No Association of the 5′ Promoter Region Polymorphism of CYP17 with Breast Cancer Risk in Japan

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To examine the association between breast cancer risk and a T-to-C substitution polymorphism at the 5′ promoter region of CYP17, a case-control study was conducted at Aichi Cancer Center Hospital in Japan. Subjects were 144 histologically confirmed breast cancer patients diagnosed in the past 4 years and 166 hospital controls without cancer. Allele frequency among controls was 44.9% (95% confidence interval; 39.5–50.2) for C allele. Odds ratio (OR) of the polymorphism relative to TT-genotype was 0.97 (0.58–1.64) for TC-genotype and 0.81 (0.39–1.68) for CC-genotype. Subgroup analyses revealed that the OR was not statistically significant for the subgroups stratified by interval after diagnosis, age at menarche, age at first birth, menopausal status, body mass index, and mother/sisters’ history of breast cancer. Consistent with previous studies conducted in other countries, the 5′ promoter region polymorphism of CYP17 affected breast cancer risk of Japanese women to a limited extent. Although this is not a large-scale case-control study with population controls, these findings provide enough information to discourage further studies on the association between this polymorphism and breast cancer risk in Japan at large, and suggest that this polymorphism is useless for breast cancer risk estimation.

Key words: Breast cancer — CYP17 — Polymorphism — Case-control study

CYP17 gene located on chromosome 10q24.3 encodes an enzyme, cytochrome p450c17, which has two different roles in steroid hormone metabolism; 17α-hydroxylase and 17,20-lyase activities. The former converts pregnenolone to 17-hydroxyprogrenolone and progesterone to 17-hydroxyprogesterone, and the latter further converts the metabolites to dehydroepiandrosterone and androstenedione, respectively. Since this enzyme is essential for estrogens synthesis, increased or decreased activities are speculated to modify the risk of breast cancer. A single nucleotide polymorphism (T-to-C transition) of CYP17 at 34 bp upstream of the translation initiation site in the 5′ untranslated region was reported to create an additional Sp1-type (CCACC box) promoter site, which suggested an increased rate of transcription and possibly a consequent increase in activity of estrogens. To date, several studies have examined the association of the polymorphism with breast cancer risk, but the results were inconsistent.1–11

Although the incidence of breast cancer in Japan is only one-fifth of that in the United States, it has been increasing since 1960s.12 Second-generation Japanese Americans in Hawaii and Los Angeles have the same level of breast cancer incidence as other Americans, indicating that lifestyle plays an important role in breast cancer carcinogenesis. However, similarly important are genetic factors which modify the effects of lifestyle factors. In Japan, there have been few studies on the association between genetic polymorphisms and breast cancer risk. This is a case-control study to examine the association of the 5′ promoter region polymorphism of CYP17 with breast cancer risk. It was conducted at a cancer hospital, Aichi Cancer Center Hospital, where patients with cancer represented about 20% of the first-visit patients, and the great majority of non-cancer patients were visitors who sought scrutiny after cancer screening and were found to have no disease.13

SUBJECTS AND METHODS

Case and control subjects Cases were female breast cancer patients aged 30 to 69 years histologically confirmed at Aichi Cancer Center Hospital, who were diagnosed in the past 4 years. Controls were female outpatients without cancer who visited outpatients clinics at the hospital. This study was openly announced to female outpatients at the reception desk for first-visit patients and in the waiting room of the breast surgery clinic. In addition, doctors in the breast surgery clinic asked eligible patients to participate in the study. Controls were enrolled mainly from the gastroenterology clinic, breast surgery clinic, and gynecol-
ogy clinic. It was not rare that outpatients visited two or more clinics, and the clinics they had visited were not confirmed systematically in this study. In the hospital, 70% of non-cancer patients were disease-free, having visited for the purpose of annual health checkup or examining a positive result of screening at worksite/screening facilities. The subjects were enrolled between March and December in 1999 in the framework of HERPACC (Hospital-based Epidemiologic Research Programs at Aichi Cancer Center). Those who gave informed consent were asked to complete a self-administered questionnaire and to donate a 7 ml blood sample from a peripheral vein.

**Laboratory methods** DNA was extracted from buffy coat fractions by using a Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA). The PCR amplification for CYP17 was conducted using the primers 5′-CAT TCG CAC TCT GGA GTC-3′ and 5′-AGG CTC TTG GGG TAC TTG-3′. Genomic DNA (30 to 100 ng) was used in a volume of 50 µl with 0.2 mM dNTPs, 100 pmol of each primer, 1.25 units of AmpliTaq Gold, and 5 µl of GeneAmp 10× PCR Buffer including 15 mM MgCl₂ (Perkin-Elmer Corp., Foster City, CA). Amplification conditions were 10 min of initial denaturation at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 57°C, and 1 min at 72°C, then a 5 min final extension at 72°C. The amplified products were incubated with MspA1 (New England Biolabs, Inc., Beverly, MA) for 3 h at 37°C, and digested fragments were visualized on a 2.5% agarose gel with ethidium bromide staining. Genotyping was distinguished as follows; 419 bp for homozygous wild type (TT, denoted as A1/A1 in other papers), 419, 295, and 124 bp for heterozygous type (TC or A1/A2), and 295 and 124 bp for homozygous mutated type (CC or A2/A2), as shown in Fig. 1.

**Statistical analysis** Odds ratios (OR) and 95% confidence intervals (CI) were calculated by an unconditional logistic model, using the SAS statistical program package. Mean age at menarche was compared among the genotypes by ANOVA. The probability for Hardy-Weinberg equilibrium was examined by STATA.

**RESULTS**

During the enrollment period, 151 breast cancer patients and 166 non-cancer patients entered the study. Seven cases were not genotyped because of the failure of DNA extraction. The remaining 144 cases and 166 controls were used for analysis. The characteristics of the subjects are shown in Table I. Age distribution was slightly lower in cases than in controls. Out of 144 cases, 52.1% participated within 1 year after their diagnosis.

As shown in Table II, the allele frequency of mutated type (C) was 44.9% (95% CI, 39.5–50.2) for controls. The allelic distribution for controls was in Hardy-Weinberg equilibrium (exact P value=0.06). Age-adjusted OR relative to TT-genotype was 0.97 (95% CI, 0.58–1.64) for TC-genotype and 0.81 (0.39–1.68) for CC-genotype, and 0.94 (0.57–1.55) for TC/CC-genotypes combined. There was no substantial difference in the estimated OR between cases 0–1 year after diagnosis and cases 2–4 years after diagnosis (Table III). The OR was not significant for the subgroups stratified by age at menarche, age at first birth, and breast cancer risk.

![Fig. 1. Gel showing the three genotypes for the 5′ promoter region polymorphism of CYP17. Lane 1 is the TT-genotype (419 bp), lane 2 the TC-genotype (419, 295, 124 bp), and lane 3 the CC-genotype (295, 124 bp).](image)

**Table I. Characteristics of Subjects**

| Characteristics                  | Cases (n=144) | Controls (n=166) |
|----------------------------------|--------------|------------------|
| Age at diagnosis for cases or at interviews for controls | 30–39        | 13 (7.0)         | 12 (7.2)          |
| 30–39                            | 40–49        | 55 (38.2)        | 37 (22.3)         |
| 50–59                            | 60–69        | 45 (31.3)        | 67 (40.4)         |
| 60–69                            | 0–1          | 31 (21.5)        | 50 (30.1)         |
| 14–15                            | 14–15        | 62 (43.1)        | 83 (50.0)         |
| 14–15                            | 16–17        | 69 (47.9)        | —                 |
| 16–17                            | 15 (9.0)     | 12 (7.2)         | 37 (22.3)         |
| Can not remember                 | 43 (29.9)    | 40 (25.8)        | 51 (30.7)         |
| Age at first birth               | 24–25        | 45 (31.3)        | 64 (38.6)         |
| 25–29                            | 26–45        | 16 (11.1)        | 14 (8.4)          |
| Menopause                        | Premenopausal | 78 (54.2)        | 79 (47.6)         |
| Postmenopausal                   | 66 (45.8)    | 87 (52.4)        | —                 |
| Body mass index                  | –19          | 31 (20.7)        | 36 (21.4)         |
| at diagnosis for cases           | 20–23        | 78 (54.2)        | 86 (51.8)         |
| at interviews for cont.          | 24–28        | 28 (17.4)        | 41 (24.7)         |
| Family history of breast cancer  | No           | 128 (88.9)       | 157 (94.6)        |

* Percentages in parentheses.

a) Breast cancer history of mother and sister(s).
menopausal status, body mass index, and mother/sisters’ history of breast cancer. Non-significant, but elevated OR of CC-genotype was found for those with age at menarche ≥14 years (OR = 1.80, 95% CI, 0.66–4.95) and those with age at first birth <25 years (OR = 1.55, 95% CI, 0.48–5.06).

Mean age at menarche was compared for controls. No difference was found among the three genotypes; 13.8 years (standard error, 1.7 years) for TT-genotype, 13.8 years (1.8 years) for TC-genotype, and 13.3 years (1.5 years) for CC-genotype (ANOVA, \( P = 0.35 \)).

**DISCUSSION**

Biological plausibility is an essential component for studies on genetic polymorphisms and risk of disease. There are many epidemiological and biological findings which support the idea that estrogens are related to breast cancer risk.\(^{17}\) Recent cohort studies showed that women with a higher estrogen concentration had a higher risk of breast cancer.\(^{18,19}\) Accordingly, polymorphisms with a potential to elevate estrogen levels are expected to be related to breast cancer risk.

This polymorphism creates a Sp-1-type promoter site, which suggests that estrogen activities might be higher for those harboring CC-genotype. But, there was a report that a recombinant Sp-1 protein which combined with human embryonic \( e \) globin gene did not combine with the created site of the CC-genotype \textit{in vitro}.\(^{8}\) Notwithstanding, it was reported that nulliparous women with CC-genotype showed higher levels of serum estradiol and progesterone at 11th day and 22nd day of menstruation,\(^{20}\) and that post-

| Table II. Allele Frequency and Genotypes of CYP17 |
|---------------------------------------------------|
| **n** | **T** | **C** | **n** | **TT** | **TC** | **CC** |
|-------|-------|-------|-------|-------|-------|-------|
| **Cases** | 288 | 165 (57.3) | 123 (42.7) | 144 | 41 (28.5) | 83 (57.6) | 20 (13.9) |
| **Controls** | 332 | 183 (55.1) | 149 (44.9) | 166 | 44 (26.5) | 95 (57.2) | 27 (16.3) |

% in parentheses.

| Table III. Age-adjusted Odds Ratios and 95% Confidence Intervals for TC- and CC-Genotypes relative to TT-Genotype |
|---------------------------------------------------------------|
| **Subjects** | **Cases/cont.** | **Genotype** | **TT** | **TC** | **CC** |
|----------------|-----------------|--------------|-------|-------|-------|
| All subjects | 144/166 | 1 | 0.97 (0.58–1.64) | 0.81 (0.39–1.68) |
| Interval after diagnosis | | | | | |
| < 2 years | 75/166 | 1 | 1.27 (0.66–2.43) | 0.81 (0.32–2.08) |
| 2–4 years | 69/166 | 1 | 0.74 (0.39–1.41) | 0.78 (0.33–1.87) |
| Menarche | | | | | |
| < 14 years | 62/83 | 1 | 0.89 (0.41–1.96) | 0.34 (0.10–1.07) |
| ≥ 14 years | 81/83 | 1 | 1.10 (0.53–2.25) | 1.80 (0.66–4.95) |
| First birth | | | | | |
| < 25 years | 65/65 | 1 | 1.66 (0.72–3.80) | 1.55 (0.48–5.06) |
| ≥ 25 years or no birth | 79/101 | 1 | 0.69 (0.35–1.38) | 0.57 (0.22–1.46) |
| Menopause | | | | | |
| Premenopausal | 78/79 | 1 | 1.24 (0.60–2.56) | 0.50 (0.17–1.43) |
| Postmenopausal | 66/87 | 1 | 0.77 (0.36–1.64) | 1.27 (0.45–3.57) |
| Body mass index | | | | | |
| < 22 | 75/89 | 1 | 1.01 (0.49–2.09) | 0.68 (0.24–1.90) |
| ≥ 22 | 69/77 | 1 | 0.96 (0.45–2.07) | 1.09 (0.38–3.13) |
| Family history of breast cancer | | | | | |
| Yes | 16/9 | 1 | 0.53 (0.05–6.31) | 0.43 (0.01–16.4) |
| No | 128/157 | 1 | 0.98 (0.57–1.69) | 0.87 (0.41–1.82) |

\(^{a}\) Breast cancer history of mother and sister(s).
menopausal women with the genotype had a higher serum estrone, estradiol, androstenedione, and dehydroepiandrosterone concentrations.\(^7\) Epidemiologic associations of this polymorphism with sex-hormone-related diseases such as polycystic ovaries, premature male-pattern baldness,\(^2, 21\) and prostate cancer,\(^22\) may also support a biological role of the polymorphism.

Possible biological complications are 1) the expression of CYP17 gene by Sp-1 promoter may be influenced by some genetic and/or environmental factors, 2) tissue-specific transcription factors other than the ubiquitous Sp-1 may play a role in the elevation of estrogens, and 3) metabolism of estrogens is also regulated by other genes, such as CYP11A, CYP19, EDH17B2, CYP1A1, CYP1A2, CYP1B1, CYP3A4, and COMT,\(^23, 24\) so that the association between estrogens and CYP17 polymorphism may be modified by genetic background. The above findings suggest that the strength of the association, if any, may vary among ethnic groups with different genetic traits and with lifestyle factors related to breast cancer.

To our knowledge, ten case-control studies including this study have been conducted for female breast cancer throughout the world, as shown in Table IV. A significantly elevated risk was observed for regional/metastatic breast cancer among Asian, African, and Latin American,\(^3\) and young Swedish women.\(^9\) The association was not observed for Caucasians in the United States,\(^4, 5, 7\) in the United Kingdom,\(^6, 10\) and in Norway,\(^8\) or Chinese in Taiwan.\(^11\) The present study adds the finding that breast cancer risk is not high for the CC-genotype among Japanese.

Table IV. Case-control Studies on the Association between the Polymorphism of CYP17 and Breast Cancer Risk

| Authors (country, year) | Subjects | Cases/cont.\(^b\) | CC type in cont. (%) | Age-ethnicity-adjusted OR (95% confidence interval) |
|-------------------------|----------|------------------|----------------------|---------------------------------------------------|
| Feigelson et al.\(^3\) (US, 1997) | All | 174/285 | 15.4 | ND\(^a\) | ND | 1.32 (0.83–2.00) |
| Weston et al.\(^4\) (US, 1998) | Regional/metastatic | 40/285 | ND | ND | 1.52 (1.07–5.94) |
| | All | 123/240 | 15.4 | ND | ND | 0.80 (0.45–1.43) |
| | Caucasian | 76/148 | 16.9 | ND | ND | 1.40 (0.44–4.38) |
| | African | 20/35 | 5.7 | ND | ND | 1.36 (0.07–2.51) |
| | Hispanic | 27/57 | 17.5 | ND | ND | 1.36 (0.34–5.01) |
| Helzlsouer et al.\(^3\) (US, 1998) | Premenopausal | 24/25 | 16.0 | 0.38 (0.09–1.63) | 0.70 (0.15–3.19) | ND |
| | Postmenopausal | 85/88 | 15.9 | 0.69 (0.34–1.39) | 0.99 (0.39–2.49) | ND |
| Dunning et al.\(^6\) (England, 1998) | All | 463/619 | 15.2 | 0.84 (0.65–1.11) | 0.97 (0.67–1.41) | 0.87 (0.68–1.13) |
| | Premenopausal | 64/70 | ND | ND | 0.51 (0.24–1.06) |
| | Postmenopausal | 357/505 | ND | ND | 0.94 (0.70–1.25) |
| Haiman et al.\(^7\) (US, 1999) | Premenopausal | 64/70 | ND | ND | 0.51 (0.24–1.06) |
| | Postmenopausal | 357/505 | ND | ND | 0.94 (0.70–1.25) |
| Kristensen et al.\(^8\) (Norway, 1999) | All | 510/201 | 12.9 | No association (odds ratio not calculated) |
| Bergman-J. et al.\(^9\) (Sweden, 1999) | All | 109/117 | 13.8 | 1.91 (1.0–3.4) | 2.8 (1.0–7.8) | 2.0 (1.1–3.5) |
| Young et al.\(^10\) (US, 1999) | All | 39/58 | 12.1 | No association (odds ratio not calculated) |
| Huang et al.\(^11\) (Taiwan, 1999) | All | 123/126 | 27.8 | 0.96 (0.48–1.94) | 1.41 (0.66–3.01) | 1.28 (0.73–2.27) |
| | Premenopausal | 52/50 | 28.0 | 1.25 (0.41–3.87) | 1.46 (0.42–5.06) | 1.18 (0.50–2.83) |
| | Postmenopausal | 68/75 | 28.0 | 0.74 (0.28–1.95) | 1.38 (0.49–3.85) | 1.31 (0.59–2.90) |
| Hamajima et al.\(^11\) (This study) | Premenopausal | 78/79 | 19.0 | 1.24 (0.60–2.56) | 0.50 (0.17–1.43) | 1.03 (0.51–2.07) |
| | Postmenopausal | 66/87 | 13.8 | 0.77 (0.36–1.64) | 1.27 (0.45–3.57) | 0.85 (0.41–1.77) |

\(a\) Controls.
\(b\) Not described.
\(c\) Odds ratio for C allele.
\(d\) Matched for age, race, menopausal status, day of menstrual cycle, and date of blood donation.
\(e\) Crude odds ratio.
\(f\) Adjusted for age, menopausal status, postmenopausal hormone use, data and time of blood draw, and fasting status.
\(g\) Adjusted for age, family history of breast cancer, age at menarche, age at first full-term pregnancy, and history of hormone replacement therapy.
A meta-analysis of four studies concluded that the relative risk was 1.5 or smaller for the CYP17 polymorphism on average. When the ten studies in Table IV are considered, it can be similarly concluded that the effect of the CYP17 polymorphism is limited and that there is no substantial difference among races.

Feigelson et al. reported that in mean age at menarche was significantly higher in TT-genotype than in those carrying C allele (13.4 years versus 13.0 years). In this study, those with CC-genotype had a 0.5 years lower mean age than those with TT/TC-genotypes, but this was not significant. Other studies reported no difference among the genotypes. It is not clear whether CYP17 influences age at menarche.

There was no published data on the frequency of this polymorphism among Japanese. This study found that the frequency of CC-genotype among Japanese (16.3%) was similar to those for Caucasians, as shown in Table IV. It was interesting that CC-genotype in this study was less common than in Chinese; 27% for 110 unrelated full-term maternity patients, and 28% for the 126 controls by Huang et al. In the case of CYP17, the genotype frequency does not affect the variation of breast cancer incidence among countries, even though it increases the risk of breast cancer.

In conclusion, the effect of this CYP17 polymorphism on breast cancer risk was limited for Japanese women, though there is still a possibility that it may modify the breast cancer risk through gene-gene interactions. Although this study was not a large-scale case-control study with population controls, the finding obtained in this study provides enough information to discourage further studies on the association between this polymorphism and breast cancer risk in Japan at large, suggesting that this genotyping alone is of little value for breast cancer risk estimation.

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