GA, USA ⁶Genomic Molecular Pathology, Department of Pathology, University of Chicago, Chicago, IL, USA ⁷IBCSG Coordinating Center and Central Pathology Office, Bern, Switzerland ⁸IBCSG Central Pathology Office, Division of Pathology and Laboratory Medicine, European Institute of Oncology, University of Milan, Milan, Italy ⁹Division of Pathology and Laboratory Medicine, European Institute of Oncology, Milan, Italy ¹⁰Breast Center, Kantonsspital, St. Gallen, Switzerland ¹¹Swiss Group for Clinical Cancer Research (SAKK), Bern, Switzerland ¹²Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark ¹³Department of Oncology, Odense University Hospital, Odense, Denmark ¹⁴Auckland City

Correspondence to: Brian Leyland-Jones, bleylandj@aol.com.

On behalf of the BIG 1–98 Collaborative Group.

Members of the BIG 1–98 Collaborative Group who submitted tumor blocks are listed in Supplementary Appendix.

Electronic supplementary material: The online version of this article (doi:10.1007/s10549-015-3378-3) contains supplementary material, which is available to authorized users.

Compliance with ethical standards: Experiments comply with the current laws of the country in which they were performed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The BIG 1–98 trial was funded by Novartis, who contracted with the IBCSG for provision of services related to the conduct and management of the trial, and provided partial support for collection and review of tumor blocks. Drs. Goldhirsch and Coates are responsible for the scientific management of the IBCSG. Dr. Viale is responsible for management of the IBCSG Central Pathology Office. Dr. Thürlimann owns stock in Novartis. Informed consent was obtained from all individual participants included in the study.

Conflict of interest: The remaining authors have no conflicts to declare.

Hospital, Auckland, New Zealand ¹⁵Department of Oncology, KU Leuven (University of Leuven), Leuven, Belgium ¹⁶Multidisciplinary Breast Center, University Hospitals Leuven, Leuven, Belgium ¹⁷Centre Léon Bérard, Lyon, France ¹⁸Herlev Hospital, Herlev, Denmark ¹⁹IBCSG Coordinating Center, Bern, Switzerland ²⁰IBCSG Statistical Center, Frontier Science and Technology Research Foundation, Boston, MA, USA ²¹International Breast Cancer Study Group and University of Sydney, Sydney, Australia ²²European Institute of Oncology, Milan, Italy ²³Institute of Oncology of Southern Switzerland (IOSI), Swiss Group for Clinical Cancer Research (SAKK) and IBCSG Ospedale Italiano, Viganello, Switzerland ²⁴University of Michigan Comprehensive Cancer Center, Ann Arbor, MI, USA ²⁵Harvard Medical School, Boston, MA, USA

Abstract

To determine whether *CYP19A1* polymorphisms are associated with abnormal activity of aromatase and with musculoskeletal and bone side effects of aromatase inhibitors. DNA was isolated from tumor specimens of 4861 postmenopausal women with hormone receptor-positive breast cancer enrolled in the BIG 1–98 trial to receive tamoxifen and/or letrozole for 5 years. Tumors were genotyped for six *CYP19A1* polymorphisms using PCR-based methods. Associations with breast cancer-free interval (BCFI), distant recurrence-free interval (DRFI), musculoskeletal and bone adverse events (AEs) were assessed using Cox proportional hazards models. All statistical tests were two-sided. No association between the *CYP19A1* genotypes and BCFI or DRFI was observed overall. A reduced risk of a breast cancer event for tamoxifen-treated patients with rs700518 variants was observed (BCFI CC/TC vs. TT: HR 0.53, 95 % CI 0.34–0.82, interaction $P = 0.08$), but not observed for letrozole-treated patients. There was an increased risk of musculoskeletal AEs for patients with rs700518 variants CC/TC versus TT (HR 1.22, 95 % CI 1.03–1.45, $P = 0.02$), regardless of treatment. Tamoxifen-treated patients with rs4646 variants had a reduced risk of bone AEs (AA/CA vs. CC: HR 0.76, 95 % CI 0.59–0.98), whereas an increase of minor allele (C) of rs10046 was associated with an increased risk of bone AEs (HR 1.28, 95 % CI 1.07–1.52). rs936308 variants were associated with a reduced risk of bone AEs in letrozole-treated patients (GG/GC vs. CC: HR 0.73, 95 % CI 0.54–0.99), different from in tamoxifen-treated patients (GG/GC vs. CC: HR 1.32, 95 % CI 0.92–1.90, interaction $P = 0.01$). *CYP19A1* rs700518 variants showed associations with BCFI, DRFI, in tamoxifen treated patients and musculoskeletal AEs regardless of treatment. SNPs rs4646, rs10046, and rs936308 were associated with bone AEs.

Keywords

CYP19A1; Aromatase inhibitor; Musculoskeletal side effects; Bone side effects; Letrozole; Tamoxifen

Introduction

The *CYP19A1* gene encodes the enzyme aromatase, which is responsible for the final step in the biosynthesis of estrogens. *CYP19A1* is a complex gene with many polymorphic and splice variants. Some polymorphisms have been related to abnormal activity of aromatase [1], breast cancer risk [2], aromatase inhibitor-associated arthralgia [3], and bone mineral

density [4–6]. Relationships between genetic variants of estrogen receptors (ERs) and their ligand, the hormone estrogen, and the enzymes that synthesize it, are not well understood. Genetically determined variants in sex steroid hormone pathways have recently been related to several measures of health status (e.g., circulating hormone concentrations, menstrual cycle profiles, lipids, diabetes mellitus, depressive symptoms, measures of cognition, bone mineral density, and vasomotor symptoms) in a community-based population of premenopausal women [7].

There is also evidence that genetic variation may explain the difference in the degree of side effects among women receiving aromatase inhibitors (AIs) for early breast cancer. Ingle et al. found that four single nucleotide polymorphisms (SNPs) (rs7158782, rs7159713, rs2369049, and rs6637820), that were closest to the T-cell leukemia 1A (TCL1A) gene, were associated with some degree of musculoskeletal symptoms using a genome-wide association case–control study [8]. In another study, a *CYP19A1* SNP was associated with a decrease in bone mineral density in postmenopausal women with ER-positive breast cancer treated with AIs [9], while in a third study, an association was found between a *CYP19A1* polymorphism and AI-associated arthralgia in postmenopausal women with ER-positive breast cancer [3].

We investigated the clinical relevance of *CYP19A1* SNPs in the Breast International Group (BIG) 1–98 trial comparing adjuvant treatment with letrozole and tamoxifen, alone or in sequence, in postmenopausal women. We hypothesized that *CYP19A1* genotypes that affect enzyme activity would be associated with worse disease outcomes among postmenopausal hormone receptor-positive breast cancer patients, and that the association may vary by type of endocrine therapy. We also investigated potential associations between *CYP19A1* SNPs and musculoskeletal adverse events (AEs) (arthralgia and myalgia) during treatment, and bone AEs (osteoporosis and bone fractures) during and subsequent to treatment.

Methods

Patients

The BIG 1–98 study [10] was a randomized, double-blind phase III trial for postmenopausal women with hormone receptor-positive early breast cancer randomly assigned to receive 5 years of tamoxifen (20 mg daily) monotherapy, 5 years of letrozole (2.5 mg daily) monotherapy, or 2 years of treatment with one agent followed by 3 years of treatment with the other, reported after median follow-up of 8.1 years [11]. BIG 1–98 enrolled 8010 women between March 1998 and May 2003. Patient visits were at baseline, every 6 months for 5 years, and yearly thereafter. Case report forms collected date of onset and severity of AEs during treatment (including bone fractures, osteoporosis, arthralgia, and myalgia) and after treatment (bone fractures and osteoporosis) using the National Cancer Institute Common Toxicity Criteria version 2.0.

Tissue collection, DNA extraction, and *CYP19A1* genotyping

Between 1998 and 2010, the International Breast Cancer Study Group (IBCSG) and the Danish Breast Cancer Collaborative Group carried out retrospective tissue collection in

accordance with institutional guidelines and national laws. 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 patients were assessed for availability of invasive tumor material for translational research, as described previously [12]. In 5166 tumor blocks, 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 4861 of 8010 trial patients (Fig. 1) and was potentially available for genotyping. Genomic DNA was extracted using the QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA) according to the 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, Männedorf, Switzerland) and aliquoted at a concentration of 10 ng/µL.

DNA samples were genotyped for six targeted *CYP19A1* SNPs, selected based on location and/or whether they had been evaluated in prior studies: two non-synonymous SNPs, rs700519(G>A) and rs28757184(G>A); one synonymous SNP, rs700518(T>C); two SNPs in the 3'UTR, rs4646(C>A) and rs10046(C>T); and one intronic SNP, rs936308(C>G). All samples were processed using polymerase chain reaction (PCR)-based methods in a Clinical Laboratory Improvement Amendments (CLIA)-compliant laboratory. Two SNPs, rs4646 and rs10046, were genotyped on the 7900HT Fast Real-Time 200 PCR System (Applied Biosystems, Foster City, CA) using Taqman allele-specific assays with both providing a 95 % genotype call rate. Four SNPs were genotyped on the GenomeLab SNPstream Genotyping System (Beckman Coulter, Brea, CA). 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. Optimization was performed using Applied Biosystems' TaqMan probes, in conjunction with the KlearKall Mastermix (KBioscience, Beverly, MA) or TaqMan Universal Master Mix II. For additional genotype quality assurance, sixteen samples plus four DNA controls and four no template controls were assayed in triplicate and allele calls were 100 % concordant. Comprehensive Hardy–Weinberg equilibrium testing did not reveal statistically significant violations across most sites providing tissue samples (Supplementary Table S1). Additionally, in order to rule out the possibility of tumor-derived DNA contributing to genotype measurement errors, we compared genotypes derived from tumor versus whole blood DNA in 120 matched samples as an extension of our previous report [13]. Five of the *CYP19A1* genotypes tested (rs10046, rs700518, rs700519, rs936308, and rs28757184) showed very high to complete concordance between tumors and whole blood (97.3–100 %), suggesting that tumor-derived genotypes accurately represented patients' germline genotypes.

Statistical analyses

To investigate the association of *CYP19A1* genotypes with disease outcomes among endocrine therapy-treated patients, all assessable patients were included. To investigate whether the association may vary by type of endocrine therapy, analyses were limited to

patients assigned to letrozole or tamoxifen monotherapy. Two disease endpoints were analyzed: (1) the breast cancer-free interval (BCFI), defined as time from randomization to first occurrence of invasive breast cancer at a local, regional, or distant site, or in the contralateral breast; and (2) the distant recurrence-free interval (DRFI), defined as 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 [10]. The analyses used Cox proportional hazards modeling, stratified by randomization option (2 or 4-arm) and prior chemotherapy (and by treatment assignment for analyses that included all patients). The models were also adjusted for the following characteristics at randomization: local therapy, tumor grade, tumor size, nodal status, peritumoral vascular invasion, HER2 status, and Ki-67 labeling index. The proportional hazards assumption was assessed by testing for genotype-by-time interaction and using Schoenfeld residuals.

We similarly investigated musculoskeletal and bone AEs. The endpoints were time to musculoskeletal AE, defined as time from randomization to first report of arthralgia or myalgia during protocol treatment; and time to bone AE, defined as time from randomization to first report of grade 3–4 osteoporosis or any grade bone fracture during or after protocol treatment. For musculoskeletal AEs, the Cox models were adjusted for age, body mass index (BMI), prior hormone replacement therapy use and year of randomization. For bone AEs, the models were adjusted for age, BMI, prior hormone replacement therapy use, smoking history, history of osteoporosis, bone fracture or bisphosphonate use, and for bisphosphonate use since randomization as a time-varying covariate.

Without pre-defined hypotheses, we first assessed SNP variant effects in a genotype model using logrank test and an additive model ($df = 1$) that compared 0 versus 1 versus 2 minor alleles using a trend test. If appropriate, dominant and recessive effects of the variant (rare) allele were also estimated. With a range of 2100–4200 assessable patients, 13–32 % event rates, and 5.5–50 % prevalence of variant genotypes, there was 80 % power (two-sided $\alpha = 0.05$) to detect hazard ratios ranging from 1.2 to 2.1, comparing variant versus wild-type groups.

Results are presented in accordance with REMARK criteria [14]. All statistical tests were two-sided. No multiple comparison adjustments were implemented, and P values < 0.05 in the overall cohort, or interaction P values < 0.10 , were considered statistically significant.

Results

DNA tumor samples for 4861 postmenopausal patients with hormone receptor-positive breast cancer were genotyped for *CYP19A1* (Fig. 1). Patients with DNA available for genotyping were representative of the BIG 1–98 population characteristics and clinical outcomes (Table 1). Most (98 %) patients were white, 43 % had lymph node-positive disease, and 77 % had no previous chemotherapy. *CYP19A1* genotyping was assessable for 4580 DNA samples. Characteristics of the *CYP19A1* SNPs with respect to allele and genotype frequencies are shown in Supplementary Table S1.

Association between *CYP19A1* genotype variants and disease outcomes

There were no associations between either BCFI or DRFI and any of the six SNPs, including the two most studied (rs4646 and rs10046), in the overall cohort of patients treated with letrozole or tamoxifen, alone or in sequence (Tables 2, S2). In the subset of 2653 patients assigned to a monotherapy regimen, there was a statistically significant differential treatment effect (letrozole vs. tamoxifen) for the association between BCFI and SNP rs700518(T>C) genotype variants (treatment-by-genotype interaction $P = 0.08$; Table 2; Fig. 2). For patients treated with tamoxifen, having the rare allele C of rs700518, either homozygous or heterozygous, was associated with a 47 % reduced hazard of a breast cancer event (HR 0.53, 95 % CI 0.34–0.82), whereas for patients treated with letrozole, the hazard of a breast cancer event was not reduced (HR 1.20, 95 % CI 0.73–1.96). The results were consistent for DRFI (interaction $P = 0.07$; Table S2).

Association between *CYP19A1* genotype variants and musculoskeletal and bone adverse events

Over the 5-year period of treatment with tamoxifen or letrozole, alone or in sequence, musculoskeletal AEs were reported for 30 % of patients. Patients with homozygous or heterozygous SNP rs700518(T>C) variants (CC or TC) were at an increased risk of reported musculoskeletal AEs (HR 1.22, 95 % CI 1.03–1.45, $P = 0.02$), compared with patients who had the wild-type (TT) genotype (Table 3; Fig. 3). No associations were observed for the other five SNPs, and there were no indications of differential associations between SNP variants and musculoskeletal AEs according to treatment assignment.

Over the 5-year period of treatment or subsequent to treatment with tamoxifen or letrozole, alone or in sequence, bone AEs were reported for 32 % of patients. No associations between *CYP19A1* genotype variants and bone AEs were observed in the cohort overall (Table 4). Heterogeneity was observed in the association of genotype with bone AEs between treatment groups (Fig. 4). For patients assigned to tamoxifen monotherapy, having the rare homozygous (AA), or heterozygous (CA) variant of SNP rs4646 was associated with a 24 % reduced hazard of reporting bone AEs, compared with the wild-type group (HR 0.76, 95 % CI 0.59–0.98), which was not observed for patients assigned to letrozole monotherapy (HR 1.02, 95 % CI 0.84–1.25; interaction $P = 0.09$). Each minor allele (C) of rs10046 was associated with a significantly increased risk of bone AEs (HR 1.28, 95 % CI 1.07–1.52) among patients assigned to tamoxifen monotherapy, which was not observed for patients assigned to letrozole monotherapy (HR 1.0, 95 % CI 0.88–1.15; interaction $P = 0.03$). In contrast, for patients assigned to letrozole monotherapy, having the rare homozygous (GG) or heterozygous (GC) variant of SNP rs936308 was associated with a 27 % reduced hazard of experiencing a bone AE, compared with the wild-type genotype (HR 0.73, 95 % CI 0.54–0.99), which was different from that observed among patients assigned to tamoxifen monotherapy (HR 1.32, 95 % CI 0.92–1.90; interaction $P = 0.01$).

Discussion

Contrary to our hypothesis, we found no statistically significant association between any of the *CYP19A1* SNP variants and BCFI or DRFI among postmenopausal, endocrine therapy-

treated patients in the BIG 1–98 trial. However, we observed that patients with SNP rs700518 variants CC and TC, compared with those with the wild-type TT, had better BCFI and DRFI in the tamoxifen group but not in the letrozole group. This SNP has not been previously linked to breast cancer outcome. Interestingly, there was also an association between SNP rs700518 variants CC and TC and an increased risk of musculoskeletal AEs during endocrine therapy.

In distinct contrast, we found that three of the six *CYP19A1* SNPs had statistically significant differential associations with bone AEs in patients randomized to tamoxifen versus letrozole. In the tamoxifen group, patients with the rare homozygote or heterozygote (AA/CA) variant of SNP rs4646 had a reduced risk of experiencing bone AEs, while patients with each minor allele (C) of SNP rs10046 had an increased risk of reporting a bone AE. On the other hand, patients with the rare homozygote or heterozygote (GG/GC) variant of SNP rs936308 had a reduced risk of bone AEs in the letrozole group, in contrast to an increased risk in the tamoxifen group.

Of note, we did not find any association between the three most studied SNPs, rs4646, rs10046, and rs700519, and BCFI or DRFI outcomes. In a number of studies in which these three SNPs have been investigated, conflicting results have been reported, raising the question of whether these SNPs are prognostic or predictive factors in endocrine therapy-treated breast cancer patients [15–21]. In one study, [19] only rs4646 was found to be associated with greater efficacy in 67 letrozole-treated advanced hormone receptor-positive breast cancer patients. Patients with the rs4646 SNP variant had median time to progression that was three times that of patients with the wild-type aromatase gene; but whether rs4646 was a prognostic or predictive factor could not be assessed [19]. In another study, 95 postmenopausal women with stage II–III ER-positive breast cancer were treated with neoadjuvant letrozole, and only rs4646 was associated with a poor response ($P = 0.03$) [17]; progression-free survival was reduced in patients carrying (CA/AA) variants compared with those with the reference CC variant [17]. In other recent studies, rs4646 was shown to have no association with breast cancer outcome or risk [18, 20, 22]. In addition, rs700519 SNP variants in exon 7, when compared with the wild-type genotype, have been associated with an increased risk of breast cancer among Hawaiian, Japanese [23], and Korean women [24]. However, in several other reports [25–28], no association was found. Overall, in studies that were not underpowered, there were no associations found between these three SNPs and disease outcomes.

Very few studies have evaluated the role that *CYP19A1* SNPs play with respect to either musculoskeletal or bone AEs in postmenopausal women with endocrine-responsive breast cancer. In one study, 390 Caucasian postmenopausal women were genotyped for several SNPs and one 7 and one 8-repeat allele [3]; 50.8 % reported having an AI-associated arthralgia and women carrying at least one 8-repeat allele had a significantly lower risk of having an AI-associated arthralgia. They also confirmed previous results [29] that women who went into menopause more recently (within 5 years) were significantly more likely to report having an AI-associated arthralgia than those greater than 10 years post menopause. Napoli et al. [9] investigated 97 postmenopausal women with ER-positive breast cancer who were treated with AIs and observed that women with the AA genotype for the *CYP19A1*

SNP rs700518 (G/A at Val80) developed significant bone loss at both the lumbar spine and total hip at 12 months relative to patients with GA/GG variants. We did not find any associations between bone AEs and *CYP19A1* SNP rs700518 genotype variants. The reason for the discrepancy between their study and ours may be related to the small sample size in their study and the subsequently few bone AEs recorded over the study period. A recent study found that the *CYP19A1* SNP rs934635 was associated with increased musculoskeletal AEs and vasomotor symptoms in patients treated with exemestane [30]. Unfortunately, we were unable to compare these results with ours because we did not test for rs934635, and it was not in linkage disequilibrium with any of the *CYP19A1* SNPs examined in our cohort.

Our study has some limitations. Testing more than six additional aromatase gene SNPs might have provided additional informative variants. There may be possible 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 population. Nevertheless, we cannot rule out the possibility that the observed association with *CYP19A1* genotypes was due to linkage disequilibrium with another causative gene and/or was the result of background genetic factors confined to an ethnically defined subgroup.

The ultimate goal of breast cancer-related pharmacogenomics studies is to use the information so patients with particular genotype variants receive the optimal therapy, either selective ER modulators (SERMs) or AIs, for the best outcome with the fewest AEs. The combination of genetic and hormonal information may help clinicians to maximize benefits, minimize AEs and determine which patients deserve close follow-up during long-term SERM or AI therapy. Our study, together with previous data, provides increasing evidence that genes involved in estrogen synthesis may modify outcomes and the risk of AEs, and enhance both quality of life and survival in women with breast cancer. Prospective investigations in larger and independent cohorts are needed to confirm these associations between *CYP19A1* SNPs and breast cancer outcomes and AEs in postmenopausal endocrine-responsive breast cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The BIG 1–98 trial is registered at ClinicalTrials.gov (Identifier NCT00004205). 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, and statistical support; 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. 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 (1R01GM099143 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).

References

1. Ma CX, Adjei AA, Salavaggione OE, Coronel J, Pelleymounter L, Wang L, Eckloff BW, Schaid D, Wieben ED, Adjei AA, Weinshilboum RM. Human aromatase: gene resequencing and functional genomics. *Cancer Res.* 2005; 65(23):11071–11082.10.1158/0008-5472.CAN-05-1218 [PubMed: 16322257]
2. Tempfer CB, Hefler LA, Schneeberger C, Huber JC. How valid is single nucleotide polymorphism (SNP) diagnosis for the individual risk assessment of breast cancer? *Gynecol Endocrinol.* 2006; 22(3):155–159.10.1080/09513590600629175 [PubMed: 16835078]
3. Mao JJ, Su HI, Feng R, Donelson ML, Aplenc R, Rebbeck TR, Stanczyk F, DeMichele A. Association of functional polymorphisms in *CYP19A1* with aromatase inhibitor associated arthralgia in breast cancer survivors. *Breast Cancer Res.* 2011; 13(1):R8.10.1186/bcr2813 [PubMed: 21251330]
4. Enjuanes A, Garcia-Giralt N, Supervia A, Nogues X, Ruiz-Gaspa S, Bustamante M, Mellibovsky L, Grinberg D, Balcells S, Diez-Perez A. A new SNP in a negative regulatory region of the *CYP19A1* gene is associated with lumbar spine BMD in postmenopausal women. *Bone.* 2006; 38(5):738–743.10.1016/j.bone.2005.10.010 [PubMed: 16344016]
5. Napoli N, Villareal DT, Mumm S, Halstead L, Sheikh S, Cagaanan M, Rini GB, Armamento-Villareal R. Effect of *CYP19A1* gene polymorphisms on estrogen metabolism and bone density. *J Bone Miner Res.* 2005; 20(2):232–239.10.1359/JBMR.041110 [PubMed: 15647817]
6. Zarrabeitia MT, Hernandez JL, Valero C, Zarrabeitia AL, Garcia-Unzueta M, Amado JA, Gonzalez-Macias J, Riancho JA. A common polymorphism in the 5'-untranslated region of the aromatase gene influences bone mass and fracture risk. *Eur J Endocrinol.* 2004; 150(5):699–704. [PubMed: 15132727]
7. 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). *Am J Med.* 2006; 119(9 Suppl 1):S103–S110.10.1016/j.amjmed.2006.07.012 [PubMed: 16949383]
8. Ingle JN, Schaid DJ, Goss PE, Liu M, Mushiroda T, Chapman JA, Kubo M, Jenkins GD, Batzler A, Shepherd L, Pater J, Wang L, Ellis MJ, Stearns V, Rohrer DC, Goetz MP, Pritchard KI, Flockhart DA, Nakamura Y, Weinshilboum RM. Genome-wide associations and functional genomic studies of musculoskeletal adverse events in women receiving aromatase inhibitors. *J Clin Oncol.* 2010; 28(31):4674–4682.10.1200/JCO.2010.28.5064 [PubMed: 20876420]
9. Napoli N, Rastelli A, Ma C, Yarramaneni J, Vattikuti S, Moskowitz G, Giri T, Mueller C, Kulkarny V, Qualls C, Ellis M, Armamento-Villareal R. Genetic polymorphism at Val80 (rs700518) of the *CYP19A1* gene is associated with aromatase inhibitor associated bone loss in women with ER + breast cancer. *Bone.* 2013; 55(2):309–314.10.1016/j.bone.2013.04.021 [PubMed: 23643682]
10. 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. *N Engl J Med.* 2005; 353(26):2747–2757.10.1056/NEJMoa052258 [PubMed: 16382061]
11. Regan MM, Neven P, Giobbie-Hurder A, Goldhirsch A, Ejlertsen B, Mauriac L, Forbes JF, Smith I, Lang I, Wardley A, Rabaglio M, Price KN, Gelber RD, Coates AS, Thurlimann B. Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1–98 randomised clinical trial at 8.1 years median follow-up. *Lancet Oncol.* 2011; 12(12):1101–1108.10.1016/s1470-2045(11)70270-4 [PubMed: 22018631]
12. 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. *J Natl Cancer Inst.* 2012; 104(6):441–451.10.1093/jnci/djs125 [PubMed: 22395644]

13. 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. [PubMed: 23958736]
14. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Statistics Subcommittee of the NCIEWGoCD. Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol.* 2005; 23(36):9067–9072.10.1200/JCO.2004.01.0454 [PubMed: 16172462]
15. Chen C, Sakoda LC, Doherty JA, Loomis MM, Fish S, Ray RM, Lin MG, Fan W, Zhao LP, Gao DL, Stalsberg H, Feng Z, Thomas DB. Genetic variation in *CYP19A1* and risk of breast cancer and fibrocystic breast conditions among women in Shanghai, China. *Cancer Epidemiol Biomarkers Prev.* 2008; 17(12):3457–3466.10.1158/1055-9965.EPI-08-0517 [PubMed: 19064562]
16. Fasching PA, Loehberg CR, Strissel PL, Lux MP, Bani MR, Schrauder M, Geiler S, Ringleff K, Oeser S, Weihbrecht S, Schulz-Wendtland R, Hartmann A, Beckmann MW, Strick R. Single nucleotide polymorphisms of the aromatase gene (*CYP19A1*), HER2/neu status, and prognosis in breast cancer patients. *Breast Cancer Res Treat.* 2008; 112(1):89–98.10.1007/s10549-007-9822-2 [PubMed: 18049890]
17. Garcia-Casado Z, Guerrero-Zotano A, Llombart-Cussac A, Calatrava A, Fernandez-Serra A, Ruiz-Simon A, Gavila J, Climent MA, Almenar S, Cervera-Deval J, Campos J, Albaladejo CV, Llombart-Bosch A, Guillem V, Lopez-Guerrero JA. A polymorphism at the 3'-UTR region of the aromatase gene defines a subgroup of postmenopausal breast cancer patients with poor response to neoadjuvant letrozole. *BMC Cancer.* 2010; 10:36.10.1186/1471-2407-10-36 [PubMed: 20144226]
18. Lunardi G, Piccioli P, Bruzzi P, Notaro R, Lastraioli S, Serra M, Marroni P, Bighin C, Mansutti M, Puglisi F, Porpiglia M, Ponzzone R, Bisagni G, Garrone O, Cavazzini G, Clavarezza M, Del Mastro L. Plasma estrone sulfate concentrations and genetic variation at the *CYP19A1* locus in postmenopausal women with early breast cancer treated with letrozole. *Breast Cancer Res Treat.* 2013; 137(1):167–174.10.1007/s10549-012-2306-z [PubMed: 23129173]
19. Colomer R, Monzo M, Tusquets I, Rifa J, Baena JM, Barnadas A, Calvo L, Carabantes F, Crespo C, Munoz M, Llombart A, Plazaola A, Artells R, Gilabert M, Lloveras B, Alba E. A single-nucleotide polymorphism in the aromatase gene is associated with the efficacy of the aromatase inhibitor letrozole in advanced breast carcinoma. *Clin Cancer Res.* 2008; 14(3):811–816.10.1158/1078-0432.CCR-07-1923 [PubMed: 18245543]
20. Ferraldeschi R, Arnedos M, Hadfield KD, A'Hern R, Drury S, Wardley A, Howell A, Evans DG, Roberts SA, Smith I, Newman WG, Dowsett M. Polymorphisms of *CYP19A1* and response to aromatase inhibitors in metastatic breast cancer patients. *Breast Cancer Res Treat.* 2012; 133(3):1191–1198.10.1007/s10549-012-2010-z [PubMed: 22418701]
21. Liu L, Bai YX, Zhou JH, Sun XW, Sui H, Zhang WJ, Yuan HH, Xie R, Wei XL, Zhang TT, Huang P, Li YJ, Wang JX, Zhao S, Zhang QY. A polymorphism at the 3'-UTR region of the aromatase gene is associated with the efficacy of the aromatase inhibitor, anastrozole, in metastatic breast carcinoma. *Int J Mol Sci.* 2013; 14(9):18973–18988.10.3390/ijms140918973 [PubMed: 24065098]
22. Clendenen T, Zeleniuch-Jacquotte A, Wirgin I, Koenig KL, Afanasyeva Y, Lundin E, Arslan AA, Axelsson T, Forsti A, Hallmans G, Hemminki K, Lenner P, Roy N, Shore RE, Chen Y. Genetic variants in hormone-related genes and risk of breast cancer. *PLoS One.* 2013; 8(7):e69367.10.1371/journal.pone.0069367 [PubMed: 23935996]
23. Haiman CA, Stram DO, Pike MC, Kolonel LN, Burt NP, Altshuler D, Hirschhorn J, Henderson BE. A comprehensive haplotype analysis of CYP19 and breast cancer risk: the Multiethnic Cohort. *Hum Mol Genet.* 2003; 12(20):2679–2692.10.1093/hmg/ddg294 [PubMed: 12944421]
24. Lee KM, Abel J, Ko Y, Harth V, Park WY, Seo JS, Yoo KY, Choi JY, Shin A, Ahn SH, Noh DY, Hirvonen A, Kang D. Genetic polymorphisms of cytochrome P450 19 and 1B1, alcohol use, and breast cancer risk in Korean women. *Br J Cancer.* 2003; 88(5):675–678.10.1038/sj.bjc.6600761 [PubMed: 12618873]
25. Miyoshi Y, Iwao K, Ikeda N, Egawa C, Noguchi S. Breast cancer risk associated with polymorphism in CYP19 in Japanese women. *Int J Cancer (Journal international du cancer).* 2000; 89(4):325–328.

26. Probst-Hensch NM, Ingles SA, Diep AT, Haile RW, Stanczyk FZ, Kolonel LN, Henderson BE. Aromatase and breast cancer susceptibility. *Endocr Relat Cancer*. 1999; 6(2):165–173. [PubMed: 10731105]
27. Watanabe J, Harada N, Suemasu K, Higashi Y, Gotoh O, Kawajiri K. Arginine-cysteine polymorphism at codon 264 of the human CYP19 gene does not affect aromatase activity. *Pharmacogenetics*. 1997; 7(5):419–424. [PubMed: 9352581]
28. Cai Q, Kataoka N, Li C, Wen W, Smith JR, Gao YT, Shu XO, Zheng W. Haplotype analyses of *CYP19A1* gene variants and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(1):27–32.10.1158/1055-9965.EPI-07-0688 [PubMed: 18199708]
29. Mao JJ, Stricker C, Bruner D, Xie S, Bowman MA, Farrar JT, Greene BT, DeMichele A. Patterns and risk factors associated with aromatase inhibitor-related arthralgia among breast cancer survivors. *Cancer*. 2009; 115(16):3631–3639.10.1002/cncr.24419 [PubMed: 19517460]
30. Fontein DB, Houtsma D, Nortier JW, Baak-Pablo RF, Kranenbarg EM, van der Straaten TR, Putter H, Seynaeve C, Gelderblom H, van de Velde CJ, Guchelaar HJ. Germline variants in the *CYP19A1* gene are related to specific adverse events in aromatase inhibitor users: a substudy of Dutch patients in the TEAM trial. *Breast Cancer Res Treat*. 2014; 144(3):599–606.10.1007/s10549-014-2873-2 [PubMed: 24590773]

Fig. 1.

Flow diagram of the Breast International Group (BIG) 1–98 trial DNA samples included in the study. Collection of tumor tissue blocks, DNA extraction, *CYP19A1* genotyping, and patient cohorts for analysis are shown. *FFPE* formalin-fixed paraffin-embedded

Fig. 2. Kaplan–Meier estimates of breast cancer-free interval (BCFI) according to *CYP19A1* SNP rs700518(T>C) variants for patients in the Breast International Group (BIG) 1–98 trial assigned to: **a** 5-year letrozole; **b** 5-year tamoxifen. Hazard ratios (HR) and confidence intervals (CI) estimated using adjusted Cox proportional hazards model in which treatment-by-genotype interaction $P = 0.08$

Fig. 3. Kaplan–Meier estimate of musculoskeletal adverse event-free interval according to *CYP19A1* SNP rs700518(T>C) variants for patients in the Breast International Group (BIG) 1–98 trial assigned to 5-year letrozole or tamoxifen. Hazard ratios (HR) and confidence intervals (CI) estimated using adjusted Cox proportional hazards model

Fig. 4. Kaplan–Meier estimates of bone adverse event-free interval according to *CYP19A1* SNP genotypes for patients in the Breast International Group (BIG) 1–98 trial assigned to 5-year letrozole or tamoxifen. Hazard ratios (HR) and confidence intervals (CI) estimated using adjusted Cox proportional hazards models. **a** SNP rs4646(C>A) letrozole; **b** SNP rs4646(C>A) tamoxifen; **c** SNP rs10046(C>A) letrozole; **d** SNP rs10046(C>A) tamoxifen; **e** SNP rs936308(C>G) letrozole; **f** SNP rs936308(C>G) tamoxifen

Table 1
Characteristics and clinical outcomes of patients in the BIG 1–98 trial according to availability of DNA for genotyping

Characteristic	DNA for <i>CYP19A1</i> Genotyping ^a	
	No (n = 3149)	Yes (n = 4861)
Four-arm randomization option (%)	66	84
Median follow-up, months	98	98
Postmenopausal (%)	100	100
White race (%)	97	98
Age, median (IQR) (year)	61 (55, 67)	61 (56, 67)
BMI, median (IQR) (kg/m ²)	26 (23, 29)	26 (23, 29)
Mastectomy (%)	46	42
Previous (neo)adjuvant chemotherapy (%)	29	23
Lymph node-positive (%)	38	43
Tumor size >2 cm (%)	35	39
Tumor grade 2 or 3 (%)	75	80
Peritumoral vascular invasion present (%)	20	19
Centrally assessed tumor features ^b		
ER-absent (%)	2	1
HER2-positive (%)	7	6
Ki-67 LI of immunostained cells, median (IQR) (%)	10 (5, 16)	12 (7, 19)
Clinical outcomes		
Breast cancer events (%)	17	17
Distant recurrences (%)	13	13
Musculoskeletal adverse events ^c (%)	25	30
Bone adverse events ^d (%)	28	32

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

CYP19A1 cytochrome P450 19A1, *IQR* interquartile range, *BMI* body mass index, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *LI* labeling index

^a Genomic DNA was isolated from formalin-fixed paraffin-embedded primary breast cancer tissue blocks from 4861 BIG 1–98 trial patients. Genotyping for six *CYP19A1* single nucleotide polymorphisms was done using polymerase chain reaction-based methods

^b Centrally assessed tumor features were available for a subset of the trial patients (1515 of 3149 patients with no DNA for genotyping; 4776 of 4861 patients with DNA available for genotyping)

^c Musculoskeletal adverse events include arthralgia and myalgia reported during the 5-year protocol treatment period

^d 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 CYP19A1 genotype variants and breast cancer-free interval, in the overall cohort and in the subset of patients assigned to a 5-year monotherapy regimen in the BIG 1–98 trial

SNP	Genetic model	Overall cohort			5-year letrozole			5-year tamoxifen			Interaction <i>P</i> ^a
		<i>N</i> patients (<i>N</i> events)	HR (95 % CI)	<i>P</i>	<i>N</i> patients (<i>N</i> events)	HR (95 % CI)	<i>P</i>	<i>N</i> patients (<i>N</i> events)	HR (95 % CI)	<i>P</i>	
rs4646	0 versus 1 versus 2 (A)	4164 (702)	1.02 (0.91, 1.14)	0.78	1236 (199)	1.00 (0.81, 1.23)		1173 (209)	1.03 (0.83, 1.28)	0.76	
rs10046	0 versus 1 versus 2 (C)	3709 (603)	1.00 (0.90, 1.10)	0.93	1084 (173)	0.93 (0.76, 1.13)		1034 (170)	1.05 (0.86, 1.28)	0.40	
rs700518	0 versus 1 versus 2 (C)	2161 (305)	0.86 (0.72, 1.03)	0.10	618 (78)	1.14 (0.81, 1.62)		587 (93)	0.64 (0.45, 0.92)	0.10	
	TT (ref) versus CC, TC		–	–		1.20 (0.73, 1.96)			0.53 (0.34, 0.82)	0.08	
rs700519	0 versus 1 versus 2 (A)	2875 (416)	1.10 (0.83, 1.44)	0.51	829 (104)	0.57 (0.27, 1.23)		809 (127)	1.37 (0.89, 2.12)	0.08	
rs936308	0 versus 1 versus 2 (G)	2434 (347)	0.81 (0.64, 1.03)	0.09	720 (94)	0.60 (0.36, 1.02)		679 (108)	0.99 (0.66, 1.47)	0.17	
rs28757184	0 versus 1 versus 2 (A)	3068 (433)	1.22 (0.89, 1.69)	0.22	875 (109)	1.58 (0.85, 2.92)		856 (135)	1.61 (0.91, 2.83)	0.94	

CYP19A1 SNPs rs4646 and rs10046 were genotyped in all 4861 patient DNA samples; other SNPs were genotyped in 3691 patient DNA samples. HRs, CIs and *P* values estimated using adjusted Cox proportional hazards models

CYP19A1 cytochrome P450 19A1, BIG Breast International Group, SNP single nucleotide polymorphism, HR hazard ratio, CI confidence interval, – = not assessed

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

Table 3
Associations between *CYP19A1* genotype variants and musculoskeletal adverse events reported over the 5-year treatment period, in the overall cohort and in the subset of patients assigned to a 5-year monotherapy regimen in the BIG 1–98 trial

SNP	Genetic model	Overall cohort			5-year letrozole			5-year tamoxifen			Interaction <i>P</i> ^a
		<i>N</i> patients (<i>N</i> events)	HR (95 % CI)	<i>P</i>	<i>N</i> patients (<i>N</i> events)	HR (95 % CI)	<i>P</i>	<i>N</i> patients (<i>N</i> events)	HR (95 % CI)	<i>P</i>	
rs4646	0 versus 1 versus 2 (A)	4164 (1228)	1.03 (0.95, 1.12)	0.42	2080 (268)	1.00 (0.84, 1.20)		2084 (475)	1.11 (0.98, 1.26)	0.4	
rs10046	0 versus 1 versus 2 (C)	3709 (1107)	0.99 (0.92, 1.07)	0.89	1848 (245)	0.99 (0.84, 1.17)		1861 (439)	1.02 (0.90, 1.15)	0.84	
rs700518	0 versus 1 versus 2 (C)	2161 (637)	1.07 (0.95, 1.21)	0.24	1074 (152)	1.05 (0.82, 1.36)		1087 (243)	1.19 (0.98, 1.43)	0.47	
	TT (ref) versus CC, TC		1.22 (1.03, 1.45)	0.02		–			–		–
rs700519	0 versus 1 versus 2 (A)	2875 (849)	1.01 (0.83, 1.24)	0.91	1435 (210)	0.88 (0.57, 1.35)		1440 (316)	1.01 (0.72, 1.42)	0.59	
rs936308	0 versus 1 versus 2 (G)	2434 (725)	1.02 (0.87, 1.18)	0.83	1204 (172)	1.10 (0.81, 1.50)		1230 (279)	0.91 (0.71, 1.18)	0.37	
rs28757184	0 versus 1 versus 2 (A)	3068 (911)	0.82 (0.63, 1.07)	0.14	1532 (216)	0.79 (0.43, 1.45)		1536 (341)	0.94 (0.64, 1.39)	0.55	

CYP19A1 SNPs rs4646 and rs10046 were genotyped in all 4861 patient DNA samples; other SNPs were genotyped in 3691 patient DNA samples. HRs, CIs, and *P* values estimated using adjusted Cox proportional hazards models

CYP19A1 cytochrome P450 19A1, *BIG* Breast International Group, *SNP* single nucleotide polymorphism, *HR* hazard ratio, *CI* confidence interval, – = not assessed

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

Table 4
Associations between *CYP19A1* genotype variants and bone adverse events reported during or subsequent to the 5-year treatment period, in the overall cohort and in the subset of patients assigned to a 5-year monotherapy regimen in the BIG 1–98 trial

SNP	Genetic model	Overall cohort			5-year letrozole			5-year tamoxifen			Interaction <i>P</i> ^a
		<i>N</i> patients (<i>N</i> events)	HR (95 % CI)	<i>P</i>	<i>N</i> patients (<i>N</i> events)	HR (95 % CI)		<i>N</i> patients (<i>N</i> events)	HR (95 % CI)		
rs4646	0 versus 1 versus 2 (A)	4164 (1324)	0.99 (0.91, 1.07)	0.72	1236 (399)	0.99 (0.85, 1.15)		1173 (250)	0.80 (0.66, 0.99)		0.12
	CC (ref) versus CA, AA		–	–		1.02 (0.84, 1.25)			0.76 (0.59, 0.98)		0.09
rs10046	0 versus 1 versus 2 (C)	3709 (1194)	0.99 (0.92, 1.07)	0.89	1084 (356)	1.00 (0.88, 1.15)		1034 (218)	1.28 (1.07, 1.52)		0.03
	TT(ref) versus TC, CC		–	–		1.02 (0.82, 1.28)			1.42 (1.05, 1.93)		0.09
rs700518	0 versus 1 versus 2 (C)	2161 (754)	1.01 (0.90, 1.13)	0.91	618 (219)	0.93 (0.76, 1.14)		587 (127)	0.92 (0.70, 1.22)		0.96
rs700519	0 versus 1 versus 2 (A)	2875 (991)	1.02 (0.85, 1.23)	0.82	829 (287)	1.23 (0.89, 1.69)		809 (170)	1.09 (0.72, 1.63)		0.52
rs936308	0 versus 1 versus 2 (G)	2424 (855)	0.89 (0.77, 1.02)	0.10	720 (249)	0.77 (0.59, 1.01)		679 (146)	1.25 (0.91, 1.71)		0.02
	CC (ref) versus GC, GG		–	–		0.73 (0.54, 0.99)			1.32 (0.92, 1.90)		0.01
rs28757184	0 versus 1 versus 2 (A)	3068 (1061)	1.15 (0.94, 1.41)	0.17	875 (299)	0.95 (0.64, 1.42)		856 (183)	0.58 (0.28, 1.19)		0.27

CYP19A1 SNPs rs4646 and rs10046 were genotyped in all 4861 patient DNA samples; other SNPs were genotyped in 3691 patient DNA samples. HRs, CIs, and *P* values estimated using adjusted Cox proportional hazards models

CYP19A1 cytochrome P450 19A1, *BIG* Breast International Group, *SNP* single nucleotide polymorphism, *HR* hazard ratio, *CI* confidence interval, – = not assessed

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