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ORIGINAL ARTICLE

Single nucleotide polymorphisms of CYP19A1 predict clinical outcomes and adverse events associated with letrozole in patients with metastatic breast cancer

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Abstract
Purpose The *CYP19A1* gene encodes the aromatase enzyme involved in the peripheral conversion of androgen to estrogen. We evaluated the efficacy of the aromatase inhibitor letrozole in patients with metastatic breast cancer (MBC) as related to DNA polymorphisms of *CYP19A1*.
Patients and methods One hundred and nine patients with hormone receptor–positive MBC were treated with letrozole alone or in combination with a GnRH agonist. DNA was isolated from peripheral blood and genotyped for 46 single nucleotide polymorphisms (SNPs) of *CYP19A1*.
Results Among 46 SNPs examined, rs700518, rs10459592, and rs4775936 were significantly associated with higher clinical benefit rate (CBR, CR + PR + SD $\geq$ 6 months) (OR = 2.61 [95% CI; 1.13–6.03], $P = 0.025$; OR = 2.45 [95% CI; 1.06–5.65], $P = 0.036$; OR = 2.60 [95% CI; 1.12–6.02], $P = 0.026$, respectively). Median time to progression (TTP) was improved without statistical significance in patients having an over-dominant form of rs700518. In haplotype analysis, the specific haplotypes M_1_3 and M_2_1 showed a strong association with CBR (OR = 3.37 [95% CI 1.43–7.90], $P = 0.005$; OR = 5.33 [95% CI 1.63–17.45], $P = 0.006$, respectively). There was a statistically significant difference in TTP in patients with haplotype M_1_3 (5.61 months [95% CI 0.00–11.45] vs. 11.08 months [95% CI 6.75–15.42], $P = 0.040$) and M_2_1 (7.31 months [95% CI 4.63–9.99] vs. 12.95 months [95% CI 9.27–16.63], $P = 0.038$). Haplotypes M_3_5 (OR = 11.25 [95% CI 1.17–108.28], $P = 0.01$) and M_5_3 (OR = 4.12, [95% CI 1.09–15.61], $P = 0.03$) were associated with side effects of arthralgia and hot flash, respectively.
Conclusion The genetic variations of *CYP19A1* were significantly associated with clinical efficacy, suggesting potential predictive markers for letrozole treatment in patients with metastatic breast cancer.

Keywords Breast cancer · CYP19A1 · Polymorphisms · Letrozole

Introduction

Endocrine therapy is probably the most important systemic therapy for hormone receptor (HR)-positive breast cancer. Aromatase inhibitors (AIs), usually administered to postmenopausal patients, have proven efficacy in both early and advanced HR-positive breast cancer [1–3]. Recently, we also demonstrated that the clinical efficacies in premenopausal patients with metastatic breast cancer were comparable to those in postmenopausal patients when goserelin therapy was combined with letrozole [4]. The biomarker for response to these agents is HR positivity, and

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patients with HR-positive advanced breast cancer achieved better clinical outcome with AI treatment than with anti-estrogen (tamoxifen) treatment as their first-line therapy [5, 6]. Despite HR positivity, a significant number of patients fail to attain a durable response with AI [7]. The identification of additional biomarkers capable of predicting the clinical efficacy of AIs is widely researched, including genetic study of candidate markers.

The target of AIs is the cytochrome P450 enzyme aromatase, which is encoded by the gene *CYP19A1*. In addition to ovary, testis, adipose tissue, skin, and placenta, breast tissue normally expresses aromatase and its expression is regulated through alternative promoters [8]. *CYP19A1* is a single gene encoding the aromatase protein that plays a key role in estrogen production and is a distinct target of AIs; however, its pharmacogenomic associations with drug efficacy and toxicity are poorly understood. Most previous studies evaluated the relationship of *CYP19* polymorphisms with breast cancer risk in healthy women [9, 10], or prognosis of patients with breast cancer [11, 12].

Ma et al. studied aromatase polymorphisms and reported 88 *CYP19* polymorphisms, resulting in 44 haplotypes in 60 patients from the ethnic groups of Caucasian Americans, Mexican Americans, Han-Chinese Americans, and African-Americans [13]. Aside from its large ethnic variations in both allele frequencies and types, activities of aromatase varied and inhibitor constant for letrozole was significantly increased compared with that of wild type, suggesting relative resistance to letrozole depending on the genotypic variation. More recently, Colomer et al. described the association of one *CYP19A1* single nucleotide polymorphisms (SNPs), rs4646 in the common 3′-untranslated region (UTR), with improved complete response rate and time to progression (TTP) in 67 postmenopausal women with HR-positive metastatic breast cancer [14]. Although only three SNPs were tested in a limited number of patients in this trial, it suggested that genetic variation could affect patient response to AIs.

From those previous studies, we hypothesized that *CYP19A1* gene polymorphisms could be related to the efficacy of letrozole in patients with HR-positive metastatic breast cancer. We also analyzed the relationship of these polymorphisms to letrozole side effects of bone/joint pain and hot flash.

Materials and methods

Study population and treatment

Patients with estrogen receptor (ER)- and/or progesterone receptor (PgR)-positive metastatic breast cancer were enrolled. Inclusion criteria were as follows: (1) received letrozole as first- or second-line hormonal therapy after tamoxifen in the metastatic setting, (2) at least 18 years old, (3) whole blood sample provided, (4) known ER, PgR, and human epidermal growth factor receptor-2 (HER2) status, and (5) written informed consent for study participation. Exclusion criteria were as follows: patients treated with aromatase inhibitor in the metastatic setting, life-threatening disease requiring chemotherapeutic intervention, uncontrolled CNS metastasis, or more than three prior chemotherapeutic regimens for metastatic disease.

The study included 109 consecutive patients treated at the National Cancer Center Hospital, Korea between January 2003 and December 2008. Postmenopausal patients received letrozole (2.5 mg, oral, once daily) alone and premenopausal patients received letrozole combined with goserelin (3.6 mg, subcutaneous, once a month) for metastatic breast cancer until disease progression or unacceptable toxicity occurred. Tumor assessment was made at baseline and repeated every 8 weeks. Treatment response was evaluated according to Response Evaluation Criteria In Solid Tumor version 1.0 (RECIST) for both measurable and evaluable lesions [15]. Patients achieving complete response (CR), partial response (PR), or stable disease for more than 6 months (SD $\geq$ 6 months) were allocated to the clinical benefit group. Patients having progressive disease (PD) or stable disease <6 months on letrozole treatment were placed in the non-clinical benefit group. Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Event version 3 (NCI-CTCAE) [16]. The Institutional Review Board for Human Research of the National Cancer Center (IRB No. NCCCTS-06198) approved this study.

SNP selection and genotyping

Candidate SNPs of the *CYP19A1* gene were identified using the National Center for Biotechnology Information (NCBI) gene and SNP databases (available at: http://www.ncbi.nlm.nih.gov/projects/SNP/, accessed April 25, 2009). Among a total of 961 SNPs registered for *CYP19A1*, we selected 49 SNPs that had a minor allele frequency (MAF) >0.05 in Han-Chinese or Japanese population or had published functional data even if their MAF was less than 0.05 or unknown. Additionally, we also selected all SNPs in coding region including synonymous SNPs (Supplement Table 1). For assay design and multiplexing, MassARRAY Assay Design (ver.3.1.2.5, Sequenom, USA) was used. For two SNPs, rs11540804 and rs4493496, there was a failure to generate suitable primers because of high primer dimer potential. Thus, 47 SNPs were successfully designed for genotyping in four assay groups (Supplement Table 2).

Genomic DNA was prepared from peripheral blood samples using MaqAttract DNA Blood kit (Qiagen, Germany) following the manufacturer's protocol. The purity and concentration of isolated DNA were determined using Quant-iT PicoGreen dsDNA Assay kit (Molecular Probes, Inc. USA), and 10 ng per sample was used for genotype analysis. Genotyping was performed by iPLEX Gold assay on the Mass ARRAY® platform (Sequenom, USA) based on matrix-associated laser desorptional ionization, time-of-flight mass (MALDI-TOF) spectrometry by manufacturer's instruction, and resulting genotype data were collected by Typer v4.0 [17].

Statistical methods

We tested for an association between genetic variants in the aromatase gene, *CYP19A1*, and treatment efficacy of the aromatase inhibitor, letrozole. Clinical benefit rate (CBR) was the primary end point, and it was defined as a proportion of patients with complete response, partial response, or stable disease ≥6 months in total study population. Time to progression (TTP) was calculated from the first date of letrozole treatment to the date of progression, or death related to breast cancer. TTP was estimated using the Kaplan–Meier method and compared using the log-rank test.

Chi-square test for genotype distribution was conducted to evaluate the deviation from Hardy–Weinberg equilibrium for all SNPs, and Bonferroni correction was applied to correct for multiple testing. For association between clinical efficacy and each SNP, univariate and multivariate logistic regression models were used with the SNP genotype tested under models of over-dominance. When appropriate, 95% confidence intervals (95% CI) for the clinical efficacy were calculated. All statistical analyses were done using STATA version 10.0 (Stata Corp College Station, TX, USA), and all *P*'s were for two-sided test. Linkage disequilibrium (LD) among pair-wise SNPs was measured using D' index [18], and frequencies of haplotypes consisting of multiple SNPs found in the CYP19A1 were estimated using PLEM (Partition Ligation EM) algorithm with SNPAnalyzer software (ISTECH, Inc., Seoul, Korea, http://www.istech21.com/) [19].

Results

Baseline patient characteristics

Of the total 109 patients, 76 patients were classified into the clinical benefit (CB) group and 33 patients into the non-clinical benefit (non-CB) group. In the CB group, 7 (6.4%) patients achieved CR, 21 (19.3%) patients PR, and 48 (44%) patients had a stable disease more than 6 months. Table 1 shows the demographic profile of each group. The two groups were well balanced by disease-free interval (DFI), performance status, number of metastatic lesions, and palliative treatment. Patients in the non-CB group were younger (median 45 years vs. 49 years, $P = 0.014$) and more frequently had HER2-positive tumors (21.2% vs. 5.5%, $P = 0.033$) than the CB group.

Among 47 SNPs selected for our study, one SNP that failed to generate enough data and 10 SNPs that were found to be monomorphic for the Korean population were excluded from further analysis. Consequently, after trimming the data using Hardy–Weinberg Equilibrium test, 36 SNPs were chosen for further analysis (Supplement Table 3).

Identification of SNPs associated with clinical benefit of letrozole treatment

Three SNPs were found to be associated with clinical benefit of letrozole treatment in an over-dominant model; rs10459592 (T/G) and rs4775936 (C/T) located in intron I of *CYP19A1* and rs700518 (T/C) located in exon 3. Table 2 shows the observed percentage of cases with three SNPs variations. In these SNPs, homozygous types (T/T and C/C for rs700518, T/T and G/G for rs10459592; C/C and T/T for rs4775936) were more common in patients having clinical benefit with letrozole treatment (65.8% vs. 42.4%, $P = 0.034$ for rs700518; 67.1% vs. 45.5%, $P = 0.034$ for rs10459592; 68.4% vs. 45.5%, $P = 0.024$ for rs4775936; Table 2). The crude and adjusted odds ratios (OR) for clinical benefit of letrozole by genotype are shown in Table 2. There was a strong association between these homozygous genotypes of three SNPs and a higher clinical benefit rate of letrozole (OR = 2.61 [95% CI 1.13–6.03], $P = 0.025$ for rs700518; OR = 2.45 [95% CI 1.06–5.65], $P = 0.036$ for rs10459592; OR = 2.60 [95% CI 1.12–6.02], $P = 0.026$ for rs4775936). Logistic regression analysis revealed that these three individual SNPs were associated with a higher clinical benefit rate of letrozole treatment after adjusting for age, HER2 positivity, number of metastatic lesions, and liver metastasis (Table 2). Aside from rs700518, rs10459592, and rs4775936 variant, older age (OR = 1.06 [95% CI 1.01–1.11], $P = 0.018$) and HER2 positivity (OR = 0.22 [95% CI 0.06–0.80], $P = 0.021$) were found to be independent predictive factors for response to letrozole treatment.

During a median follow-up of 31.7 months (range, 4.0–107.4 months), there was no significant difference in median TTP according to genetic variants (rs700518, 12.07 months [95% CI 8.67–15.46] vs. 7.54 months [95% CI 6.53–8.55], $P = 0.097$; rs4775936, 11.93 months [95% CI 8.83–15.04] vs. 7.54 months [95% CI 6.57–8.51],

Table 1 Baseline patient characteristics

	CB group ($N = 76$)	Non-CB group ($N = 33$)	P value*
Age (median, range)	49 (31–70)	45 (32–69)	0.014
ER positive	76 (100%)	33 (100%)	1.000
PgR positive	62 (83.8%)	31 (93.9%)	0.218**
HER2 positive	4 (5.5%)	7 (21.2%)	0.033**
Adjuvant chemotherapy	47 (62.7%)	24 (72.7%)	0.310
Adjuvant hormonal therapy	39 (52.0%)	21 (63.6%)	0.262
DFI ≥2 years	41 (54.0%)	20 (60.6%)	0.520
Premenopausal status	33 (43.4%)	14 (42.4%)	0.923
Postmenopausal status	43 (56.6%)	19 (57.6%)	
ECOG PS 0 or 1	70 (98.6%)	31 (93.9%)	0.626**
Number of metastatic site			
1	27 (35.5%)	15 (45.4%)	0.065
2	21 (27.6%)	7 (21.2%)	
≥3	28 (36.9%)	11 (33.4%)	
Metastatic sites			
Liver	11 (14.5%)	7 (21.2%)	0.384
Lung	28 (36.8%)	15 (45.5%)	0.398
Bone	48 (63.2%)	22 (66.7%)	0.726
Soft tissue	40 (52.6%)	16 (48.5%)	0.691
Number of palliative chemotherapeutic regimen			
0	57 (75.0%)	19 (57.6%)	0.193**
1	13 (17.1%)	11 (33.3%)	
2	5 (6.6%)	3 (9.1%)	
≥3	1 (1.3%)	0	
Letrozole treatment			
First line	73 (96.1%)	32 (97.0%)	1.00**
Second line	3 (3.9%)	1 (3.0%)	

CB clinical benefit of letrozole treatment, *ER* estrogen receptor, *PgR* progesterone receptor, *HER2* human epithelial receptor 2, *DFI* disease-free interval, *PS* performance status

* P value was determined using the Wilcoxon rank sum test for non-parametric variables and the chi-square test for categorical variables

** P value was determined using Fisher's exact test

Table 2 Genotype and allelic frequencies of rs700518, rs10459592 and rs4775936, and Odds Ratios (OR) for clinical benefit of letrozole treatment by genotype

Gene/SNP	CB group ($N = 76$)	Non-CB group ($N = 33$)	P*	Crude		Adjusted**	
				OR (95% CI)	P	OR (95% CI)	P
rs700518							
T/T or C/C	50 (65.8%)	14 (42.4%)	0.034	2.61 (1.13–6.03)	0.025	2.52 (1.02–6.20)	0.044
T/C	26 (34.2%)	19 (57.6%)		Reference			
rs10459592							
G/G or T/T	51 (67.1%)	15 (45.5%)	0.034	2.45 (1.06–5.65)	0.036	2.61 (1.06–6.46)	0.038
G/T	25 (32.9%)	18 (54.5%)		Reference			
rs4775936							
C/C or T/T	52 (68.4%)	15 (45.5%)	0.024	2.60 (1.12–6.02)	0.026	2.89 (1.16–7.22)	0.023
C/T	24 (31.6%)	18 (54.5%)		Reference			

CB clinical benefit of letrozole treatment, *OR* odds ratio, *CI* confidence interval

* Two-way analysis (homozygous vs. heterozygous)

** Individually adjusted for age, HER2 positivity, number of metastatic lesions, and liver metastasis

$P = 0.205$; rs10459592, 11.93 months [95% CI 8.66–15.21] vs. 7.74 months [95% CI 6.51–8.97], $P = 0.176$; Fig. 1).

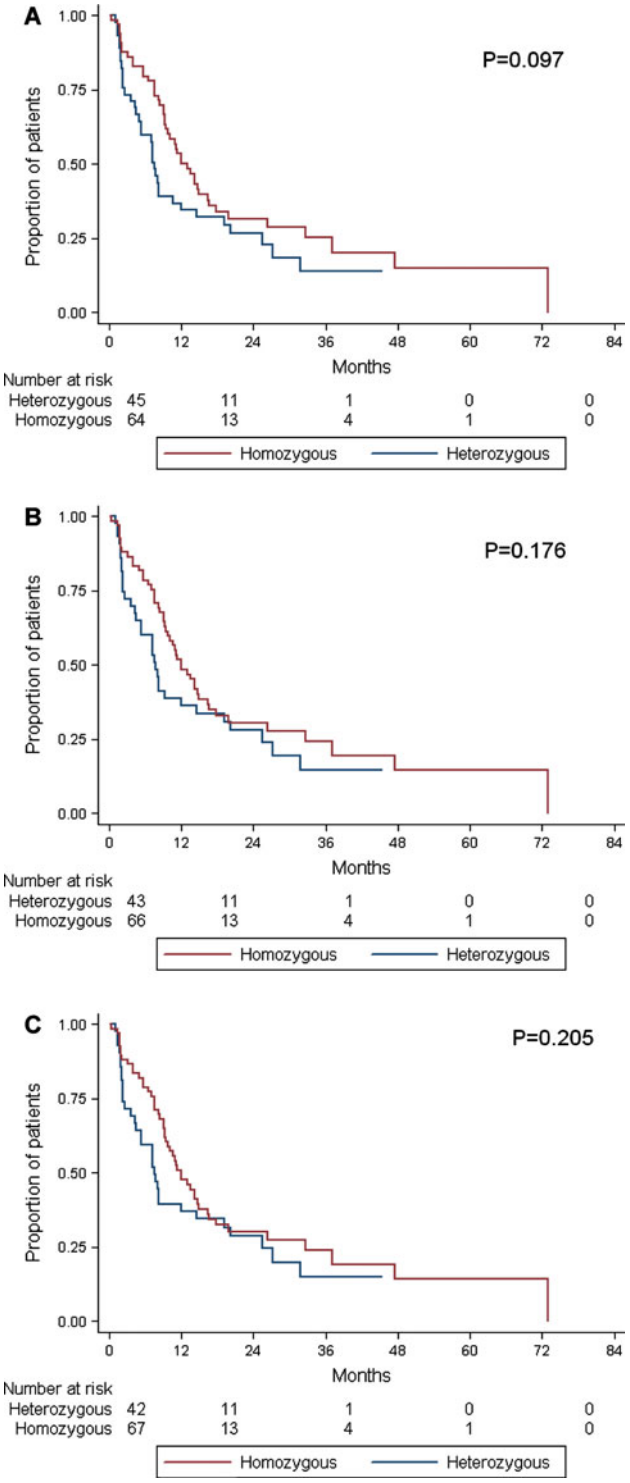

Fig. 1 Time to progression on letrozole treatment by genotype of **a** rs700518, **b** rs10459592, **c** rs4775936

Haplotypes associated with clinical benefit and side effects of letrozole treatment

The linkage disequilibrium and haplotypes were analyzed (Supplement Fig. 1 and 2). We investigated the clinical efficacy of letrozole in the association of specific haplotypes with a frequency >1%. Haplotype analysis showed that there were 14 major haplotypes derived from all 36 SNPs tested included and, of those, haplotype M_1_3 was significantly associated with clinical benefit of letrozole treatment (OR = 5.33, [95% CI 1.63–17.45], $P = 0.006$; Table 3). Of 8 haplotypes derived from the 8 SNPs, M_2_1 also showed a significant association with clinical benefit of letrozole treatment under the over-dominant model (OR = 3.37, [95% CI 1.43–7.90], $P = 0.005$; Table 4). Kaplan–Meier estimates indicated that the patients carrying those relevant haplotypes had significantly longer TTP (11.08 months [95% CI 6.75–15.42] vs. 5.61 months [95% CI 0.00–11.45], $P = 0.040$ for M_1_3; 12.95 months [95% CI 9.27–16.63] vs. 7.31 months [95% CI 4.63–9.99], $P = 0.038$ for M_2_1; Fig. 2).

Of the total 109 patients, 66 were analyzed for the presence of joint/bone pain and hot flash during letrozole treatment. We investigated the association between these side effects and genetic variations of *CYP19A1*. While none of the individual SNPs in a *CYP19A1* gene displayed significant association with hot flash or arthralgia, the specific haplotype M_5_3 was significantly associated with hot flash (OR = 4.12, [95% CI 1.09–15.61], $P = 0.03$) and M_3_5 was associated with arthralgia (OR = 11.25, [95% CI 1.17–108.28], $P = 0.01$; Table 4). Among clinical variables, the presence of hot flash was strongly related to premenopausal status (78.1% vs. 44.1%, $P = 0.006$). In multivariate analysis, premenopausal status remained a significant risk factor for increasing hot flash (premenopausal status; OR = 4.24, [95% CI 1.38–12.98], $P = 0.012$: M_5_3; OR = 3.57, [95% CI 0.87–14.63], $P = 0.077$).

Discussion

In the present study, we evaluated the relationship between genetic variation of the *CYP19A1* gene and the efficacy of letrozole in patients with metastatic breast cancer. All 109 patients were from a homogeneous ethnic group, Korean, and the majority received letrozole as first-line hormonal therapy. According to our results, the SNP variants rs700518, rs10459592, and rs4775936 were associated with variability in letrozole clinical efficacy.

Previously, Colomer et al. reported that among three SNPs tested (rs4646, rs10046, and rs727479), the rs4646 variant located in the 3′-untranslated region (UTR) was significantly associated with prolonged TTP in

Table 3 Association between clinical benefit of letrozole treatment and CYP19A1 haplotypes under the over-dominant model analysis

Haplotype	Haplotype composition[a]	CB(+)[b] $N = 76$	CB(−)[a] $N = 33$	OR (95% CI)	*P*
M_1_1	CTCAGCCCAATGAAGAATCAGTGCCATAAATTGCCA	48	25	0.55 (0.19–1.48)	0.200
M_1_2	CCAGGATTCACTGGCAGCGGTCGCCATAAACTGCTC	57	21	1.71 (0.64–4.48)	0.227
M_1_3	CTCAGCCCAATGAAGAATCAGTGCCATAAACTGCTC	71	24	5.33 (1.63–17.45)	0.006
M_1_4	CTCAGCTTAACTGGCGATCAGTAGTAGCAACTGCTA	65	31	0.38 (0.04–1.92)	0.213
M_1_5	TCCGAATTAACTGGCGACCGTCAGTGGCAACTGCTC	69	31	0.64 (0.06–3.62)	0.583
M_1_6	CCAGGATTCACTGGCAGCGGTCGCCATAAATTGCCA	70	32	0.36 (0.01–3.22)	0.341
M_1_7	CTCAGCCCAATGAAGAATGGTCAGTAGCAACCATTC	72	31	1.16 (0.10–8.58)	0.867
M_1_8	CTCAGCCCAACTAAGAATCAGTGCCATAAACTGCTC	74	31	2.39 (0.16–34.00)	0.382
M_1_9	TCCGAATTAACTGGCGACCGTCAGTGGCATCCATTC	74	31	2.39 (0.16–34.00)	0.382
M_1_10	CCAGGATTCACTGGCAGCGGTCGCCATAGTCCATTC	74	31	2.39 (0.16–34.00)	0.382
M_1_11	CTCAGCTTAACTGGCGATCAGTAGTAGCAATTGCCA	74	32	1.16 (0.02–22.93)	0.907
M_1_12	TCCGAATTAACTGGCGACCGTCAGTGGCGTCCATTC	74	32	1.16 (0.02–22.93)	0.907
M_1_13	CTCAGATTCACTGGCAGCGGTCGCCATAAACTGCTC	74	33	–[c]	–[c]
M_1_14	CTCAGCCCAATGAAGAATCAGTGCCATAGACTGCTC	76	31	–[c]	–[c]
M_2_1	CTCAGCCC	50	12	3.37 (1.43–7.90)	0.005
M_2_2	CCAAGGATT	47	17	1.53 (0.61–3.77)	0.314
M_2_3	TCCGAATT	61	29	0.56 (0.12–1.99)	0.336
M_2_4	CTCAGCTT	60	28	0.67 (0.17–2.18)	0.473
M_2_5	CTCAGATT	73	31	1.57 (0.12–14.35)	0.628
M_2_6	TCCGGATT	72	33	–[c]	–[c]
M_2_7	TCCGACTT	75	33	–[c]	–[c]
M_2_8	CCAGGCCC	76	32	–[c]	–[c]

CB(+) patients having clinical benefit of letrozole treatment, *CB(−)* patients having no clinical benefit of letrozole treatment, *OR* odds ratio, *CI* confidence interval

[a] Haplotypes deriving from the following SNPs in order: all 36 SNPs for M_1; rs2255192, rs12148604, rs4646, rs10046, rs700519, rs4324076, rs700518, rs3759811 for M_2

[b] The number of patients having an over-dominant form of given haplotype

[c] Exact confidence levels were not possible with zero count cells

Table 4 Association between side effects of letrozole treatment—bone pain and hot flashes—and *CYP19A1* haplotypes under the over-dominant model analysis

	Haplotype	Haplotype composition[a]	Haplotype frequency	OR (95% CI)	*P*
Hot flashes	M_5_3	AGTGGC	0.121	4.12 (1.09–15.61)	0.03
Bone pain	M_3_5	TCAGATTCCTGGCAGC	0.022	11.25 (1.17–108.28)	0.01

M_5_3: rs1902586, rs7181886, rs936306, rs1902582, rs16964254, rs28566535

M_3_5: rs12148604, rs4646, rs10046, rs700519, rs4324076, rs700518, rs3759811, rs727479, rs4775936, rs10459592, rs767199, rs10519297, rs1062033, rs2008691, rs1008805, and rs17523527

[a] Haplotypes deriving from the following SNPs in order

postmenopausal women treated with letrozole in the metastatic setting [14]. According to their other independent study, the rs4646 variants were also associated with poor response to letrozole in the neoadjuvant setting [20]. We analyzed 46 SNPs including all known non-synonymous SNPs and specifically rs4646, rs10046, and rs727479, after consideration of all *CYP19A1* SNPs with MAF >0.05. Potential importance lies in the ethnic differences among patient population, since genetic variability of *CYP19A1* as well as AI treatment response is reportedly affected by ethnicity [21], and this may explain the conflicting results between the present study and Colomer's.

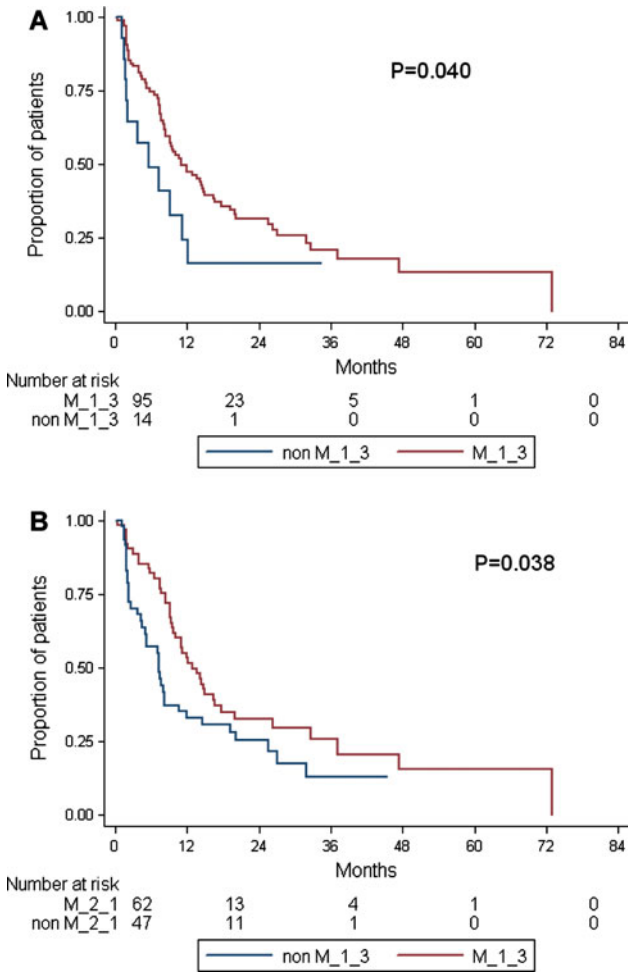

Fig. 2 Time to progression on letrozole treatment by relevant haplotype, **a** M_1_3, **b** M_2_1

We constructed common haplotypes in the *CYP19A1* gene and investigated the association with clinical efficacy of letrozole treatment. Relevant haplotypes M_2_1 and M_1_3 showed more significant association with letrozole clinical efficacy than individual SNPs in terms of TTP and clinical benefit rate. Haplotype M_1_3 was derived from all 36 SNPs described in this study. The other relevant haplotype, M_2_1, was constructed from 8 SNPs, which included rs70018. In our results, not all SNPs included in relevant haplotypes showed an individual association with clinical efficacy of letrozole. This indicates that SNPs with small individual functional effects may cumulatively influence phenotype. This kind of cumulative effect of haplotypes formed by SNPs with weak or no association has been recently reported [22, 23].

For the analysis of letrozole side effects, we focused on joint/bone pain and hot flash, which were presumably associated with the change in circulating estrogen level. Even when individual SNP did not show a statistically significant relation with these side effects, two specific haplotypes, M_3_5 and M_5_3, were strongly associated with bone/joint pain and hot flash, respectively. We suggest that the associations of these specific haplotypes with symptoms might be based on the combinations of those alleles rather than the influence of individual SNPs. In the current study, we did not conduct functional characterization of these *CYP19A1* variants. While several previous studies demonstrated variations in circulating estrogen levels with different polymorphisms of estrogen synthesizing and metabolism genes, including *CYP19A1* [24, 25], no definite correlation between circulating hormonal levels and response to aromatase inhibitors was observed. Letrozole is a potent suppressor of total body aromatization, suppressing over 99% [26], and reduces plasma estradiol to a nearly not detectable level in most patients [27]. Therefore, in terms of differences in efficacy of letrozole, genetic polymorphisms of *CYP19A1* would be associated with the local over-production of aromatase, especially within the tumor and its microenvironment, rather than circulating plasma estrogen levels. Thus far, two mechanisms of regulation of aromatase expression in breast cancer tissue have been addressed [8]. The altered cellular composition in breast cancer and the change in affinity of transcriptional enhancers to the upstream of aromatase promoter I.3/II were known to be responsible for estrogen over-production in breast cancer tissue [28, 29]. Wang et al. revealed from their genotype–phenotype study that the genetic variation of *CYP19A1*, especially rs6493497 and rs7176005 in the 5′-flanking region of *CYP19* exon I.1, affected the transcriptional activity of aromatase in the tumor tissue and, thus, increased transcriptional level, resulting in higher aromatase activity [30].

The two SNPs, rs10459592 and rs4775936, found to be significantly associated with letrozole treatment efficacy in our study are located in intron I, and the relevant haplotype M_2_1 was included in the regulatory region of gene expression. Therefore, investigation of the role of genetic polymorphisms in the regulation of aromatase expression in breast cancer should be addressed in future studies.

In summary, we found that genetic variants of the *CYP19A1* gene—rs70018, rs10459592, and rs4775936—were associated with the clinical efficacy of letrozole treatment in Korean, HR-positive metastatic breast cancer patients. We report here for the first time the association of *CYP19A1* haplotypes with clinical response and side effects of letrozole. Further studies addressing the function of these SNPs for prospective validation in the clinical setting will be essential to improve therapeutic guidance of endocrine therapy in patients with metastatic breast cancer.

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