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Original Article

Possible Difference in Frequencies of Genetic Polymorphisms of Estrogen Receptor α, Estrogen Metabolism and P53 Genes Between Estrogen Receptor-positive and -negative Breast Cancers

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Objective: Genetic polymorphisms associated with breast cancer risk are likely to differ among ethnic and molecular subtypes. The ability to identify genetic polymorphisms affecting the risk of estrogen receptor (ER)-positive breast cancer may lead to the more efficient selection of candidates for chemoprevention with endocrine agents. We focused on identifying common genotypes for ER-positive breast cancer in premenopausal Japanese women.
Methods: We compared genetic polymorphisms of ERα, estrogen metabolism genes (CYP17A1, CYP19A1, HSD17B1 COASY, CYP1B1 and COMT), and p53 between ER-positive and -negative female Japanese breast cancer patients, and analyzed whether these polymorphisms affected the frequency of ER-positive breast cancer.
Results: Carriers of the G allele of ERα (rs6905370) were more frequent in ER-positive breast cancer than in ER-negative breast cancer especially in those under 50-year old. Pairwise analysis showed that combinations of the ERα G allele with the homozygous Trp genotype of CYP19A1 codon 39 (rs2236722), the methionine (Met) allele of COMT codon 158 (rs4680) or Pro allele of p53 codon 72 (rs1042522) were more frequent in ER-positive than ER-negative breast cancer, especially in patients less than 50-year old. The frequencies of these combinations were even higher in patients with strongly ER-positive tumors (Allred's scores of 7 or 8).
Conclusion: Our study demonstrated genetic polymorphisms of ERα, CYP19A1, COMT and p53 genes frequently occur in ER-positive breast cancer in premenopausal Japanese women.

Key words: breast cancer – genetic polymorphisms – estrogen receptor

INTRODUCTION

Recent gene expression-based molecular classification has revealed that breast cancer is not one disease but a collection of several biologically different diseases (1,2). There are large-scale molecular differences between estrogen receptor (ER)-positive and -negative cancers that reach far beyond the presence or absence of ER. Moreover, since recent trials with tamoxifen and raloxifene showed that the risk-reducing effect of tamoxifen was limited to ER-positive breast cancer (3,4), it is necessary to establish predictive factors to assess the risk of ER-positive breast cancer in order to select candidates for chemoprevention with endocrine agents more efficiently.

Estrogen plays a crucial role in the carcinogenesis and progression of breast cancers, and special attention has been focused on polymorphisms in the ERα gene and in estrogen biosynthesis and metabolism genes. Although a few studies

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demonstrated statistically significant associations between ERα polymorphisms and breast cancer, most studies have not shown linkage or association between ERα polymorphisms and breast cancer risk (5–7). Recently, Gold et al. identified several haplotypes in Ashkenazi Jews in both ERα and ERβ genes that may elevate susceptibility to breast cancer (8). In addition, genetic polymorphisms in estrogen biosynthesis (CYP17A1, CYP19A1, HSD17B1) and metabolism genes (CYP1B1, COMT) are also expected to affect the synthesis or degradation of estrogen, and, consequently, the risk of breast cancer (9–15). The COASY gene is located 3′ of the HSD17B1 gene, and rs598126 of COASY is a haplotype tagging single nucleotide polymorphism (SNP) of HSD17B1 (15). It has been reported that this SNP is significantly associated with ER-negative tumors.

The tumor suppressor protein, p53, is a principal modulator of multiple cellular functions, such as gene transcription, DNA synthesis and repair, cell cycle regulation, cell senescence and apoptosis. We recently found that p53 protein accumulation predicts resistance to endocrine therapy and shorter post-relapse survival in metastatic breast cancer (16). In addition to acquired mutations that alter its function, p53 is subject to polymorphisms, especially an Arg72Pro variant, which is located in a proline-rich region that is essential for p53-mediated apoptosis, and for which there appears to be sound biological evidence of functional differences between the Arg and Pro forms (17,18). Since it is well established that inactivation of p53 is involved in the pathogenesis of breast cancer, it seems reasonable to assume that the p53 genetic polymorphism that affects the function of p53 might have an influence on breast cancer risk (19,20).

In this study, we compared genetic polymorphisms of ERα, estrogen metabolism genes and p53 between ER-positive and -negative female Japanese breast cancer patients, and analyzed whether these polymorphisms affected the risk of ER-positive breast cancer.

PATIENTS AND METHODS

Patients

Eligible cases were 453 female breast cancer patients who were treated at Nagoya City University Hospital between 1992 and 2006. The study protocol was approved by the institutional review board and conformed to the guidelines of the 1975 Declaration of Helsinki. All patients had undergone surgical treatment for primary breast cancer (either mastectomy or lumpectomy). The median age of the patients was 57.1 years (range, 25–89 years). There were 313 patients who were 50 or more than 50-year old, and 140 patients were less than 50-year old.

Genotyping

Genomic DNA for genotyping was extracted from the whole blood samples using the Wizard SV Genomic DNA purification system (Promega, Madison, USA) according to the manufacturer's instructions. A total of 12 SNPs were analyzed in the ESR1gene (ERα): rs6905370, rs2077647, rs827421; in the CYP17A1 gene: rs743572; in the CYP19A1 gene (aromatase): rs2236722, rs10046; HSD17B1 gene: rs676387; COASY gene: rs598126; CYP1B1 gene: rs1800440, rs1056836; COMT gene: rs4680; and p53 gene; rs1042522 (Table 1). All genotyping was carried out by using TaqMan PCR assays (Applied Biosystems, Warrington, UK) in 96-well arrays that included blank wells as negative controls, according to the manufacturer's instructions (19). TaqMan® Pre-Designed SNP Genotyping assays and TaqMan® MGB probes were used. TaqMan PCR and genotyping analyses were performed on Applied Biosystems 9600 Emulation System (Applied Biosystems). The reaction mixtures were amplified in 1 μl of genomic DNA (10 ng/μl), 5 μl of 2× TaqMan® Universal Master Mix, 0.5 μl of 20× primer/probe mix, 3.5 μl of ddH_2O in a volume of 10 μl. PCR cycling conditions were as follows: one cycle at 60°C for 1 min as initial step; one cycle at 95°C for 20 min, 40 cycles at 92°C for 3 min and at 60°C for 30 s; and one cycle at 60°C for 1 min as annealing step. The results were analyzed on Applied Biosystems 9600 Emulation System using the allelic discrimination assay program.

Immunohistochemical Evaluation of ERα Status

Primary invasive breast cancer specimens were obtained by surgical excision, and immunohistochemical staining of ERα was done using monoclonal mouse antihuman ERα antibody (1D5; Dako Denmark A/S, Glostrup, Denmark) at 1:100 dilution for ERα as a primary antibody as previously described (21). The expression status of ERα was assessed according to the estimated proportion of nuclear staining of tumor cells that were positively stained. Tumors with 10% or more positive cells were considered to be positive, and tumors with 0% positive cells were considered to be negative for ERα expression. Patients whose tumors showed 1–9% positive cells were excluded from this study.

Statistical Analysis

The χ^2-test was used for statistical analysis for the differences in the genotype distributions between ERα-positive and -negative breast cancers. Differences were considered significant when a P value of less than 5% was obtained.

RESULTS

Genotype Frequencies Between ER-Positive and -Negative Breast Cancer Patients

We first analyzed individual SNP types in ER-positive and -negative breast cancer patients (Table 2). There were 312 patients with ER-positive tumors and 141 patients with ER-negative tumors. There was no significant difference in

Table 1. Genes and SNP details

Gene	dbSNP ID	SNP type	Context sequence
	Assay ID	Amino acid change	
ESR1 (ERα)	rs6905370	Intron 1	CCAAGGCAAGTAGTCACTACAAGGC[A/G]
	C328969		AGTTTTGTTCTGTCTATCCCAAGGC
ESR1 (ERα)	rs2077647	Silent mutation	CCATGACCCTCCACACCAAAGCATC[C/T]
	C11414978	Ser10Ser	GGGATGGCCCTACTGCATCAGATCC
ESR1 (ERα)	rs827421	Intron 1	AACGACAAGGTATTCAAAGGAGAAC[A/G]
	C11920506		TTGTACTTTATGACAGTTCTTTGGG
CYP17A1	rs743572	Promoter region	GGTGCCGGCAGGCAAGATAGACAGC[A/G]
	C2852784	34 bp upstream of the initiation codon	GTGGAGTAGAAGAGCTGTGGCAACT
CYP19A1	rs2236722	Mis-sense mutation	ATTGAGGATGTGCCCTCATAATTCC[A/G]
	C15954948	Trp39Arg	CACCAAGAGAAAAAGGCCAGTGAGG
CYP19A1	rs10046	3′ non-coding region	CTACTGATGAGAAATGCTCCAGAGT[A/G]
	C8234731		GGTACTGACCAGCCTTCTCTAGTGT
HSD17B1	rs676387	Intron 1	CCGCGTTTCAAATGTTCTGGTTATC[A/C]
	C2769181		CCAGCGCCCTTTCCGCCTCACTTCC
COASY	rs598126	Silent mutation	AGCTGCTGAAGGACCTCAGACATAC[A/G]
	C2350909	Thr324Thr	GAGAATGAAGAGGACAAAGTCAGCT
CYP1B1	rs1800440	Mis-sense mutation	CATCACTCTGCTGGTCAGGTCCTTG[C/T]
	C11642651	Asn453Ser	TGATGAGGCCATCCTTGTCCAAGAA
CYP1B1	rs1056836	Mis-sense mutation	AAGTTCTCCGGGTTAGGCCACTTCA[C/G]
	C3099976	Leu432Val	TGGGTCATGATTCACAGACCACTGG
COMT	rs4680	Mis-sense mutation	CCAGCGGATGGTGGATTTCGCTGGC[A/G]
	C25746809	Met158Val	TGAAGGACAAGGTGTGCATGCCTGA
P53	rs1042522	Mis-sense mutation	CCCAGAATGCCAGAGGCTGCTCCCC[C/G]
	C2403545	Arg72Pro	CGTGGCCCCTGCACCAGCAGCTCCT

SNP, single nucleotide polymorphism; Ser, serine; Trp, tryptophan; Arg, arginine; Thr, threonine; Asn, asparagine; Leu, leucine; Val, valine; Met, methionine; Pro, proline; ER, estrogen receptor.

genotype frequencies among the individual SNP types analyzed in this study. The reported data from the HapMap project showed that the significant differences were observed in the genotypes of rs6905370, rs598126, rs1800440, rs1056836, rs4680 and rs1042522 between Europeans and Japanese (Table 2). The relationship between the polymorphisms of the genes and clinicopathological factors such as tumor size, number of positive lymph nodes and grade were analyzed. No significant association was found among those factors (data not shown).

Genotype Frequencies of ERα Gene Between ER-Positive and -Negative Breast Cancer Patients

We compared the frequencies of the AA type and G allele (AG+GG) genotypes of ERα (rs6905370) in ER-positive and -negative breast cancer patients, and found that G allele carriers were more frequent in the former group (76.9 versus 67.1%, $P = 0.029$), especially in patients who were less than 50-year old (77.3 versus 52.5%, $P = 0.004$) (Table 3). Moreover, the difference was even larger when patients with strongly ER-positive tumors (Allred's score of 7 or 8) were compared with those with ER-negative tumors (82.7% G allele carriers versus 67.1%, $P = 0.001$) (Table 4). Interestingly, G allele carriers are more frequent in Europeans than in Japanese according to HapMap data ($P < 0.0001$) (Table 2). We conclude that the G allele of ERα (rs6905370) is associated with an increased likelihood that breast cancer will be ER-positive in premenopausal Japanese women.

Genotype Frequencies of Estrogen Metabolism Genes Between ER-Positive and -Negative Breast Cancer Patients

We then analyzed genetic frequencies of CYP genes, CYP19A1 (rs2236722), CYP19A1 (rs10046), HSD17B1 (rs676387) and COMT (rs4680). When we compared

Table 2. Association between genotypes and ER status in breast cancer patients

	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	HapMap		
				Japanese % ($n = 90$)	European % ($n = 120$)	*P* value
ERα (rs6905370)						
AA	72 (23.1)	46 (32.9)		22.2	8.3	
AG	155 (49.7)	64 (45.7)	0.077	60.0	38.3	<0.0001
GG	85 (27.2)	30 (21.4)		17.8	53.3	
ERα (rs2077647)						
CC	19 (18.4)	12 (16.2)				
CT	50 (48.5)	36 (48.6)	0.91			
TT	34 (33.0)	26 (35.1)				
ERα (rs827421)						
GG	52 (25.5)	12 (16.0)		15.6	21.7	
GA	98 (48.0)	37 (49.3)	0.17	41.1	46.7	0.69
AA	54 (26.5)	26 (34.7)		33.3	31.7	
CYP17A1 (rs743572)						
AA	93 (29.8)	38 (27.0)				
AG	147 (47.1)	71 (50.4)	0.74			
GG	72 (23.1)	32 (22.7)				
CYP19A1 (rs2236722)						
CC	0 (0)	1 (0.7)		0	0	
CT	19 (6.1)	10 (7.3)	0.29	6.8	0	
TT	291 (93.9)	126 (92.0)		93.2	100	
CYP19A1 (rs10046)						
GG	88 (28.2)	32 (23.2)		11.1	18.2	
GA	172 (55.1)	76 (55.1)	0.32	60.0	52.7	0.35
AA	52 (16.7)	30 (21.7)		28.9	29.1	
HSD17B1 (rs676387)						
AA	71 (22.9)	23 (16.3)		24.3	15.4	
AC	141 (45.5)	79 (56.0)	0.12	40.5	0.12	36.5
CC	98 (31.6)	39 (27.7)		35.1	48.1	
COASY (rs598126)						
AA	99 (31.7)	39 (27.7)		29.5	18.3	
GA	137 (43.9)	77 (54.6)	0.11	52.3	43.3	0.004
GG	76 (24.4)	25 (17.7)		18.2	38.3	
CYP1B1 (rs1800440)						
TT	110 (98.2)	76 (98.7)		100	70.0	
TC	2 (1.8)	1 (1.3)	0.79	0	26.7	<0.0001
CC	0 (0)	0 (0)		0	3.3	
CYP1B1 (rs1056836)						
CC	4 (1.6)	2 (1.5)		2.3	21.7	
CG	57 (22.9)	35 (26.9)	0.19	13.6	45.0	<0.0001
GG	188 (75.5)	93 (71.5)		84.1	33.3	

Continued

Table 2. *Continued*

	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	HapMap		
				Japanese % ($n = 90$)	European % ($n = 120$)	*P* value
COMT (rs4680)						
AA	56 (17.9)	23 (16.3)		4.5	25.0	
AG	129 (41.3)	57 (40.4)	0.85	38.6	53.3	<0.0001
GG	127 (40.7)	61 (43.3)		56.8	21.7	
p53 (rs1042522)						
GG	35 (11.3)	24 (17.0)		20.5	8.3	
CG	140 (45.3)	68 (48.2)	0.11	36.4	30.0	0.01
CC	134 (43.4)	49 (34.8)		43.2	61.7	

Table 3. Genotype frequencies between ER-positive and ER-negative breast cancer patients

	All patients			Age <50 years			Age ≥50 years		
	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value
ERα (rs6905370)									
AA	72 (23.1)	46 (32.9)	0.029*	22 (22.7)	19 (47.5)	0.004*	50 (23.5)	27 (27.6)	0.44
AG+GG (G carrier)	240 (76.9)	94 (67.1)		75 (77.3)	21 (52.5)		163 (76.5)	71 (72.4)	
CYP19A1 codon 39 (rs2236722)									
TT (Trp homo)	291 (93.9)	126 (92.0)	0.46	89 (92.7)	38 (90.5)	0.66	200 (94.3)	87 (92.6)	0.55
CT+CC (Arg carrier)	19 (6.1)	11 (8.0)		7 (7.3)	4 (9.5)		12 (5.7)	7 (7.4)	
CYP19A1 (rs10046)									
GG	88 (28.2)	32 (23.2)	0.27	32 (33.0)	5 (12.2)	0.012*	56 (26.0)	24 (25.0)	0.85
GA+AA (A carrier)	224 (71.8)	106 (76.8)		65 (67.0)	36 (87.8)		159 (74.0)	72 (75.0)	
HSD17B1 (rs676387)									
AA	71 (22.9)	23 (16.3)	0.15	23 (24.0)	4 (9.3)	0.044*	48 (22.6)	20 (20.4)	0.66
AC+CC (C carrier)	239 (77.1)	118 (83.7)		73 (76.0)	39 (90.7)		164 (77.4)	78 (79.6)	
COMT codon 158 (rs4680)									
AA (Met homo)	56 (17.9)	23 (16.3)	0.67	20 (20.6)	6 (14.3)	0.38	35 (16.4)	17 (17.3)	0.84
AG+GG (Val carrier)	256 (82.1)	118 (83.7)		77 (79.4)	36 (85.7)		178 (83.6)	81 (82.7)	
p53 codon 72 (rs1042522)									
GG (Arg homo)	35 (11.3)	24 (17.0)	0.97	7 (7.4)	11 (26.2)	0.003*	27 (12.7)	13 (13.3)	0.9
CG+CC (Pro carrier)	274 (88.7)	117 (83.0)		87 (92.6)	31 (73.8)		185 (87.3)	85 (86.7)	

*$P<0.05$.

ER-positive and -negative breast cancer patients who were under 50-year old, we found that the ER-positive group showed a lower frequency of A allele carriers of CYP19A1 (rs10046) (67.0 versus 87.8%, $P = 0.012$) and of C allele carriers of HSD17B1 (rs676387) (76.0 versus 90.7%, $P = 0.044$) (Table 3). G allele carriers [Valine (Val) carriers] of COMT codon 158 (rs4680) were more frequent in strongly ER-positive (Allred's score 7 or 8) than ER-negative breast cancer (92.0 versus 83.7%, $P = 0.026$) (Table 4). Interestingly, this allele is more frequent in Japanese than in Europeans, according to HapMap data ($P < 0.0001$) (Table 2).

Genotype Frequencies of p53 Gene Between ER-Positive and -Negative Breast Cancer Patients

Analysis of genotype frequencies between a GG type (Arg homo-type) and C allele carriers (Pro carriers) of p53 codon 72 (rs1042522) in patients who were under 50-year old showed that C allele carriers (Pro carriers) were more frequent

Table 4. Genotype frequencies between ER-positive (score 7 or 8) and ER-negative breast cancer patients

	All patients			Age <50 years			Age ≥50 years		
	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value
ERα (rs6905370)									
AA	28 (17.3)	46 (32.9)	0.001*	8 (19.0)	19 (46.3)	0.008*	20 (16.5)	27 (27.6)	0.048*
AG+GG (G carrier)	134 (82.7)	94 (67.1)		34 (81.0)	21 (52.5)		101 (83.5)	71 (72.4)	
CYP19A1 codon 39 (rs2236722)									
TT (Trp homo)	152 (92.7)	126 (92.0)	0.82	37 (88.1)	38 (90.5)	0.72	115 (94.3)	87 (92.6)	0.61
CT+CC (Arg carrier)	12 (7.3)	11 (8.0)		5 (11.9)	4 (9.5)		7 (5.7)	7 (7.4)	
CYP19A1 (rs10046)									
GG	44 (26.8)	32 (23.2)	0.47	14 (33.3)	5 (12.2)	0.022*	30 (24.6)	24 (25.0)	0.94
GA+AA (A carrier)	120 (73.2)	106 (76.8)		28 (66.7)	36 (87.8)		92 (75.4)	72 (75.0)	
HSD17B1 (rs676387)									
AA	34 (20.9)	23 (16.3)	0.31	8 (19.0)	4 (9.3)	0.2	26 (21.5)	20 (20.4)	0.85
AC+CC (C carrier)	129 (79.1)	118 (83.7)		34 (81.0)	39 (90.7)		95 (78.5)	78 (79.6)	
COMT codon 158 (rs4680)									
AA (Met homo)	13 (8.0)	23 (16.3)	0.026*	1 (2.4)	6 (14.3)	0.048*	12 (10.0)	17 (17.3)	0.11
AG+GG (Val carrier)	149 (92.0)	118 (83.7)		41 (97.6)	36 (85.7)		108 (90.0)	81 (82.7)	
p53 codon 72 (rs1042522)									
GG (Arg homo)	14 (8.6)	24 (17.0)	0.028*	2 (4.8)	11 (26.2)	0.007*	12 (10.0)	13 (13.3)	0.45
CG+CC (Pro carrier)	148 (91.4)	117 (83.0)		40 (95.2)	31 (73.8)		108 (90.0)	85 (86.7)	

*$P<0.05$.

in ER-positive than ER-negative breast cancer (92.6 versus 73.8%, $P = 0.003$) (Table 3). Comparison of strongly ER-positive and -negative cases showed a higher frequency of C allele carriers (Pro carriers) in the former group at all ages (91.4 versus 83.0%, $P = 0.028$), but the difference was greater in patients who were under 50-year old (95.2 versus 73.8%, $P = 0.007$) (Table 4). This allele is more frequent in Europeans than in Japanese, according to HapMap data ($P < 0.0001$) (Table 2). We conclude that C allele (Pro) of the p53 codon 72 is associated with an increased likelihood that breast cancer will be ER-positive in premenopausal Japanese women.

Combination Analysis of Genotype Frequencies Using ERα Gene Polymorphisms

We then performed combination analysis to identify better risk indicators than ERα gene (rs6905370) genotypes alone. Analysis of ERα (rs6905370) and CYP19A1 codon 39 (rs2236722) polymorphisms showed that the combination of a G allele genotype for ERα and Trp homo genotype for CYP19A1 codon 39 was more frequent in ER-positive breast cancer than in ER-negative breast cancer (72.7 versus 62.2%, $P = 0.027$), and the difference was greater in patients under 50-year old (70.8 versus 50.0%, $P = 0.02$) (Table 5). The difference was even more pronounced (76.1 versus 62.2%, $P = 0.0095$) when patients with strongly ER-positive tumors were compared with those who were ER-negative (Table 6).

Similarly, analysis of ERα (rs6905370) and COMT codon 158 (rs4680) genotypes showed that the combination of the ERα G allele and COMT codon 158 Met allele was more frequent in ER-positive breast cancer than in ER-negative breast cancer (62.8 versus 57.6%, $P = 0.01$) (Table 5), and this difference was much greater when strongly ER-positive tumors were compared (77.0 versus 57.6%, $P = 0.0003$), regardless of age (Table 6). In case of the classification of COMT codon 158, it is different between the single (Tables 3 and 4) and the combination (Tables 5 and 6) analyses. Because Val carriers of COMT codon 158 were more frequent in strongly ER-positive (Allred's score 7 or 8) than ER-negative breast cancer (92.0 versus 83.7%, $P = 0.026$) (Table 4), we used this classification in the single (Tables 3 and 4) analysis. However, we apply Met carriers of COMT codon 158 in the combination analysis, since significance was greater in Met carriers than in Val carriers in the combination (Tables 5 and 6) analysis.

Analysis of ERα (rs6905370) and p53 codon 72 (rs1042522) genotypes demonstrated that the combination of ERα G allele and p53 codon 72 Pro allele was more frequent in ER-positive than ER-negative breast cancer (67.3 versus 56.1%, $P = 0.02$), with a greater difference seen in patients

Table 5. Combination analysis of genotype frequencies using ERα gene polymorphisms

	All patients			Age <50 years			Age ≥50 years		
	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value
ERα(rs6905370)/CYP19A1 codon 39 (rs2236722)									
G carrier/Trp homo	224 (72.7)	84 (62.2)	0.027*	68 (70.8)	20 (50.0)	0.02*	154 (73.3)	63 (67.0)	0.26
Others	84 (27.3)	51 (37.8)		28 (29.2)	20 (50.0)		56 (26.7)	31 (33.0)	
ERα (rs6905370)/COMT codon 158 (rs4680)									
G carrier/Met carrier	201 (62.8)	80 (57.6)	0.01*	62 (63.9)	18 (45.0)	0.04*	138 (65.4)	61 (62.2)	0.59
Others	119 (37.2)	59 (42.4)		35 (36.1)	22 (55.0)		73 (34.6)	37 (37.8)	
ERα (rs6905370)/p53 codon 72 (rs1042522)									
G carrier/Pro carrier	208 (67.3)	78 (56.1)	0.02*	65 (69.1)	18 (45.0)	0.0084*	142 (66.7)	59 (60.2)	0.27
Others	100 (32.7)	61 (43.9)		29 (30.9)	22 (55.0)		71 (33.3)	39 (39.8)	

*$P<0.05$.

Table 6. Combination analysis of genotype frequencies between ER-positive (score 7 or 8) and ER-negative breast cancer

	All patients			Age <50 years			Age ≥50 years		
	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value	ER-positive [*n* (%)]	ER-negative [*n* (%)]	*P* value
ERα (rs6905370)/CYP19A1 codon 39 (rs2236722)									
G carrier/Trp homo	124 (76.1)	84 (62.2)	0.0095*	29 (69.0)	20 (50.0)	0.079	95 (78.5)	63 (67.0)	0.06
Others	39 (23.9)	51 (37.8)		13 (31.0)	20 (50.0)		26 (21.5)	31 (33.0)	
ERα (rs6905370)/COMT codon 158 (rs4680)									
G carrier/Met carrier	124 (77.0)	80 (57.6)	0.0003*	34 (81.0)	18 (45.0)	0.0007*	90 (75.6)	61 (62.2)	0.033*
Others	37 (23.0)	59 (42.4)		8 (19.0)	22 (55.0)		29 (24.4)	37 (37.8)	
ERα (rs6905370)/p53 codon 72 (rs1042522)									
G carrier/Pro carrier	123 (75.5)	78 (56.1)	0.0004*	32 (76.2)	18 (45.0)	0.0038*	91 (75.2)	59 (60.2)	0.02*
Others	40 (24.5)	61 (43.9)		10 (23.8)	22 (55.0)		30 (24.8)	39 (39.8)	

*$P<0.05$.

under 50-year old (69.1 versus 45.0%, $P = 0.0084$) (Table 5). The frequency of this combination was even higher in strongly ER-positive cases, and the difference between this group and the ER-negative category was significant, regardless of age (75.5 versus 56.1%, $P = 0.0004$) (Table 6).

DISCUSSION

We compared genetic polymorphisms of ERα, estrogen metabolism genes and p53 between ER-positive and -negative female Japanese breast cancer patients, and analyzed whether genetic polymorphisms of these genes affected the risk of ER-positive breast cancer. Our results indicated that the G allele of ERα (rs6905370) is associated with an increased likelihood that breast cancer will be ER-positive in premenopausal Japanese women, and that the combination of this allele with certain genotypes of estrogen metabolism genes or p53 increased the probability of ER-positive breast cancer.

Recent genome-wide association studies revealed that several SNPs such as FGFR2, are associated with breast cancer risk; the population studies were mostly on Europeans and Americans. Moreover, Stacey et al. identified common SNPs associated with ER-positive breast cancer (22). However, frequencies of the variants varied markedly between ethnicities and the common SNPs they identified in Europeans were not associated with breast cancer risk in Japanese Americans (22,23). Another notable difference between these populations is that Japanese women experience the highest incidence of breast cancer in their 40s, whereas the incidence increases with age in Europeans and Americans (23). Furthermore, molecular profiling has revealed that breast cancer is not one disease but a collection of several biologically different diseases, and that there are large-scale molecular differences between ER-positive and

-negative breast cancers (1,2). Therefore, SNPs associated with breast cancer risk would be expected to differ among ethnic groups and molecular subtypes. In this study, we focused on identifying common genotypes for ER-positive breast cancer in premenopausal Japanese women.

It has been reported that polymorphisms in the ERα gene interact with ER status in affecting breast cancer survival in Chinese patients (24). Another study showed that genotypes of the ERα gene, rs6905370, rs2077647 and rs827421 were significantly associated with breast cancer risk in Ashkenaji Jews (8). Our data also indicated that G allele of ERα (rs6905370) was associated with ER-positive breast cancer especially below age 50. Biological mechanisms behind the interaction with ERα genotypes remain to be clarified, since the functional effects of these ERα polymorphisms have not been reported.

CYP19 (aromatase) catalyzes the conversion of androgens into estrogens. CYP19A1 polymorphism at codon 39 (rs2236722) is accompanied by the substitution of amino acid Trp with Arg, and Japanese women with the variant allele Arg have significantly lower risk of developing a breast cancer (10). An experimental study showed that the Arg 39 mutant was unable to synthesize estrogens (25). Our study indicated that both G allele carriers of ERα and Trp homo carriers of CYP19A1 codon 39 were more frequent in ER-positive breast cancer than in ER-negative breast cancer, and that the frequency was even higher in patients with strongly ER-positive tumors, suggesting that the Trp allele of CYP19A1 codon 39 might affect ER-positive breast cancer risk in Japanese women.

The bioavailability of hormones is partially controlled by catabolism, and catechol estrogens (2 hydroxy-estrogens) are the major breakdown products of estrogens. Catechol *O*-methyl transferase (COMT) methylates catechol-estrogens during their conjugation and inactivation. A G–A transition at codon 158 of the COMT gene, which leads to a substitution of methionine (Met) for valine (Val), has been linked to a reduced COMT activity (26). Though several studies have examined this variant in relation to breast cancer risk, the reported results are inconsistent (14). Our data demonstrated that patients carrying both a G allele of ERα and Met allele of COMT codon 158 were more frequent in ER-positive than ER-negative breast cancer patients, and even more frequent in the strongly ER-positive group. Thus, the Met allele of COMT codon 158 might affect ER-positive breast cancer risk in Japanese women.

The p53 Arg72Pro polymorphism has been well characterized in both functional analyses and association studies (9,18). A previous study showed that this polymorphism was associated with ER-positive breast cancer risk in Japanese women (27). Our results also indicated that Pro carriers were more frequent in ER-positive breast cancer than in ER-negative breast cancer, especially in those below age 50, suggesting that this variant is associated with an increased likelihood that breast cancer will be ER-positive in premenopausal Japanese women.

Our study demonstrated genetic polymorphisms of ERα, estrogen metabolism genes, and the p53 gene associated with premenopausal, ER-positive breast cancer in Japanese women. The possibility that these polymorphisms might be involved in the development of ER-positive breast cancer needs to be analyzed prospectively in the future study. Identifying genetic polymorphisms that can help to assess the risk of ER-positive breast cancer may make it possible to select candidates for chemoprevention with endocrine agents more efficiently.

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Conflict of interest statement

None declared.

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