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Association of genetic polymorphisms in *CYP19* and *CYP1A1* with the oestrogen receptor-positive breast cancer risk

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Abstract

Since tamoxifen has been shown to reduce the risk of oestrogen receptor (ER)-positive, but not ER-negative, breast cancers in a chemoprevention trial (P-1), it is important to develop assays to assess risk factors for ER-positive breast cancer in order to appropriately select candidates for chemoprevention with tamoxifen. Thus, the significance of genetic polymorphisms of genes involved in oestrogen biosynthesis (*CYP19*) and metabolism (*CYP1A1*) as a risk factor for ER-positive breast cancers was evaluated. A case-control study was conducted with 257 breast cancer patients and 191 healthy female controls. Two polymorphisms, *CYP19* (*TTTA repeats*) in intron 4 and *CYP1A1* $^{6235}C/T$ in the 3′ non-coding region, and their association with the breast cancer risk after adjustment for the other epidemiological risk factors were examined. *CYP19* $(TTTA)_{7(-3bp)}$ allele carriers showed a significantly ($P<0.05$) increased risk of ER-positive breast cancers (Odds Ratio (OR) = 1.72, 95% Confidence Interval (CI) 1.10–2.69), but not ER-negative breast cancers. *CYP1A1* ^{6235}C allele carriers showed a non-significant ($P=0.06$) trend towards a decreased risk of ER-positive breast cancers (OR = 0.65, 95% CI 0.42–1.02), but not ER-negative breast cancers. The combination of these two polymorphisms was found to be more useful in the assessment of the ER-positive breast cancer risk (OR = 3.00, 95% CI = 1.56–5.74) than the *CYP19* $(TTTA)_{7(-3bp)}$ polymorphism alone. The combination of *CYP19* $(TTTA)_{7(-3bp)}$ and *CYP1A1* $^{6235}C/T$ polymorphisms is associated with an ER-positive, but not ER-negative, breast cancer risk, and, thus, would be useful in the selection of candidates for chemoprevention with tamoxifen.

Keywords: *CYP19*; *CYP1A1*; Polymorphism; Breast cancer; Oestrogen receptor

1. Introduction

The demonstration in the NSABP P-1 trial that tamoxifen reduces breast cancer risk by 49% in high-risk women [1] seems to have opened the door for a new era where the prevention of breast cancer is given as much emphasis as treatment of an established breast cancer. Currently, tamoxifen is the only agent approved by Food and Drug Administration (FDA) for prophylactic use in order to reduce breast cancer risk. Risk/benefit models suggest that higher benefits with lower adverse effects are obtained with tamoxifen use in women aged 35–49 years with a 5-year Gail risk of 1.67% or greater, in the hysterectomised women aged 50 years and older with a 5-year Gail risk of 2.5% or greater, and in the non-hysterectomised women aged 50 years and older with a 5-year Gail risk of greater than 5.0% [2].

When tamoxifen use is considered, the risk assessment of breast cancer is the most important step. For such a purpose, the risk prediction model developed by Gail and colleagues is presently the most widely used [3]. In this model, the risk of each woman is calculated on the basis of epidemiological risk factors such as age, parity, family history, age at menarche, breast biopsy history and race. The Gail model was developed in order to predict the risk of breast cancer regardless of the oestrogen receptor (ER) status and, thus, it does not differentiate the risk of ER-positive and ER-negative breast cancers. Since the NSABP P-1 trial clearly showed that the risk-reducing effect of tamoxifen was limited to ER-positive breast cancer [1], a new model to

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assess the risk of ER-positive breast cancer needs to be established for selecting candidates for chemoprevention with tamoxifen more efficiently.

Recently, studies on the association between genetic polymorphisms and breast cancer risk have attracted considerable attention [4,5]. Oestrogens play an important role in carcinogenesis and the progression of breast cancer, and special attention has focused on polymorphisms in oestrogen biosynthesis and metabolising genes. Such polymorphisms are expected to affect the synthesis or degradation of oestrogens and, consequently, the risk of breast cancer. It is speculated that a hormonal milieu that is favourable for breast carcinogenesis (i.e. a high oestrogen level) associated with a certain genetic polymorphism would stimulate the development of ER-positive breast cancer rather than ER-negative breast cancer because a high oestrogen level is thought to stimulate carcinogenesis and the development of breast cancer through the ER. Moreover, the fact that women with hormone replacement therapy are more likely to develop ER-positive breast cancer [6] seems to support this speculation. In this regard, genetic polymorphisms in the oestrogen biosynthetic and degradation genes, such as *CYP19* and *CYP1A1*, respectively, may act as a predictor of the ER-positive breast cancer risk. Although several reports have demonstrated a significant association between genetic polymorphisms in these genes and breast cancer risk [7–14], until now, no report has been available investigating the relationship between the genetic polymorphisms and the ER-positive breast cancer risk. Therefore, in our study, we have studied the association of these polymorphisms with ER-positive breast cancer. In addition, we have evaluated the usefulness of risk assessment from a combination of these polymorphisms.

2. Patients and methods

2.1. Cases and controls

Eligible cases were 257 Japanese women living in Osaka who were treated with mastectomy or breast-conserving surgery for primary breast cancer in the Osaka University Hospital from February 1998 to March 2001. They women were consecutively recruited to this study. A histological diagnosis of breast cancer was confirmed for each case (Infiltrating ductal carcinoma; 232, non-infiltrating ductal carcinoma; 6, invasive lobular carcinoma; 10, and other types; 9). One hundred ninety-one healthy Japanese females who lived in Osaka and attended the breast cancer screening programme at the affiliated institutes in Osaka were consecutively recruited as controls from April 2001 to March 2002. All the women were confirmed to be free from breast cancer by a physical examination and mammography. Written informed consent was obtained from all cases and controls.

2.2. Genotype analysis

DNAs were extracted from peripheral blood mononucleated cells. Genomic DNA (0.1 μg each) was used for the polymerase chain reaction (PCR) amplifications using a pair of primers designed for each polymorphism. The genotypes were determined using single-strand conformation polymorphism analysis for *CYP1A1*, as previously described in Ref. [14]. PCR for *CYP19* was carried out using a non-labelled primer 5′-AGCAGGTACTTAGTTAGCTAC-3′ and a Rhodamine-labelled primer 5′-TTACAGTGAGCCAAGGTCGT-3′. The PCR was carried out in a total volume of 20 μl containing 0.1μg of genomic DNA using the GeneAmp PCR system 9600 (Perkin-Elmer, Foster City, CA, USA). The reaction was as follows; after initial denaturation at 94 °C for 5 min, 35 cycles of amplification at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s. Amplified samples were diluted in loading buffer, and electrophoresed onto a 6% polyacrylamide gel containing 7.66 M urea at 30 W for 4 h. Each band was visualised by scanning with the FMBIO II image analyser (Takara, Otsu, Japan).

2.3. ER assay

ER level in the breast cancers was measured using an enzyme immunoassay and a kit provided by Abbott Research Laboratories (Chicago, IL, USA). The cut-off level for ER was defined as 5 fmol/mg protein.

2.4. Serum oestrogen analysis

Serum samples for oestrone (E1) and oestradiol (E2) assays were obtained from 72 postmenopausal healthy controls. Serum E1 and E2 levels were measured by a radioimmunoassay, without a prior separation step, using the kits provided by Diagnostic Systems Laboratories (DSL-8700, Webster, TX, USA) and by CIS biointernational (ESTR-US-CT, Cedex, France), respectively. These kits detect E1 and E2 so sensitively that the detection limits of E1 and E2 were 1.2 and 1.5 pg/ml, respectively.

2.5. Statistical analysis

The relationship between genotype and breast cancer risk was determined using a logistic regression method to obtain the Odds Ratio (OR) and 95% Confidential Interval (CI), and adjusted for the other epidemiological risk factors such as age, family history, age at first live birth, and body mass index (BMI). These risk factors were categorised as follows; family history of first-degree relatives (yes or no), age at menarche (12 years or

younger, 13–14 years and 15 years or older), parity (first live birth at 25 years or younger, 26–29 years, 30 years or older, or nulliparity), and BMI (under 20 kg/m^2, 20 to <23 kg/m^2, and 23.0 or more kg/m^2). The allele frequency of TTTA repeat polymorphisms in *CYP19* between cases and controls was assessed using the χ^2-test and the association between the polymorphisms and the serum E1 and E2 levels was assessed by the Student *t*-test. A *P* value of <0.05 was considered significant.

3. Results

3.1. Genetic polymorphisms in CYP19 and CYP1A1 and breast cancer risk

The age distribution between cases (51.3±9.9 years, mean±standard deviation (S.D.), range 27–78 years) and controls (49.7±8.8 years, range 23–69 years) were not significantly different. However, frequencies of women with a family history, first live birth at 30 years or older, or high BMI (23.0 or more kg/m^2) were significantly higher in cases than in controls (data not shown). As for previous breast biopsies, there was no significant difference between the cases and controls. The allele frequency of the *CYP19* polymorphism was compared between the cases and controls (Table 1). Since the lowest *P* value ($P=0.05$) was obtained for *CYP19* $(TTTA)_{7(-3bp)}$ allele (7 repeats with additional 3-bp deletion), this allele was analysed further (Table 2). Carriers of the *CYP19* $(TTTA)_{7(-3bp)}$ allele showed a significantly ($P<0.05$) increased risk of breast cancer compared with non-carriers (OR = 1.51, 95% CI 1.02–2.25). However, there was no significant association with the *CYP1A1* polymorphism and the breast cancer risk.

Table 1
Allele frequency of tetranucleotide (TTTA) repeat polymorphism in the *CYP19*

(TTTA) repeats no.	Cases (514)[a]	Controls (382)[a]	*P* value
13	0.002	0.003	0.83
12	0.091	0.102	0.59
11	0.276	0.285	0.77
10	0.008	0	0.08
9	–	–	–
8	0.008	0.005	0.64
7	0.335	0.382	0.14
7(−3)	0.280	0.223	0.05

[a] Total number of alleles.

Secondly, we studied the relationship between genetic polymorphisms (*CYP19* and *CYP1A1*) and ER-positive or ER-negative breast cancer risk (Table 3). *CYP19* $(TTTA)_{7(-3bp)}$ allele carriers showed a significantly ($P<0.05$) increased risk for the ER-positive breast cancers (OR = 1.72, 95% CI = 1.10–2.69), but not the ER-negative breast cancers (Table 3). In addition, a trend towards a decreased risk ($P=0.06$) of ER-positive breast cancers (OR = 0.65, 95% CI 0.42-1.02), but not ER-negative breast cancers, was observed in the *CYP1A1* ^{6235}C allele carriers.

3.2. Combination analysis of ER-positive breast cancer risk using CYP19 and CYP1A1 polymorphisms

Since the *CYP19* $(TTTA)_{7(-3bp)}$ allele carriers showed a higher risk of ER-positive breast cancer than non-carriers, *CYP19* $(TTTA)_{7(-3bp)}$ allele carriers were designated as a *CYP19* high-risk genotype and non-carriers were designated as a *CYP19* low-risk genotype. Similarly, the *CYP1A1* variant allele carriers were designated as *CYP1A1* low-risk genotype and non-carriers were designated as *CYP1A1* high-risk genotype because the former genotype was associated with a high risk and the latter genotype was associated with a low risk of ER-positive breast cancer (Table 3). Then, the cases and controls were classified into the three categories according to the number of high-risk genotypes of *CYP19* and *CYP1A1* polymorphisms (Table 4), i.e., 0 = no high-risk genotype, 1 = either *CYP19* or *CYP1A1* high-risk genotype, and 2 = both *CYP19* and *CYP1A1*

Table 2
Relationship between *CYP19* and *CYP1A1* polymorphisms and breast cancer risk

	Cases ($n=257$)	Controls ($n=191$)	OR (95% CI)[a]	OR (95% CI)[b]
CYP19 $(TTTA)_{7(-3bp)}$ allele				
Non-carrier	136 (53)[c]	117 (61)	1.00	1.00
Carrier	121 (47)	74 (39)	1.45 (0.99–2.13)[d]	1.51 (1.02–2.25)[e]
CYP1A1 ^{6235}C allele				
Non-carrier	120 (47)	74 (39)	1.00	1.00
Carrier	137 (53)	117 (61)	0.74 (0.51–1.09)	0.73 (0.49–1.08)

[a] Odds Ratio (OR) and 95% Confidence Interval (CI) adjusted for age.
[b] Odds Ratio and 95% Confidence Interval adjusted for age, family history, parity and body mass index (BMI).
[c] Numbers in parentheses are percentages.
[d] $P<0.1$.
[e] $P<0.05$.

Table 3
Relationship between *CYP19* and *CYP1A1* polymorphisms and breast cancer risk according to ER status

ER-positive breast cancer	Cases ($n=156$)[a]	Controls ($n=191$)	OR (95% CI)[b]	OR (95% CI)[c]
CYP19 $(TTTA)_{7(-3bp)}$ allele				
Non-carrier	77 (49)[d]	117 (61)	1.00	1.00
Carrier	79 (51)	74 (39)	1.68 (1.09–2.59)[e]	1.72 (1.10–2.69)[e]
CYP1A1 ^{6235}C allele				
Non-carrier	77 (49)	74 (39)	1.00	1.00
Carrier	79 (51)	117 (61)	0.67 (0.44–1.04)[f]	0.65 (0.42–1.02)[f]
ER-negative breast cancer	**Cases ($n=88$)[a]**	**Controls ($n=191$)**	**OR (95% CI)[b]**	**OR (95% CI)[c]**
CYP19 $(TTTA)_{7(-3bp)}$ allele				
Non-carrier	53 (60)[d]	117 (61)	1.00	1.00
Carrier	35 (40)	74 (39)	1.05 (0.63–1.77)	1.11 (0.65–1.90)
CYP1A1 ^{6235}C allele				
Non-carrier	37 (42)	74 (39)	1.00	1.00
Carrier	51 (58)	117 (61)	0.89 (0.53–1.48)	0.88 (0.51–1.51)

ER, oestrogen receptor.
[a] ER status was unknown in 13 cases.
[b] Odds Ratio (OR) and 95% Confidence Interval (CI) adjusted for age.
[c] Odds Ratio and 95% Confidence Interval adjusted for age, family history, parity and body mass index (BMI).
[d] Numbers in parentheses are percentages.
[e] $P<0.05$.
[f] $P<0.1$.

Table 4
Relationship between the combination of *CYP19* and *CYP1A1* polymorphisms and ER-positive breast cancer risk

No. of high-risk genotypes[a]	Cases ($n=156$)	Controls ($n=191$)	OR (95% CI)[b]	OR (95% CI)[c]
All women				
0	46 (29)[d]	67 (35)	1.00	1.00
1	64 (41)	100 (52)	0.92 (0.56–1.50)	0.89 (0.53–1.48)
2	46 (29)	24 (13)	2.75 (1.48–5.13)[e]	3.00 (1.56–5.74)[e]
Premenopausal				
0	29 (32)	34 (37)	1.00	1.00
1	37 (40)	46 (51)	0.92 (0.47–1.79)	0.87 (0.43–1.77)
2	26 (28)	11 (12)	2.58 (1.08–6.16)[f]	2.29 (0.92–5.68)[g]
Postmenopausal				
0	17 (27)	33 (33)	1.00	1.00
1	27 (42)	54 (54)	1.06 (0.47–2.38)	1.28 (0.54–3.03)
2	20 (31)	13 (13)	3.90 (1.44–10.61)[h]	5.37 (1.74–16.63)[e]

[a] High-risk genotypes are $TTTA_{7(-3)}$ carriers for *CYP19* and ^{6235}C non-carriers for *CYP1A1*.
[b] Odds Ratio (OR) and 95% Confidence Interval (CI) adjusted for age.
[c] Odds Ratio and 95% Confidence Interval adjusted for age, family history, parity and BMI.
[d] Numbers in parentheses are percentages.
[e] $P<0.005$.
[f] $P<0.05$.
[g] $P<0.1$.
[h] $P<0.01$.

high-risk genotypes. Women with both *CYP19* and *CYP1A1* high-risk genotypes were associated with a significantly ($P<0.005$) increased risk of ER-positive breast cancer compared with those without them (OR = 3.00, 95% CI 1.56–5.74). Such an association was also observed in the premenopausal (OR = 2.29, 95% CI 0.92–5.68) and postmenopausal (OR = 5.37, 95% CI 1.74–16.63) subsets (Table 4). Genotypic distributions of *CYP19* and *CYP1A1* were not significantly different between the ER-positive and ER-negative cases. However, the distribution was significantly different after the combination of both genotypes (Table 5).

Table 5
Genotype frequencies between ER-positive and ER-negative breast cancer patients

	ER-positive ($n=156$)	ER-negative ($n=88$)	P value
CYP19 $(TTTA)_{7(-3bp)}$ allele			
Non-carrier	77 (49)[a]	53 (60)	0.10
Carrier	79 (51)	35 (40)	
CYP1A1 ^{6235}C allele			
Non-carrier	77 (49)	37 (42)	0.27
Carrier	79 (51)	51 (58)	
No. of high-risk genotypes			
0	46 (29)	29 (33)	0.03
1	64 (42)	46 (52)	
2	46 (29)	13 (15)	

[a] Numbers in parentheses are percentages.

3.3. *CYP19* and *CYP1A1* polymorphisms and serum oestrogen levels in postmenopausal controls

Serum oestrogens (E1 and E2) levels were compared in the postmenopausal controls between the high-risk and low-risk genotypes of *CYP19* or *CYP1A1* polymorphisms (Fig. 1a and b). Neither *CYP19* nor *CYP1A1* polymorphisms were significantly associated with the serum oestrogens levels. Furthermore, the combination of *CYP19* and *CYP1A1* polymorphisms was also not significantly associated with the serum oestrogen levels (Fig. 1c).

4. Discussion

Many reports have been published on a variety of risk factors for breast cancer, but these studies have exclusively investigated the risk of breast cancer irrespective of the ER status of tumours. However, it is likely to be clinically important to identify the risk factors for ER-positive breast cancer in order to select properly the candidates for chemoprevention with tamoxifen as the breast cancer prevention trial (P-1 trial) demonstrated that tamoxifen reduces the risk of ER-positive, but not ER-negative, breast cancer [1]. Therefore, in our study, we attempted to identify the risk factors associated with ER-positive breast cancer by analysing the polymorphisms of genes involved in oestrogen biosynthesis (*CYP19*) and metabolism (*CYP1A1*). We found that *CYP19* $(TTTA)_{7(-3bp)}$ allele carriers were significantly associated with an increased risk of ER-positive, but not ER-negative, breast cancers when compared with non-carriers. In addition, we found that, although *CYP1A1* $^{6235}C/T$ polymorphism is not significantly associated with ER-positive breast cancer risk by itself, this polymorphism is useful in a prediction of ER-positive breast cancer risk in combination with the *CYP19* $(TTTA)_{7(-3bp)}$ polymorphism with more accuracy ($P<0.005$, OR = 3.00) than the *CYP19* $(TTTA)_{7(-3bp)}$ polymorphism alone ($P<0.05$, OR = 1.72).

Since *CYP19* and *CYP1A1* polymorphisms are expected to affect the synthesis or degradation of oestrogens, we speculated that a high oestrogen level associated with these high-risk genotypes would be favourable for ER-positive breast carcinogenesis. However, we could not show an association between the high-risk genotypes of *CYP19* or *CYP1A1* with elevated serum oestrogen levels in postmenopausal controls. A combination of the *CYP19* and *CYP1A1* high-risk genotypes also failed to show an association with the serum oestrogen level, suggesting that polymorphisms of these genes are unlikely to affect oestrogen production originating from the adrenal glands in postmenopausal women. Since we did not study the association between *CYP19* and *CYP1A1* polymorphisms and the serum oestrogen level in premenopausal women, we cannot say whether the *CYP19* and *CYP1A1* polymorphisms affect oestrogen biosynthesis and metabolism in the ovaries in these cases. In addition, recent studies have suggested the *in situ* synthesis of oestrogens in the breast tissue [14–16]. Because both *CYP19* and *CYP1A1* are expressed in breast tissue [17,18], it is possible that polymorphisms in these genes affect their expression in the breast and, consequently, the *in situ* synthesis of oestrogens. However, CYP1A1 is known to be involved in the metabolism of environmental carcinogens, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which has been reported to induce CYP1A1 through the aryl hydrocarbon receptor (AhR) in breast cancer cell lines [19]. Since *in vitro* experiments have shown that upregulation of *CYP1A1* mRNA is much greater in ER-positive cells than in ER-negative cells, probably due to cross-talk between the ER and AhR systems [20], it is possible that the *CYP1A1* polymorphism contributes only to the ER-positive breast cancer risk because its expression level is much higher in ER-positive than ER-negative tumours. The observation that there was no

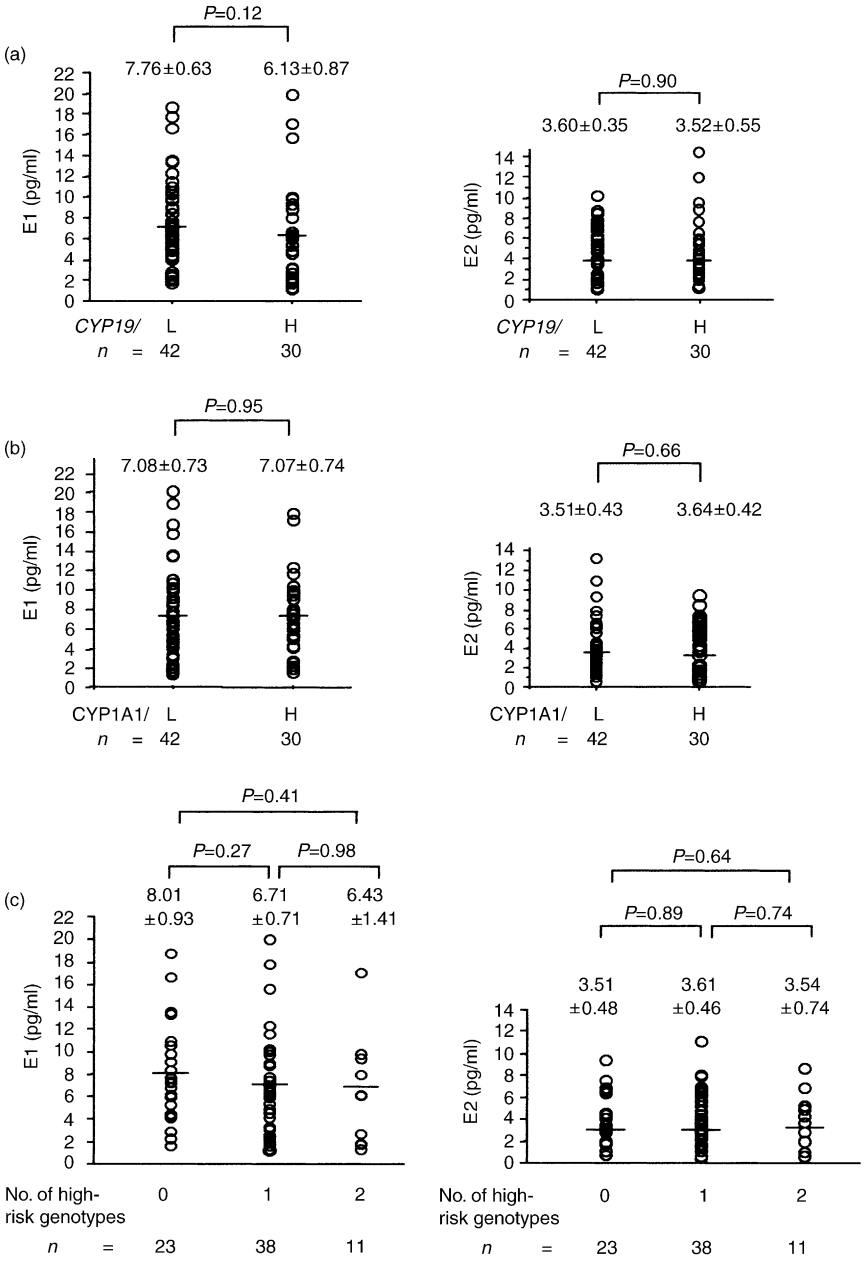

Fig. 1. Serum E1 and E2 levels in postmenopausal controls according to genotypes of *CYP19* and *CYP1A1* polymorphisms: (a) *CYP19* polymorphism; (b) *CYP1A1* polymorphism; (c) combination of *CYP19* and *CYP1A1* polymorphisms. Bars, mean (±standard error of the mean (SEM)). L, low-risk; H, high-risk.

significant association between the *CYP1A1* polymorphism and the serum oestrogen level may suggest that this polymorphism contributes to carcinogenesis through metabolism of environmental carcinogens rather than that of oestrogens. These possibilities need to be investigated in the future studies.

Case–control studies conducted on Caucasians have mostly shown that *CYP19* $(TTTA)_7$, $(TTTA)_{10}$, or $(TTTA)_{12}$ allele carriers are associated with a breast cancer risk [7–9]. However, in our study, we failed to show such an association in Japanese women, and, instead, showed that *CYP19* $(TTTA)_{7(-3bp)}$ allele carriers are significantly associated with the breast cancer risk (especially the ER-positive breast cancer risk). The reason for this discrepancy is currently unknown, but racial differences as well as genetic and environmental risk factors might partly explain the differences observed.

In conclusion, we have demonstrated that *CYP19* $(TTTA)_{7(-3bp)}$ allele carriers are associated with a significantly increased risk of ER-positive, but not ER-negative, breast cancers. Our data suggests that the combination of *CYP19* $(TTTA)_{7(-3bp)}$ and *CYP1A1* $^{6235}C/T$ polymorphisms would be more useful in the

assessment of the ER-positive breast cancer risk than the *CYP19* $(TTTA)_{7(-3bp)}$ polymorphism alone. The possibility that these polymorphisms might be involved in the *in situ* synthesis of oestrogens in the breast tissue, together with a functional analysis of these polymorphisms, needs to be investigated further.

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