# The *CYP19* Gene and Associations with Androgens and Abdominal Obesity in Premenopausal Women

*Fariba Baghaei,* Roland Rosmond,* Lars Westberg,† Monica Hellstrand,† Elias Eriksson,† Göran Holm,* and Per Björntorp**

**Abstract**

BAGHAEI, FARIBA, ROLAND ROSMOND, LARS WESTBERG, MONICA HELLSTRAND, ELIAS ERIKSSON, GÖRAN HOLM, AND PER BJÖRNTORP. The *CYP19* gene and associations with androgens and abdominal obesity in premenopausal women. *Obes Res.* 2003;11: 578-585.

***Objective:*** Elevated androgens in women are associated with type 2 diabetes and are dependent on the conversion to estrogens by aromatase cytochrome P450. Polymorphisms of a tetranucleotide repeat $[TTTA]_n$ in the fourth intron of the *CYP19* gene are associated with endocrine-dependent diseases and were examined in relation to hormone levels and disease risk factors in premenopausal women.

***Research Methods and Procedures:*** A population sample of women born in 1956 ($n = 270$) were genotyped for this polymorphism and the results set in relation to steroid hormones, including saliva cortisol, anthropometric variables, estimates of insulin, glucose and lipid metabolism, and blood pressure.

***Results:*** Seven tetranucleotide repeat $[TTTA]_n$ alleles were detected with allelic sizes of 168 to 195 bp, with a TCT deletion/insertion (168/171 bp) upstream of this microsatellite. Smoking was associated with elevated androgens ($p = 0.005$ to 0.019). Using the median (average stretch, 177.5 bp) as a dividing line, nonsmoking women with the shorter microsatellite had higher free testosterone ($p = 0.018$) and lower sex hormone binding globulin ($p = 0.033$). These differences were pronounced with the 168-bp allele. Such women were also characterized by a less-substantial decrease of morning cortisols ("unwinding"; $p = 0.035$) and central obesity (abdominal sagittal diameter, $p = 0.049$) and had waist/hip circumference ratios of borderline significance ($p = 0.064$).

***Discussion:*** The results indicate that, in premenopausal women, a short microsatellite in the fourth intron of the *CYP19* gene, caused by a TCT deletion upstream the $[TTTA]_n$ tract, is associated with elevated androgens, perturbed regulation of the hypothalamic-pituitary-adrenal axis, and abdominal obesity.

***Key words*: aromatase gene, polymorphism, hyperandrogenicity, risk factors, type 2 diabetes**

## Introduction

"Diabetes in women with beard" was first described in the 1920s (1). Severe hyperandrogenicity in women in defined clinical entities such as polycystic ovary syndrome is associated with insulin resistance, visceral obesity, dyslipidemia, and hypertension (2). Similar associations are also present in the highest quintile of serum androgens in women in the population with androgen values within conventional normal limits (3,4). These women are at increased risk for developing type 2 diabetes, myocardial infarction, hypertension, and endocrine-dependent cancers and for having a premature mortality (3–5). In the lowest quintile of sex hormone binding globulin (SHBG),[1] one of five women developed type 2 diabetes during a period of 12 years (3), probably indicating elevated androgens. From this study, the population attributable risk to develop type 2 diabetes by

Received for review October 9, 2002.
Accepted in final form January 10, 2003.
*The Cardiovascular Institute, Sahlgrenska University Hospital, Göteborg, Sweden; and †Department of Pharmacology, University of Göteborg, Göteborg, Sweden.
Address correspondence to Dr. Fariba Baghaei, MD, The Cardiovascular Institute, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden.
E-mail: fariba.baghaei@hjl.gu.se


[1] Nonstandard abbreviations: SHBG, sex hormone binding globulin; WHR, waist/hip circumference ratio; T, testosterone; E2, 17-$\beta$ estradiol; FSH, follicle stimulating hormone; LH, luteinizing hormone; ACTH, adrenocoticotropic hormone; freeT, free testosterone; DHEAS, dehydroepiandrosterone sulfate; IGF I, insulin-like growth factor I; [TTTA], tetranucleotide repeat polymorphism; HPA, hypothalamic-pituitary-adrenal.

elevated androgens can be estimated to be >50% (3). Androgens are, consequently, one of the major risk factors for type 2 diabetes in women.

These findings make it of interest to try to trace the origin of androgens in the higher part of the distribution of androgen values in the population. In women, these hormones are secreted mainly by the ovaries and adrenals. Another pathophysiological prospect is a reduced conversion of androgens to estrogens by the aromatase enzyme (6,7).

The role of microsatellites in genes involved in steroid hormone metabolism and action is a novel dimension of molecular genetic research (8,9). The allelic state of the microsatellite in intron 4 of the *CYP19* gene has recently been shown to be associated with increased risk for the development of breast and endometrial cancer and osteoporosis in women (10–14). In this study, we addressed the hypothesis that this microsatellite is also associated with the elevation of androgens in women and is related to disease risk factors. Because smoking women are known to have elevated androgens (15), we primarily examined nonsmoking women to elucidate associations among aromatase gene alleles, hormones, and risk factors.

## Research Methods and Procedures

### *Population*

In the present study, we recruited the subjects from an ongoing cohort study of women ($n = 1137$), all born in 1956 on odd-numbered days of the month and living in Göteborg (16). The study was initiated in 1996. The waist/hip circumference ratio (WHR) was self-measured after careful instructions. From these values, a subgroup of 450 women was selected who did not differ in WHR from the total cohort. In this subgroup, the WHR values were apparently normally distributed. During the period from November 1997 to December 1998, these women were invited to a health examination at the laboratory, and 270 (60%) volunteered to participate. Women were excluded from the study if they were postmenopausal, defined as no menstruations during the past 6 months. All examinations were performed in the follicular phase of the menstruation cycle (days 5 to 10). Women using birth control pills ($n = 25$) or injections ($n = 1$), or using hormone-containing intrauterine devices ($n = 22$), were excluded from the analyses. Nonsmokers were defined as women who had never smoked and those who had quit smoking for at least 1 year before the clinical examination. Smokers were defined as current smokers. This left 193 women for the study; 142 were nonsmokers.

All women gave written informed consent before participating in the study, which was approved by the Ethics Committee of the University of Göteborg.

### *Anthropometry*

These measurements were repeated in the laboratory after the women had been fasting overnight. Body weight was measured to the nearest 0.1 kg, with the women in underwear, height was measured to the nearest 0.01 m, and the BMI was calculated. The waist circumference was measured halfway between the lower rib and the iliac crest, the hip circumference was measured over the widest part of the gluteal region, and the WHR was calculated. These values correlated closely ($r > 0.80$) with the self-reported values. The abdominal sagittal diameter was recorded as the distance between the examination table and the highest point of the abdomen in the recumbent position and is an anthropometric estimation of intra-abdominal fat mass (17).

### *Cortisol Measurements*

Cortisol was measured by radioimmunoassay (Orion Diagnostica Cortisol RIA; Orion Corp., Espoo, Finland) in saliva collected by a device with a small cotton plug, which the women chewed on for 60 s and then placed in a test tube for subsequent centrifugation (Salivette; Sarstedt, Landskrona, Sweden). Collections were performed several times during a random day of the week. A sample was obtained in the morning on awakening, at 11:45 AM, 30, 45, and 60 minutes after a standardized lunch at noon, 5:00 PM, and just before bedtime. The lunch was provided by the laboratory and contained 430 kcal (protein, 16 g; carbohydrate, 74 g; fat, 8 g). The salivettes were stored in the refrigerator and delivered to the laboratory. Controls certified that this storage did not affect the results. Comparisons with serum cortisols showed *r* values >0.85. This method was used to avoid confounding factors associated with an unfamiliar hospital milieu and venipuncture.

In calculations of the cortisol values, each time-point was analyzed separately as well as the sum of all measurements, providing a total cortisol secretion. Furthermore, the sum of the cortisols at 30, 45, and 60 minutes after lunch are presented as lunch-stimulated cortisol. The decrease ("unwinding") of cortisol from the morning values to that determined at 11:45 AM was called slope 1, and the decrease to the bedtime value was slope 2.

### *Dexamethasone Suppression Test*

This test was performed on the day after the cortisol measurements. The women obtained a 0.5-mg tablet with dexamethasone (Decadron; MSD, Stockholm, Sweden) and collected saliva two mornings as described above. At 10:00 PM, the dexamethasone tablet was taken, and another saliva sample was taken the next morning. The suppression was calculated as the difference between the mean of the two morning cortisols before and the cortisol on the morning after dexamethasone.

### *Hormones and Lipids in Serum and Blood Glucose*

These determinations were performed in venous blood, obtained after an overnight fast. Total serum testosterone (T), 17-$\beta$ estradiol (E2), SHBG, and follicle stimulating

hormone (FSH) were analyzed using chemoluminescent enzyme immunoassays (Diagnostic Products, Los Angeles, CA). Luteinizing hormone (LH), adrenocoticotropic hormone (ACTH), free testosterone (freeT), androstenedione, and dehydroepiandrosterone sulfate (DHEAS) were determined by radioimmunoassays, using assay kits from Sereno Diagnostics (LH) and Diagnostic Products (all others). Insulin was measured by radioimmunoassay (Phadebas, Pharmacia Insulin RIA 100; Kabi-Pharmacia Diagnostics, Uppsala, Sweden), and insulin-like growth factor I (IGF I) was determined by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano CA). Glucose was determined enzymatically, and serum lipids were determined as described previously (18).

### *Blood Pressure*

Before blood sampling, two blood pressure measurements were recorded on the right arm, with automatic digital blood pressure measuring equipment (UA-751; A&D Co., Tokyo, Japan) The women were sitting; the first blood pressure was measured after a 5-minute rest, the second after another 5-minute rest, and the values were averaged. Heart rate was recorded simultaneously.

### *Determination of Genotypes*

DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen Inc. GmbH, Hilden, Germany). The tetranucleotide polymorphism in intron 4 of the aromatase gene was genotyped employing a modified protocol of Polymeropoulos et al. (19). The forward primer, 5′-GCAGGTACTTAGTTAGCTAC, fluorescently labeled with 6-fluorescein, was used together with the reverse primer, 5′-TTACAGTGAGCCAAGGTCGT. The polymerase chain reaction also contained 1 U AmpliTaq Gold DNA polymerase (Perkin-Elmer, Stockholm, Sweden)/25 $\mu$L and 0.3 uM of each primer. The temperature profile was 95 °C for 12 minutes followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, followed by a final incubation at 72° for 7 minutes. The fluorescently labeled DNA fragments were analyzed by size with automated capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer).

### *Data Analysis*

All data analyses were performed with SPSS for Windows, release 10.0 (SPSS Inc., Chicago, IL). The tetranucleotide repeat polymorphisms ranged from 7 to 13 repeats $[TTTA]_{7–13}$, with allelic sizes of 168 to 195 bp. The $[TTTA]_7$ repeat polymorphism contained two different alleles depending on a TCT insertion/deletion 50-bp upstream of the $[TTTA]_n$ tract, resulting in base pair products of 168 and 171 bp, respectively (11).

At first the subjects were divided into two groups based on the average level of their two alleles, with the median length of the [TTTA] repeats as the cut-off limit (177.5 bp). Alleles containing ≤177.5 bp were defined as short alleles, and those containing >177.5 bp were defined as long alleles. Furthermore, different genotypes were evaluated using a dichotomous variable as allele carriers vs. noncarriers, e.g., those with or without a 168-bp allele ($[TTTA]_7$-TCT).

Differences between subgroups were analyzed with the Mann-Whitney *U* test. Data are presented as the mean ± SD. A two-tailed test was used throughout, and a $p < 0.05$ was considered as statistically significant.

**Table 1.** Steroid hormones in smokers and nonsmokers

| | Smokers ($n$ = 51) | Non-smokers ($n$ = 142) | $p$ Value |
|---|---|---|---|
| T (nM) | 2.41 ± 1.38 | 1.89 ± 0.87 | 0.019 |
| freeT (pM) | 5.53 ± 2.70 | 4.48 ± 2.33 | 0.012 |
| Androstenedione (nM) | 8.43 ± 3.54 | 6.91 ± 2.73 | 0.005 |
| SHBG (nM) | 45.9 ± 24.6 | 44.9 ± 18.8 | NS |
| E2 (pM) | 204.4 ± 148.0 | 201.4 ± 168.9 | NS |
| E2/T | 119.9 ± 133.2 | 142.9 ± 163.7 | NS |
| E2/freeT | 47.0 ± 41.1 | 64.1 ± 86.6 | NS |
| E2/androstenedione | 27.8 ± 24.4 | 34.8 ± 36.7 | NS |
| DHEAS ($\mu$M) | 3.93 ± 1.71 | 3.80 ± 1.59 | NS |
| FSH (IU/L) | 15.9 ± 16.9 | 13.2 ± 7.6 | NS |
| LH (IU/L) | 5.20 ± 5.03 | 3.89 ± 2.97 | 0.008 |
| IGFI ($\mu$g/L) | 191.6 ± 59.6 | 194.5 ± 50.4 | NS |

Mean ± SD.
NS, not significant.

## Results

Table 1 shows comparisons between hormone values in smokers and nonsmokers. Smokers had higher total T ($p$ = 0.019), freeT ($p$ = 0.012), androstenedione ($p$ = 0.005), and LH ($p$ = 0.008) values. Smokers also had higher WHR (0.830 ± 0.065 mM vs. 0.805 ± 0.070 mM, $p$ = 0.042) and triglycerides (1.24 ± 0.74 mM vs. 0.98 ± 0.52 mM, $p$ = 0.017) and lower high-density lipoproteins (1.48 ± 0.42 mM vs. 1.65 ± 0.38 mM, $p$ = 0.009) than nonsmokers, whereas other anthropometric and metabolic values and blood pressure were not different (data not shown).

Seven tetranucleotide repeat alleles of aromatase gene (*CYP19*) within intron 4 were identified, ranging from 7 to 13 repeats and comprising 168 to 195 bp. The distribution of both alleles of the aromatase gene is shown in Figure 1.

The nonsmoking women were divided into two groups based on the average level of their two alleles, with the median length of the [TTTA] repeats as the cut-off limit

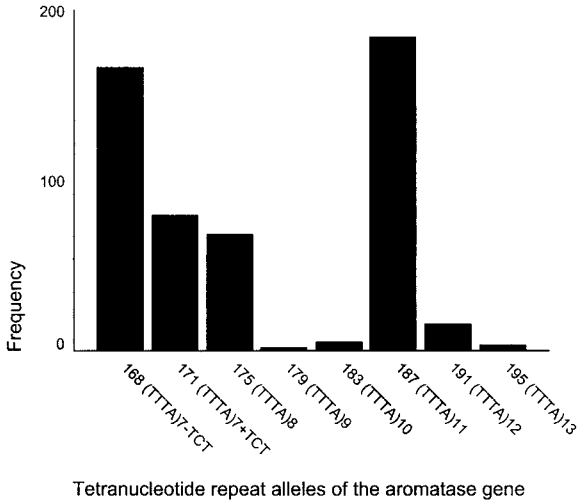


*Figure 1:* The distribution of alleles in intron 4 of the aromatase cytochrome P450 gene (*CYP19*) in all women ($n = 268$).

(177.5 bp). Hormone levels in nonsmoking women in these two groups are shown in Table 2. Women with average short [TTTA] stretches (≤177.5 bp) had higher freeT ($p = 0.018$) and lower SHBG ($p = 0.033$) and (borderline) E2/freeT ratio ($p = 0.076$) than women with long alleles (>177.5 bp). Other hormones were not different. Similar comparisons among smokers revealed no differences (data not shown).

**Table 2.** Comparisons of steroid hormones between nonsmokers with average short TTTA-repeats [(allele 1 + allele 2)/2 ≤ 177.5 bp] and those with average long TTTA-repeats [(allele 1 + allele 2)/2 > 177.5 bp] in the fourth intron of the *CYP19* gene

| | Mean alleles ≤ 177.5 bp ($n = 85$) | Mean alleles > 177.5 bp ($n = 51$) | *p* Value |
|---|---|---|---|
| T (nM) | 1.94 ± 0.89 | 1.80 ± 0.84 | NS |
| freeT (pM) | 4.83 ± 2.49 | 3.83 ± 2.00 | 0.018 |
| Androstenedione (nM) | 7.05 ± 2.78 | 6.63 ± 2.69 | NS |
| SHBG (nM) | 41.9 ± 17.0 | 49.7 ± 21.1 | 0.033 |
| E2 (pM) | 185.9 ± 134.5 | 229.2 ± 218.2 | NS |
| E2/T | 136.5 ± 168.5 | 159.1 ± 164.1 | NS |
| E2/freeT | 53.9 ± 67.6 | 84.2 ± 112.9 | 0.076 |
| E2/androstenedione | 31.8 ± 29.7 | 40.9 ± 47.2 | NS |
| DHEAS (μM) | 3.86 ± 1.67 | 3.67 ± 1.50 | NS |
| FSH (IU/L) | 13.6 ± 7.5 | 12.6 ± 8.2 | NS |
| LH (IU/L) | 3.96 ± 3.45 | 3.83 ± 2.13 | NS |
| IGFI (μg/L) | 199.5 ± 51.9 | 186.2 ± 48.9 | NS |

Mean ± SD.
NS, not significant.

**Table 3.** Hormones in nonsmoking women with at least one 168-bp allele in comparison with the rest of the women

| | With the 168-bp allele ($n = 80$) | Without the 168-bp allele ($n = 56$) | *p* Value |
|---|---|---|---|
| T (nM) | 1.99 ± 0.90 | 1.72 ± 0.81 | 0.072 |
| freeT (pM) | 4.93 ± 2.47 | 3.78 ± 2.03 | 0.004 |
| Androstenedione (nM) | 7.20 ± 2.50 | 6.46 ± 3.05 | 0.017 |
| SHBG (nM) | 41.9 ± 17.3 | 49.0 ± 20.4 | 0.072 |
| E2 (pM) | 182.3 ± 127.4 | 230.4 ± 217.5 | NS |
| E2/T | 130.2 ± 163.1 | 166.0 ± 170.7 | NS |
| E2/freeT | 53.0 ± 68.0 | 82.9 ± 109.2 | 0.035 |
| E2/androstenedione | 30.0 ± 27.0 | 42.6 ± 47.7 | NS |
| DHEAS (μM) | 4.02 ± 1.68 | 3.46 ± 1.43 | 0.043 |
| FSH (IU/L) | 12.7 ± 5.5 | 14.0 ± 10.1 | NS |
| LH (IU/L) | 3.57 ± 1.54 | 4.40 ± 4.30 | NS |
| IGFI (μg/L) | 195.7 ± 52.0 | 192.8 ± 50.1 | NS |

Mean ± SD.
NS, not significant.

Next, we compared the steroid hormone levels in nonsmoking women with or without given alleles of the microsatellite polymorphism in intron 4 of the *CYP19* gene. Women with at least one 168-bp allele ($[TTTA]_7$-TCT), in comparison with the rest of the women (Table 3), had higher values of freeT ($p = 0.004$), androstenedione ($p = 0.017$), and DHEAS ($p = 0.043$), and borderline higher T ($p = 0.072$). The E2/freeT ratio was lower ($p = 0.035$), and the SHBG values were borderline lower ($p = 0.072$) among these women. Similar comparisons regarding other allelic variations (171-,175-,187-, and 191-bp alleles) among nonsmokers showed that women with at least one 175-bp allele ($n = 33$) had lower DHEAS (3.30 ± 1.53 vs. 3.95 ± 1.60 μM, $p = 0.022$) than the rest of the women (data not shown). No other differences were found.

Analyses among smokers also showed higher freeT (6.27 ± 2.82 vs. 4.72 ± 2.26 pM, $p = 0.041$) and lower SHBG (41.0 ± 25.3 vs. 50.4 ± 23.3 nM, $p = 0.041$) in women ($n = 23$) with the 168-bp allele (data not shown).

In combination variants with the 168-bp allele, the 168/171 allele ($n = 14$) showed higher values of LH (4.16 ± 1.37 vs. 3.89 ± 3.15 IU/L, $p = 0.045$), as well as a trend toward higher freeT (5.43 ± 2.27 vs. 4.35 ± 2.35 pM, $p =$

0.058), and borderline lower IGF I values (173.1 ± 47.7 vs. 197.0 ± 51.0 μg/L, $p = 0.078$) than the rest of the women. Women with the allelic combination of 168/187 ($n = 33$) had higher values of DHEAS (4.41 ± 1.93 vs. 3.59 ± 1.44 μM, $p = 0.035$) and IGF I (213.7 ± 54.8 vs. 188.3 ± 48.4 μg/L, $p = 0.027$) and lower values of SHBG (38.2 ± 15.2 vs. 46.9 ± 19.5 nM, $p = 0.016$) than the rest of the women. Homozygotes for the 168/168-bp allele ($n = 14$) among nonsmokers showed no differences in the above measurements. Other combinations were not meaningful to analyze because of the small number.

Table 4 shows that women with the 168-bp allele had higher cortisol values at 11:45 AM ($p = 0.023$) and at bedtime ($p = 0.049$), as well as lower values of the cortisol slope 1 ($p = 0.035$). Other cortisol measurements, as well as ACTH and dexamethasone suppression test, showed no differences.

Similar comparisons were next performed for anthropometric, metabolic, and hemodynamic variables in nonsmoking women (Table 5). Women with the 168-bp allele $[TTTA]_7$-TCT had higher values of abdominal sagittal diameter ($p = 0.049$) and fasting insulin ($p = 0.034$), as well as borderline higher values of BMI ($p = 0.078$) and WHR ($p = 0.064$). No other differences in metabolic values or blood pressure in comparison with the rest of the women were revealed.

Women with the 179 ($[TTTA]_9$)-, 183 ($[TTTA]_{10}$)-, 191 ($[TTTA]_{12}$)-, and 195 ($[TTTA]_{13}$)-bp alleles, as well as homozygotes for these alleles, were generally too few to allow meaningful analyses.

**Table 4.** Saliva cortisol at indicated times (nM) and ACTH (ng/L) values in nonsmoking women with at least one 168-bp allele in comparison with the rest of the women

| | With the 168-bp allele ($n = 72$) | Without the 168-bp allele ($n = 53$) | *p* Value |
|---|---|---|---|
| Morning | 16.5 ± 12.3 | 16.4 ± 8.0 | NS |
| 11:45 AM | 8.9 ± 11.5 | 5.8 ± 1.9 | 0.023 |
| 30 minutes after lunch at noon | 9.8 ± 13.1 | 7.3 ± 2.7 | NS |
| 45 minutes after lunch at noon | 8.7 ± 9.7 | 6.9 ± 2.7 | NS |
| 60 minutes after lunch at noon | 7.9 ± 6.7 | 6.3 ± 2.5 | NS |
| 5:00 PM | 7.4 ± 15.2 | 4.9 ± 2.2 | NS |
| At bedtime | 4.8 ± 12.3 | 3.0 ± 3.9 | 0.049 |
| Morning − 11:45 AM (slope 1) | 7.6 ± 7.8 | 10.6 ± 8.6 | 0.035 |
| Morning − at bedtime (slope 2) | 11.7 ± 7.7 | 13.4 ± 8.9 | NS |
| Lunch cortisol | 26.4 ± 28.9 | 20.5 ± 7.4 | NS |
| Total cortisol (sum of all) | 64.5 ± 76.7 | 50.6 ± 14.3 | NS |
| Dexamethasone suppression | 12.0 ± 7.2 | 11.9 ± 9.0 | NS |
| ACTH | 25.6 ± 15.9 | 24.2 ± 13.0 | NS |

Mean ± SD.
Lunch cortisol: cortisol values at 30 plus 45 plus 60 minutes after lunch.
Dexamethasone suppression: sum of two morning cortisol values/2 minus the cortisol value the next morning after 0.5 mg dexamethasone at 10 PM.
NS, not significant.

## Discussion

The major findings in this study are the associations among a short [TTTA] in intron 4 of the *CYP19* gene, steroid hormones, and abdominal obesity. Several studies have recently reported associations between the *CYP19* repeat genotypes and hormone-sensitive cancers such as breast- and endometrial cancer in women and prostate carcinoma in men (10–14,20). In only a few of these studies have associations between $[TTTA]_n$ polymorphisms and hormone levels been examined, and no significant associations were found. Although in one study, the authors reported an association between the $[TTTA]_7$+TCT allele (171 bp) and decreased levels of estrone sulfate, estrone, and estradiol, and a lower estrone/androstenedione ratio, this was found among only controls and not cases (13). To our knowledge, no studies have been reported on associations between *CYP19* repeat genotypes and hormonal, anthropometric, and metabolic variables among premenopausal women from the population.

With shorter alleles, particularly the 168-bp allele, there were clear associations with elevated androgen levels and lower E2/androgen levels, which are the expected findings with an enzyme with suboptimal function in converting androgens to estrogens. In contrast, there were no such associations with the longer alleles.

Smoking is known to increase androgen production in women (15), which was confirmed in this report. The mechanism for this effect is supposed to be inhibition of aromatase function (21). With the goal of sorting out the potential effects of aromatase gene polymorphisms on androgen concentrations, we therefore examined nonsmokers. Analyses among smokers, however, also showed higher freeT and lower SHBG in women with the 168-bp allele, demonstrating that some association between androgens and the 168-bp allele can be found despite the confounding effect of smoking.

**Table 5.** Anthropometric, metabolic, and hemodynamic variables in nonsmoking women with at least one 168-bp allele in comparison with the rest of the women

| | With the 168-bp allele ($n = 80$) | Without the 168-bp allele ($n = 56$) | *p* Value |
|---|---|---|---|
| BMI (kg/m$^2$) | 25.6 ± 4.84 | 24.1 ± 3.82 | 0.078 |
| WHR | 0.815 ± 0.071 | 0.793 ± 0.071 | 0.064 |
| Sagittal abdominal diameter (cm) | 20.0 ± 3.0 | 19.0 ± 2.40 | 0.049 |
| Fasting glucose (nM) | 4.41 ± 0.43 | 4.36 ± 0.38 | NS |
| Fasting insulin (mU/L) | 8.48 ± 3.53 | 7.70 ± 4.13 | 0.034 |
| Triglycerides (mM) | 0.99 ± 0.46 | 0.98 ± 0.62 | NS |
| Total cholesterol (nM) | 5.07 ± 0.79 | 5.24 ± 0.83 | NS |
| Low-density lipoprotein cholesterol (mM) | 2.99 ± 0.73 | 3.15 ± 0.75 | NS |
| High-density lipoprotein cholesterol (mM) | 1.65 ± 0.40 | 1.63 ± 0.36 | NS |
| Systolic blood pressure (mm Hg) | 111.7 ± 14.5 | 109.1 ± 14.2 | NS |
| Diastolic blood pressure (mm Hg) | 66.0 ± 10.5 | 63.8 ± 9.3 | NS |

Mean ± SD.
NS, not significant.

Measurements of saliva cortisol also showed associations with different alleles. Women with the 168-bp allele had higher levels at 11:45 AM and, therefore, a less steep slope between morning and prelunch values (slope 1), which is a slower "unwinding" of the high morning cortisol secretion. Furthermore, DHEAS was higher. These results suggest a malfunction of the hypothalamic-pituitary-adrenal (HPA) axis. Similar changes have been described after frequently repeated or chronic challenges of the HPA axis by stress (22,23). In fact, women with elevated androgens in the examined population have been found to have psychosocial and socioeconomic handicaps and traits of depression that would be expected to expose them to frequent challenges of the HPA axis (unpublished observations).

The short allele was also associated with elevated sagittal abdominal diameter and borderline higher WHR, indicating abdominal obesity, and was associated with elevated insulin values. In a previous study of selected lean women from this cohort, a longer allele (187 bp) was found to be prevalent (24). These results indicate a dependence of abdominal obesity on the status of the aromatase gene.

There are no definitive data concerning a potential functional significance of the $[TTTA]_n$ polymorphism in the fourth intron of the *CYP19* gene and aromatase enzyme activity. However, recent studies in aromatase gene knockout mice show not only elevated androgens but also abdominal obesity and insulin resistance (25), clearly showing that aromatase function is involved in the development of abdominal obesity. We speculate that in women, the short tetranucleotide repeat allele, particularly with the presence of the TCT deletion upstream the microsatellite in question, might be indicative of a nonoptimal aromatase function with associated abdominal obesity and insulin resistance.

The explanation for elevated androgens in abdominal obesity has previously been found to be an elevated production rate of T. Furthermore, indirect measurements of aromatase activity seem to be lower in abdominal than peripheral obesity in women (26). Recent studies in the women in this report have shown that the function of the aromatase enzyme in adipose tissue is negatively proportional to androgen levels when adjusted for abdominal obesity (unpublished observations).

High freeT might be caused by low SHBG, which, in turn, is dependent either on elevated T or insulin; both interfere with SHBG synthesis in the liver (27,28). On the other hand, elevated androgens, either administered from external sources to women (29,30) or to female rats (31), or as a consequence of knockout of the aromatase gene (25), are followed by abdominal obesity and elevated insulin caused by insulin resistance, suggesting that exposure to androgens has a primary role. There is, however, a complex interplay between these factors, and it is difficult to determine cause and effect relationships.

The origin of the elevated androgens might be either or both of the two major contributors of androgens in women, the adrenals and the ovaries. Direct studies of venous outflow of androgens from the ovary or adrenals in hirsute women show that the ovarian contribution usually is higher than that from the adrenals (32). These results are, however, not necessarily applicable to the women in the population sample studied in this work and previously (3,4), where the androgen levels involved comprise the upper quintile of conventionally normal values.

The measurements of cortisol showed changes indicating an abnormal regulation of the HPA axis in the women with the short, 168-bp allele as discussed above. These findings

suggest that the elevated androgens might have an adrenal origin. Further support for this suspicion may be found in tight correlations between androgens (T and freeT) and hormones mainly or exclusively secreted by the adrenals (androstenedione and DHEAS), with correlation coefficients between 0.49 and 0.64 ($p < 0.0001$). With elevated secretion of adrenal androgens, aromatase function is particularly critical for the resulting androgen concentrations, and we speculate that a TCT deletion in the fourth intron of the aromatase gene is indicative of a lower than normal aromatase function, which might, therefore, amplify elevation of circulating androgens. The potential role of ovarian androgen production has not yet been examined.

The status of the microsatellite in the fourth intron of the gene of the aromatase enzyme has now been shown to be associated with a number of serious diseases in women, including breast and endometrial cancer and osteoporosis. All these conditions have been suspected to be associated with the estrogen/androgen balance (5,10–14,20). The results presented here clearly show that the allelic variation of the fourth intron of the *CYP19* gene is associated with this balance. When androgens are prevailing, this is a condition with powerful impact on the risk of developing type 2 diabetes and myocardial infarction (3,4). In addition, knockout of the entire gene is followed by elevated androgens, obesity, and insulin resistance (25). Although a direct influence, if any, of the polymorphisms of the aromatase gene studied is not known, the fact that associations with a number of serious prevalent diseases in women have been established demand further studies to clarify this important problem.

## Acknowledgments

This study was supported by Medical Research Council Project K 2000-72X-00,251-38B. Nils-Gunnar Pehrsson, senior statistician, is acknowledged for statistical advice and Anna Nilsson for excellent technical assistance.

### References

1. **Achard MC, Thiers MJ.** Le virilism pilaire et son association a l'insufficance glycolytique (Diabète des femmes a barbe). *Bull Med Natl Med (Paris*). 1921;86:51–66.
2. **Wild RA, Painter PC, Coulson PB, Carruth KB, Ranney GB.** Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 1985;61:946–51.
3. **Lindstedt G, Lundberg PA, Lapidus L, Lundgren H, Bengtsson C, Björntorp P.** Low sex-hormone-binding globulin concentration as independent risk factor for development of NIDDM. 12-yr follow-up of population study of women in Gothenburg, Sweden. *Diabetes*. 1991;40:123–8.
4. **Haffner SM.** Sex hormone-binding protein, hyperinsulinemia, insulin resistance and non insulin-dependent diabetes. *Horm Res*. 1996;45:233–7.
5. **Lapidus L, Helgesson Ö, Merck C, Björntorp P.** Adipose tissue distribution and female carcinomas. A 12 year follow-up of the participants in the population study of women in Gothenburg, Sweden. *Int J Obes Relat Metab Disord*. 1988; 12:361–8.
6. **Ackerman GE, Smith ME, Mendelson CR, MacDonald PC, Simpson ER.** Aromatization of androstenedione by human adipose tissue stromal cells in monolayer culture. *J Clin Endocrinol Metab*. 1981;53:412–7.
7. **Simpson ER, Clyne C, Rubin G, et al.** Aromatase: a brief overview. *Annu Rev Physiol*. 2002;64:93–127.
8. **Comings DE.** Polygenic inheritance and micro/minisatellites. *Mol Psychiatry*. 1998;3:21–31.
9. **Riggins GJ, Lokey LK, Chastain JL, et al.** Human genes containing trinucleotide repeats. *Nat Genet*. 1992;2:186–91.
10. **Berstein LM, Imyanitov EN, Suspitsin EN, et al.** CYP19 gene polymorphism in endometrial cancer patients. *J Cancer Res Clin Oncol*. 2001;127:135–8.
11. **Baxter SW, Choong DYH, Eccles DM, Campbell IG.** Polymorphic variation in CYP 19 and the risk of breast cancer. *Carcinogenesis*. 2001;22:347–9.
12. **Masi L, Becherini L, Gennari L, et al.** Polymorphism of the aromatase gene in postmenopausal Italian women: distribution and correlation with bone mass and fracture risk. *J Clin Endocrinol Metab*. 2001;86:2263–9.
13. **Probst-Hensch NM, Ingles SA, Diep AT, et al.** Aromatase and breast cancer susceptibility. *Endocr Relat Cancer*. 1999; 6:165–73.
14. **Haiman CA, Hankinson SE, Spiegelman D, et al.** A tetranucleotide repeat polymorphism in CYP 19 and breast cancer risk. *Int J Cancer*. 2000;87:204–10.
15. **Khaw KT, Chir B, Tazuke S, Barrett-Connor E.** Cigarette smoking and levels of adrenal androgens in postmenopausal women. *New Engl J Med*. 1988;318:1705–9.
16. **Rosmond R, Björntorp P.** Psychosocial and socioeconomic factors in women and their relationship to obesity and regional body fat distribution. *Int J Obes Relat Metab Disord*. 1999; 23:138–45.
17. **Kvist H, Chowdhury B, Grangård U, Tylén U, Sjöström L.** Total and visceral adipose tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. *Am J Clin Nutr*. 1988;48:1351–61.
18. **Wiklund O, Fager G, Craigh IH.** Alphalipoprotein cholesterol levels in relation to acute myocardial infarction and its risk factors. *Scand J Clin Lab Invest*. 1980;40:239–47.
19. **Polymeropoulos MH, Xiao H, Rath DS, Merril CR.** Tetranucleotide repeat polymorphism at the human aromatase cytochrome P450 gene (CYP 19). *Nucleic Acid Res*. 1991;19: 195.
20. **Latil AG, Azzouzi R, Cancel GS, et al.** Prostate carcinoma risk and allelic variants of genes involved in androgen biosynthesis and metabolism pathways. *Cancer*. 2001;92: 1130–7.
21. **Barbieri RL, McShane PM, Ryan KJ.** Constituents of cigarette smoke inhibit human granulosa cell aromatase. *Fertil Steril*. 1987;46:232–6.
22. **Dallman MF, Akana SF, Scribner KA, et al.** Stress, feedback and facilitation in the hypothalamic-pituitary-adrenal axis. *J Neuroendocrinol*. 1992;4:156–9.

23. **Björntorp P, Rosmond R.** Obesity and cortisol. *Nutrition.* 2000;16:924–36.
24. **Baghaei F, Rosmond R, Westberg L, et al.** The lean woman. *Obes Res*. 2002;10:115–21.
25. **Jones MEE, Thorburn AW, Britt KL, et al.** Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci USA*. 2000;97:12735–40.
26. **Kirschner MA, Samojlik E, Drejka M, Szmal E, Schneider G, Ertel N.** Androgen-estrogen metabolism in women with upper body versus lower body obesity. *J Clin Endocr Metab*. 1990;70:473–9.
27. **Plymate SR, Matej LA, Jones RE, Friedl KE.** Inhibition of sex hormone binding globulin in the human hepatoma (HEP G 2) cell line by insulin and prolactin. *J Clin Endocr Metab*. 1988;67:460–4.
28. **Selby C.** Sex hormone binding globulin; origin, function and clinical significance. *Ann Clin Biochem*. 1990;27:532–41.
29. **Lovejoy JC, Bray G, Bourgeois MO, et al.** Exogenous androgens influence body composition and regional fat distribution in obese postmenopausal women. A clinical research center study. *J Clin Endocrinol Metab*. 1996;81: 2189–203.
30. **Elbers JMH, Asscheman H, Seidell JC, Megens JA, Gooren LJG.** Long-term testosterone administration increases visceral fat in female to male transsexuals. *J Clin Endocrinol Metab*. 1997;82:2044–7.
31. **Holmäng A, Svedberg J, Jennische E, Björntorp P.** Effects of testosterone on muscle insulin sensitivity and morphology in female rats. *Am J Physiol*. 1990;257:E555–E60.
32. **Kirschner MA, Jacobs JB.** Combined ovarian and adrenal vein catheterization to determine the site(s) of androgen overproduction in hirsute women. *J Clin Endocrinol*. 1971;33: 199–209.