Aromatase and breast cancer susceptibility

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Abstract

Based on experimental and epidemiological evidence it is hypothesized that estrogen increases breast cancer risk by increasing mitotic activity in breast epithelial cells. Aromatase is crucial to the biosynthesis of estrogens and may therefore play a role in breast cancer development. Supporting data for an etiological role of aromatase in breast tumor biology are several-fold. First, the association between weight and postmenopausal breast cancer risk may be mediated by aromatase. Secondly, a pilot study found a higher aromatase expression in normal breast adipose tissue from breast cancer cases as opposed to healthy women. Thirdly, experimental data in animals suggest that aromatase activity predisposes mammary tissue to preneoplastic and neoplastic changes. In a multiethnic cohort study conducted in Los Angeles and on Hawaii we investigated (i) whether the plasma estrone to androstenedione (E1/A) ratio in different ethnic groups was associated with ethnic differences in breast cancer incidence, and (ii) whether genetic variation in the CYP19 gene encoding the P450 aromatase protein was associated with breast cancer risk. The age- and weight-adjusted ethnic specific E1/A ratios ×100 among women without oophorectomy were 7.92 in African-Americans, 8.22 in Japanese, 10.73 in Latinas and 9.29 in non-Latina Whites (P=0.09). The high E1/A ratio in Latina women was not associated with a high breast cancer incidence; in fact Latina women had the lowest breast cancer incidence in the cohort observed so far. We found no consistent association of an intronic (TTT A)ₙ repeat polymorphism with breast cancer risk in different ethnic groups. This polymorphism was not associated with differences in the plasma E1/A ratio in a way that would predict its functional relevance. We describe a newly identified TTC deletion in intron 5 of the CYP19 gene that is associated with the (TTTA)ₙ repeat polymorphism. Neither this polymorphism, nor a polymorphism at codon 264 in exon VII of the CYP19 gene, was associated with breast cancer. We did not identify any genetic variation in exon VIII in 54 African-American subjects. We identified rare genetic variants of unknown functional relevance in the promoter 1.4 of the CYP19 gene in 3 out of 24 Latina women. Further investigation into the role of aromatase in breast cancer etiology is important, given that the potential use of aromatase inhibitors as breast cancer chemopreventives depends on these results.

Introduction

Data from several research areas point to the involvement of sex hormones in the etiology of breast cancer. Laboratory studies have shown that estrogens control the growth of breast epithelial cells. Reducing estrogen exposure affects the course of established breast cancer. Reproductive events and obesity have a substantial impact on a woman’s lifetime risk of breast cancer, probably in part through their impact on the estrogen exposure of breast epithelial cells. Based on this evidence the
hypothetical was developed that high serum concentrations of endogenous estrogen, and specifically estradiol (E2), increase breast cancer risk (Henderson et al. 1982). The data from prospective studies assessing hormone levels and breast cancer risk in postmenopausal women have recently been combined in a systematic review and quantitative analysis by Thomas et al. (1997b). The authors in fact observed a statistically significant higher risk of breast cancer among women with higher levels of serum E2.

After menopause, estrogen in the circulation is predominantly estrone (E1), derived from the peripheral aromatization of androstenedione (Grodin et al. 1973). Plasma estrogen production is directly correlated with body weight (MacDonald et al. 1978), indicating that most of the postmenopausal, extraglandular aromatization of plasma androstenedione takes place in adipose tissue. The aromatization of androgens to estrogens is catalyzed by the enzyme aromatase P450 (Simpson et al. 1994). Aromatase activity thus has the potential to play a role in the etiology of breast cancer. Several lines of evidence support this hypothesis.

First, evidence that postmenopausal obesity as well as weight gain over the adult years are positively associated with postmenopausal breast cancer risk has been substantiated, especially for women who never used hormone replacement therapy (Huang et al. 1997). A positive association between adiposity and plasma estrogen levels in postmenopausal women has been reported quite consistently (Cauley et al. 1989, Kaye et al. 1991, London et al. 1991, Hankinson et al. 1995, Potischman et al. 1996, Thomas et al. 1997a, Madigan et al. 1998). It has been hypothesized, therefore, that the impact of obesity on breast cancer risk is mediated at least in part through the increased aromatization of androstenedione to E1 in the adipose tissue of obese women.

Secondly, data by Agarwal et al. (1996) suggest that breast cancer patients may have an inherently higher aromatase expression in breast adipose tissue when compared with healthy women. They studied 9 women undergoing reduction mammoplasty and 18 breast cancer patients undergoing mastectomy. Non-tumor bearing adipose samples from mastectomies expressed significantly more aromatase than adipose tissue from reduction mammoplasty patients, a difference that is unlikely to be due to the tumor’s influence on aromatase expression.

Thirdly, Bulun et al. (1996) showed that in the human breast the distribution pattern of aromatase P450 transcripts and adipose fibroblasts, the primary extraglandular site of aromatase P450 expression, correlates well with the most common and least common sites of carcinoma in the breast, the outer and inner regions respectively.

Fourthly, experimental data indicate an etiological role of aromatase activity in the development of breast tumors in animals. The integration site 5 of the mouse mammary tumor virus (MMTV) is within the aromatase gene. Transgenic mice that overexpress int-5/aromatase under the control of MMTV enhancer/promoter have mammary tissue predisposed to preneoplastic changes (Tekmal et al. 1996). Aromatase inhibitors are successful in preventing mammary tumors in experimental animals (Rao et al. 1985, Lubet et al. 1994, Moon et al. 1994, Gunson et al. 1995, Grubbs et al. 1996).

If the role of aromatase activity in breast cancer etiology was substantiated, aromatase inhibitors, currently used in the treatment of breast cancer, would deserve consideration as potential chemopreventives for breast cancer (Kelloff et al. 1998). To gain further insight into the role of aromatase activity in breast cancer development we investigated whether ethnic differences exist in plasma E1 and androstenedione (E1/A) ratios, a surrogate measure for aromatase activity that would correlate with ethnic differences in breast cancer incidence. In addition, pilot data on the association of breast cancer risk with genetic variation in the CYP19 gene encoding the P450 aromatase are presented.

Materials and methods

Study population

In 1993, we initiated a cohort study of individuals aged 40-75 years (Kolonel et al. 1999). The cohort consists of 215 251 men and women living in the states of Hawaii and California (primarily Los Angeles county) and being mainly of African-American, Japanese, Latino and non-Latino White ancestry. The cohort was mainly accessed from the drivers’ license files over the period from 1993 to 1996. Participants completed a 26-page, self-administered mail questionnaire that elicited information about diet, demographic variables, medical history, personal habits (e.g. smoking, drinking), physical activity, and, for women, reproductive history. Overall response rates (after three mailings) were 25.5% in African-American women, 51.3% in Japanese women, 21.3% in Latina women and 47.0% in non-Latina White women. Compared with the US Census for the entire population of the study areas, the cohort is somewhat more educated than the general population, but all levels of education are comparatively well represented. The correspondence in marital status between the cohort members and the US Census is high.

All respondents are being followed for incident cancers by passive linkage with Surveillance, Epidemiology and End Results registries and periodic matches of the cohort to the National Death Index, and the Voters’ Registration and Death Certificate files in Hawaii and
hormones were first extracted with hexane:ethyl acetate
which were previously validated in our laboratory
were measured by sensitive and specific RIA methods
Laboratory methods
As samples were collected, blood components were
separated and stored in 0.5 ml aliquots at −80 °C. DNA
was purified from buffy coats of peripheral blood samples
for all cases and controls using a rapid DNA preparation
method (Talmud et al. 1991).
Plasma levels of androstenedione, E1 and E2 lpg/ml
were measured by sensitive and specificRIA methods
which were previously validated in our laboratory
(Goeblsman et al. 1973, 1979, Stanczyk et al. 1988, Cassidenti et al. 1992). Prior to quantification, the
hormones were first extracted with hexane:ethyl acetate
(3:2) and then separated from interfering metabolites by
use of Celite column partition chromatography. The
intrasassay and interassay coefficients of variation ranged
between 5 and 10% and 10 and 15% respectively for the
three analytes.
An intrinsic tetranucleotide repeat at bp 682 in intron 5 of
the CYP19 gene was described by Polymeropoulos et al. (1991). For detection of the polymorphism, PCR
reactions were performed with one radioactively labeled
and one unlabeled primer and products were visualized on
modified acrylamide gels. The following primers were used: forward 5'-GCAGGTACCTTAGGCTAC-3',
reverse 5'-TTAGGTCAGGCTAAGAGCT-3'. The forward primer with final concentration of 1.5 pmol per
reaction was labeled with [γ-33P]ATP (New England
Nuclear, Boston, MA, USA) to a specific activity of 6000 Ci/ mmol using T4 polynucleotide kinase (New England
Biolabs, Beverly, MA, USA) according to a standard
protocol (Sambrook et al. 1989). PCR reactions were
performed in large scale using 96-well microtiter plates.
Approximately 10 ng genomic DNA per subject in a
reaction mixture were amplified on a MJ Research Inc.,
(Watertown, MA, USA) programmable thermal controller
PTC-100. At least two control samples from each allele
that was found by genotyping were directly sequenced
using an Amersham Thermosequenase kit (Amersham,
Arlington Heights, IL, USA) for confirmation of the
number of TTTA repeats.
To screen for polymorphisms in exon VIII of the
CYP19 gene, a non-isotopic RNase Cleavage Assay of
MisMatch Detect II Kit (Ambion Inc., Austin, TX, USA) was
used. Primers used for a two-stage nested PCR were:
outer-sense 5'-TTCTCCATGTCGAATTGTTTATAAAGATG-3';
outer-antisense 5'-AGAAGAAATTGGTTTTTAAAGATG-3';
nested-promoter inner-sense 5'-TATAAGCTCTACTATAGGCCCATCTGTTAATTCTTTTT-3';
nested-promoter inner-sense 5'-ATTAGGTGAACGATATAG
GAAAGAAATTTGGTTTTAAAGATG-3'.
To screen for polymorphisms in the I.4 promoter
region, a 1149 bp region from −786 to +363, relative to
the transcription start site) was amplified using primers
5'-GATCATGCTACAGTGAATGAA-3' and 5'-TTCAGC
TCCAAAAGAATGTTTCTCC-3'. From this PCR product,
several smaller overlapping segments were amplified
using nested primers. Nested PCR products were treated
with shrimp alkaline phosphatase and exonuclease
(Amersham) and directly sequenced using the Amersham
Thermosequenase kit.
Statistical analysis
The distribution of the E1/A ratio was markedly skewed.
Formal statistical testing was therefore performed on
logarithmically transformed values, and geometric mean
values are presented. The ANOVA method was used to
compare the E1/A ratio between ethnic groups and between weight categories within ethnic groups. Weight tertiles were formed based on the weight distribution in the total study population. An ANCOVA method was used to compare the E1/A ratio between ethnic groups while adjusting for the potential effect of weight and age. Linear regression analysis was used to assess the linear association between the E1/A ratio and age or weight. All \( P \) values presented are two-sided (Ott 1988).

**Results**

Selected characteristics of the study population are shown in Table 1. African-American women were most likely to have their first child under the age of 21 (51%), whereas Japanese women were least likely to have their first child under the age of 21 (11%). Weight, height and BMI were highest in African-American women (79 kg, 164 cm, 29.4 \( \text{kg/m}^2 \)), and lowest in Japanese women (55 kg, 153 cm, 23 \( \text{kg/m}^2 \)). African-American women were most likely to have had at least one ovary removed (14.5% one ovary, 16.1% two ovaries, 3.2% unknown how many), and Japanese women were least likely to have had at least one ovary removed (3.6% one ovary, 3.6% two ovaries).

In Table 2 we examined the ethnic distribution of the E1/A ratio×100. We observed no statistically significant differences in androstenedione levels among ethnicities (\( P=0.65 \) after adjustment for weight and age). The highest E1/A ratio×100 was observed among Latina women (9.68) as compared with 8.25 in African-American, 9.31 in Latina and 8.57 in non-Latina White women. Restriction of the analysis to subjects without oophorectomy revealed age- and weight-adjusted E1/A ratios×100 of 7.92 for African-American, 8.22 for Japanese, 10.73 for Latina and 9.29 for non-Latina White women (\( P=0.09 \)). Ethnic-specific, age-adjusted breast cancer incidence rates (per 100000) observed in the cohort so far are 137.1 (African-Americans), 139.0 (Japanese) 84.3 (Latina) and 131.7 (non-Latina White).

Adjustment in Table 2 for BMI (kg/m²) or adiposity (kg/m\(^{1.5}\)) instead of weight did not substantially alter any of the findings presented. We also investigated the effect of reproductive variables on the E1/A ratio. Adjustment for whether or not a woman had ever been pregnant, the

<table>
<thead>
<tr>
<th>Table 1 Characteristics of the study population by ethnicity</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>No. subjects</td>
</tr>
<tr>
<td>Mean age (years)</td>
</tr>
<tr>
<td>Mean height (cm)</td>
</tr>
<tr>
<td>Mean weight (kg)</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
</tr>
<tr>
<td>Parity (% nulliparous)</td>
</tr>
<tr>
<td>Age (years) at first birth</td>
</tr>
<tr>
<td>≤20 (%)</td>
</tr>
<tr>
<td>20–30 (%)</td>
</tr>
<tr>
<td>&gt;30 (%)</td>
</tr>
<tr>
<td>Age (years) at first period</td>
</tr>
<tr>
<td>≤12 (%)</td>
</tr>
<tr>
<td>13–14 (%)</td>
</tr>
<tr>
<td>&gt;14 (%)</td>
</tr>
<tr>
<td>Ovaries removed</td>
</tr>
<tr>
<td>None (%)</td>
</tr>
<tr>
<td>One (%)</td>
</tr>
<tr>
<td>Two (%)</td>
</tr>
<tr>
<td>At least one, but not known how many (%)</td>
</tr>
</tbody>
</table>
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The weight effect on hormone levels in different ethnic groups is presented in Table 3. Restriction of the analysis to women with intact ovaries did not alter the results. In African-American, Latina and White women the highest E1/A ratio was observed in the highest weight tertile. None of the Japanese women weighed 75 kg or more. Neither linear regression analysis nor ANOVA indicated a consistently statistically significant association between weight and the E1/A ratio in all ethnic groups.

Data on allele distribution for the intronic tetranucleotide repeat polymorphism among postmenopausal Japanese, African-American, Latina and White breast cancer cases and controls are presented in Table 4. We identified six alleles in our multiethnic study population with the numbers of TTTA repeats being 7, 8, 10, 11, 12 and 13. In African-Americans only, we observed three additional alleles that had a very low frequency of less than 1% that were not further sequenced for determination of the repeat number. No single TTTA repeat allele was consistently more prevalent in breast cancer cases in all ethnic groups. Analysis of the data by genotype instead of alleles led to the same conclusion: no single genotype was consistently more prevalent among breast cancer cases in all ethnic groups, but the data became sparse. In African-American women, the largest ethnic group in our study, we also investigated the association of different genotypes with the plasma E1/A ratio, as a surrogate measure for phenotype in a subsample of control subjects (Table 5). Those genotypes with a higher prevalence in breast cancer cases as opposed to controls were not consistently associated with a high E1/A ratio.

In intron 5 we identified additional genetic variation in the vicinity of the (TTTA)\(n\) repeat polymorphism. At 5’ of the (TTTA)\(7\) repeat polymorphism we observed a TTC deletion. The deletion was present in 36% of African-American, 22% of Japanese, 34% of Latina and 35% of White women.

### Table 2

Geometric mean plasma E1/A ratio ×100 in postmenopausal women: stratified by ethnicity

| Ethnicity       | African-American | Japanese | Latina | Non-Latina White | P-value
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>E1/A ×100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and weight adjusted</td>
<td>8.25 (n=66)</td>
<td>9.31 (n=30)</td>
<td>9.68 (n=58)</td>
<td>8.57 (n=39)</td>
<td>0.65(^a)</td>
</tr>
<tr>
<td>Age and weight adjusted, no oophorectomy</td>
<td>7.92 (n=41)</td>
<td>8.22 (n=26)</td>
<td>10.73 (n=45)</td>
<td>9.29 (n=32)</td>
<td>0.09(^b)</td>
</tr>
</tbody>
</table>

\(^a\) P value for main effects of weight and age are 0.002 and 0.07 respectively.
\(^b\) P value for main effects of weight and age are 0.003 and 0.01 respectively.

### Table 3

Geometric mean E1/A ratio ×100 by weight tertile\(^a\): stratified by ethnicity

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>African-American</th>
<th>Japanese</th>
<th>Latina</th>
<th>Non-Latina White</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1/A ×100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight tertile 1 (&lt;62 kg)</td>
<td>8 6.41</td>
<td>25 8.40</td>
<td>16 10.31</td>
<td>15 7.94</td>
</tr>
<tr>
<td>Weight tertile 2 (&lt;75 kg)</td>
<td>21 7.81</td>
<td>5 7.25</td>
<td>22 7.90</td>
<td>12 6.50</td>
</tr>
<tr>
<td>Weight tertile 3 (≥75 kg)</td>
<td>37 10.56</td>
<td>0 —</td>
<td>20 11.46</td>
<td>12 12.44</td>
</tr>
<tr>
<td>P value (ANOVA)(^b)</td>
<td>— 0.06</td>
<td>— 0.50</td>
<td>— 0.17</td>
<td>— 0.03</td>
</tr>
<tr>
<td>P value (regression)(^c)</td>
<td>— 0.01</td>
<td>— 0.70</td>
<td>— 0.38</td>
<td>— 0.17</td>
</tr>
</tbody>
</table>

\(^a\) Tertile formation based on weight distribution in the total study population.
\(^b\) P value based on one-way ANOVA.
\(^c\) P value based on linear regression of log(E1/A) on weight.
White control subjects, so that the intronic sequence around the (TTTA)$_n$ repeat polymorphism read 5'....AA TC*TTTTTTGTCTA TGAA TGTGC-CTTTTTT GAAA TCATA TTTTTAAAA TA [TTTA] 7 TTGAG....3'
where * indicates the location of the TTC deletion. This variation was not observed in subjects with a number of TTTA repeats different from seven, other than in three African-American subjects with eight TTTA repeats and the TTC deletion. We did not find this variation to be associated consistently with breast cancer risk in all ethnic groups.

Screening for polymorphisms in exon VIII did not reveal any genetic variation among African-American women we studied (27 control and 25 breast cancer cases).

Screening for polymorphisms in the promoter/exon I.4 among 16 control and 8 breast cancer subjects of Latina origin revealed a few rare variants. One woman was a T/C heterozygote at position −771 (numbering from the start site of transcription) and an A/T heterozygote at position −757. One woman was an A/G heterozygote at position −616. A third woman was a heterozygote at positions −236 (A/G) and −336 (A/G). These three Latina women exhibiting genetic variation were breast cancer cases.

**Discussion**

The E1/A ratio was measured to reflect aromatase activity. This ratio is expected to reflect the peripheral aromatization of androstenedione, since the clearance rates of both E1 and androstenedione are similar (Judd et al. 1980). We did not observe any statistically significant differences in the E1/A ratio between ethnic groups. We observed the highest E1/A ratio in Latina women, a difference that became almost statistically significant among women without oophorectomy. This finding did not correlate with ethnic differences in breast cancer incidence. In fact the age-adjusted breast cancer incidence rate in the multiethnic cohort study was lowest for Latina (84 per 100 000), followed by 137 per 100 000 for African-American, 138 per 100 000 for non-Latina White and 139 per 100 000 for Japanese women. This observation raises the following questions. First, does the

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**Table 4** Allele distribution of the (TTTA)$_n$ repeat polymorphism in intron 5 of the CYP19 gene in postmenopausal breast cancer cases and controls: stratified by ethnicity

<table>
<thead>
<tr>
<th>Allele (number of TTTA repeats)</th>
<th>African-American</th>
<th>Japanese</th>
<th>Latina</th>
<th>Non-Latina White</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>12</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>5</td>
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<tr>
<td>11</td>
<td>25</td>
<td>18</td>
<td>21</td>
<td>10</td>
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<tr>
<td>8</td>
<td>7</td>
<td>5</td>
<td>16</td>
<td>7</td>
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<tr>
<td>7</td>
<td>108</td>
<td>76</td>
<td>157</td>
<td>76</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>207</td>
<td>20</td>
<td>82</td>
</tr>
</tbody>
</table>

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**Table 5** (TTTA)$_n$ repeat polymorphism genotype in postmenopausal African-American women: association with geometric mean E1/A×100

<table>
<thead>
<tr>
<th>Genotype (number of TTTA repeats)</th>
<th>Genotype prevalence in breast cancer cases: controls (%)</th>
<th>n$_{\text{phenotyped controls}}$</th>
<th>Geometric mean plasma E1/A×100</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/7</td>
<td>64:57</td>
<td>36</td>
<td>7.79</td>
</tr>
<tr>
<td>7/8</td>
<td>4:12</td>
<td>6</td>
<td>10.42</td>
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<td>7/10</td>
<td>0:2</td>
<td>2</td>
<td>11.32</td>
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<td>20:16</td>
<td>12</td>
<td>11.36</td>
</tr>
<tr>
<td>7/12</td>
<td>1:7</td>
<td>6</td>
<td>11.92</td>
</tr>
<tr>
<td>8/11</td>
<td>6:4</td>
<td>1</td>
<td>3.03</td>
</tr>
</tbody>
</table>
E1/A ratio measured in the plasma of postmenopausal women properly reflect aromatase activity overall or in the relevant target tissue, and at the relevant time window? Second, is the impact of aromatase activity alone on breast cancer risk too subtle to explain ethnic differences in breast cancer incidence, given the multifactorial origin of breast cancer? Third, does aromatase activity not matter with regard to breast cancer etiology? It is conceivable that the interconnection between obesity, plasma estrogen levels, and breast cancer risk are merely associated with the interconversion of androstenedione to E1, but not mediated through aromatase activity.

In agreement with previous studies we found an indication of a positive association between weight and the E1/A ratio in ethnic groups other than Japanese. We did not observe a weight effect on the E1/A ratio in Japanese women. It is conceivable that Japanese women in our study lacked degrees of obesity that substantially influence the interconversion of androstenedione to E1. Our results as well as results presented by Madigan et al. (1998) indicate a non-linear weight effect on estrogen levels, with a substantial effect mainly observable at weights 80 kg and higher.

The role of aromatase in the association between weight and postmenopausal breast cancer risk must be further investigated. Exactly how obesity influences the aromatization rate is also unclear. Cleland et al. (1985) did not observe any correlation between aromatase activity of freshly prepared adipose tissue stromal cell suspensions and body weight of young women. Since obesity increases postmenopausal breast cancer risk, and if this effect is mediated by aromatase activity, Cleland’s data potentially indicate that obesity reflects an increase in the amount of adipose tissue, and thus aromatase activity in the body overall, rather than reflecting an impact on local aromatase activity in breast adipose tissue.

Unlike previous studies (Hemsell et al. 1974, Cleland et al. 1985, Bulun & Simpson 1994) we did not consistently observe an increase in the E1/A ratio with age in all ethnic groups (data not presented). Conceivably, this is due to the fact that our study population was postmenopausal. There is graphical indication in the data from previous studies that the effect on aromatase activity and expression attributed to age may in fact be a menopause effect (Hemsell et al. 1974, Cleland et al. 1985, Bulun & Simpson 1994). Although Cleland et al. (1985) found that aromatase activity in adipose stromal cells from five women under the age of 45 who had undergone a previous surgically induced menopause, and who were not being treated with estrogens, were not significantly different from an age-matched group of premenopausal women, the issue of the menopause impact on aromatase activity deserves further investigation given the small sample size of this study.

We believe our plasma hormone measurements accurately reflect hormone concentrations in blood. Single hormone measurements in postmenopausal women have been shown to be reproducible over a 2-3 year period. They should suffice to rank subjects with regard to long-term hormone concentrations (Micheli et al. 1991, Hankinson et al. 1995). Unlike previously reported data (Thomas et al. 1997b), we did not find any relationship between duration of blood sample storage and hormone concentrations. Although blood was not consistently sampled at the exact same time of the day, and adrenal androstenedione excretion is subject to a circadian rhythm with peak excretion during the very early morning hours, we do not expect this problem to influence our finding of racial differences in androstenedione and E1/A ratio. Madigan et al. (1998) found no significant differences in serum androstenedione levels depending on sample collection time.

Assessment of genetic variation in the CYP19 gene is another way to investigate the role of aromatase activity in breast cancer etiology. In principle, genotyping may offer the advantage of potentially reflecting a susceptibility history independent of short-term fluctuations in aromatase activity.

In agreement with previous data (Watanabe et al. 1997), we did not find an association between a C→T point mutation in exon VII, codon 264 and breast cancer risk (data not presented). We investigated the role of an intronic tetranucleotide repeat polymorphism in the CYP19 gene. In a group of non-Latina White women Kristensen et al. (1998) found five different alleles among 366 breast cancer cases and 252 controls. It is theoretically conceivable that the (TTTA)n repeat affects transcriptional activation of CYP19 (Kristensen et al. 1998). However several observations in our study are counter to this hypothesis. First, the allele with 12 repeats was statistically significantly more frequent in their Caucasian population, with a prevalence of 4% in breast cancer cases and 2% in controls. But in our White subjects this allele was more frequent in controls (3%) as opposed to cases (1%). Secondly, none of the six alleles observed in our study population was consistently more prevalent in cases in all ethnic groups. Thirdly, in an attempt to associate genotype with phenotype (E1/A ratio) we did not observe those genotypes with a higher prevalence in breast cancer cases to be associated with a high E1/A ratio. The role of the (TTTA)n repeat polymorphism as well as the newly identified TTC deletion in a subset of subjects with the seven-repeat allele deserves further investigation before an association with breast cancer can be assigned to it.

Because with regard to breast cancer susceptibility we are interested in subtle rather than drastic effects of genetic variation on aromatase activity, and in order to detect...
functionally more relevant variants of the *CYP19* gene, we shifted our screening focus to the promoter region of the aromatase gene. To look for functionally more relevant variants of the *CYP19* gene we started screening for further polymorphisms. We screened the region of the promoter I.4 and unspliced exon I.4, given the relevance of promoter I.4 in driving aromatase expression in normal adipose tissue, including breast adipose tissue (Zhao et al. 1995a, Agarwal et al. 1996). In our sample of Latina women, we only identified rare variants of questionable relevance to breast cancer risk, given their low prevalence, and the fact that they are not located in potentially important regulatory regions identified thus far in the same promoter (Zhao et al. 1995a).

Given the evidence suggesting the role of aromatase activity in the etiology of breast cancer, research into this issue must continue. We plan to continue systematic screening for polymorphisms and will next focus on the promoter I.3 and II regions, which were found to be relevant to aromatase expression in the adipose tissue in breast cancer patients (Agarwal et al. 1996, Zhao et al. 1997, Zhou et al. 1997). The relevance of the genetic control of steroid biosynthesis to serum hormone levels and thus breast cancer risk has recently been evidenced for a cytochrome P450c17α gene polymorphism (Spencer Feigelson et al. 1998). It is likely that this polymorphism acts in concert with genetic variation in the activity of other enzymes in the steroid biosynthesis pathway, for example aromatase.

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