Idiopathic hirsutism: local and peripheral expression of aromatase (CYP19A) and 5α-reductase genes (SRD5A1 and SRD5A2)

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Objective: To evaluate idiopathic hirsutism etiology via molecular studies testing peripheral and local aromatase and 5α-reductase expression.

Design: Assessment of the expression of messenger RNA (mRNA) for type 1 and 2,5α-reductase isoenzyme gene (SRD5A1, SRD5A2) and aromatase (CYP19A) in dermal papillae cells and peripheral blood mononuclear cells.

Setting: University hospital.

Patient(s): 28 untreated idiopathic hirsute patients and 20 healthy women (controls).

Intervention(s): Human skin biopsies and peripheral venous blood.

Main Outcome Measure(s): SRD5A1, SRD5A2, CYP19A gene expression in skin biopsies and peripheral blood.

Result(s): A statistically significant reduction of SRD5A1, SRD5A2, and CYP19A gene expression was found in the dermal papillae cells and peripheral blood mononuclear cell between the study and control group.

Conclusion(s): Further study, including protein expression and enzyme activity assays, are warranted to characterize the paradoxically low gene expression levels of local 5α-reductase and aromatase in women with idiopathic hirsutism. (Fertil Steril 2011;96:479–82. ©2011 by American Society for Reproductive Medicine.)

Key Words: 5α-reductase, aromatase, expression, idiopathic hirsutism

Received December 24, 2010; revised May 6, 2011; accepted May 13, 2011; published online June 15, 2011.

A.O.C. has nothing to disclose. M.D. has nothing to disclose. F.T. has nothing to disclose. N.A.B. has nothing to disclose. K.U. has nothing to disclose. Y.O. has nothing to disclose. M.B. has nothing to disclose. C.B. has nothing to disclose. F.K. has nothing to disclose.

Funded by Erciyes University Research Funding and the Scientific and Technological Research Council of Turkey (TUBITAK, Project No: SBAG-106S170).

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Idiopathic hirsutism (IH) is defined as the presence of hirsutism in conjunction with regular ovulation and normal androgen levels, a condition found in only 5% to 15% of consecutive hirsute women (1). Little information is available regarding the pathogenesis of IH. Increased peripheral 5α-reductase activity and androgen receptor gene polymorphisms have been postulated to explain the pathogenesis of IH (2–4). Recent studies have shown that IH may be associated with some degree of insulin resistance and an increased tendency for glucose intolerance notably in obese women with IH (5).

The dermal papilla plays a key role in the control of hair growth, and dermal papilla cells (DPCs) seem to be the primary target of an- drogen regulation of hair growth (6, 7). Therefore, an alteration in the local regulation of 5α-reductase activity could cause of idiopathic hirsutism (1). We additionally investigated peripheral and local CYP19A (aromatase cytochrome P450) gene expression to detect potential local imbalances between the androgen and estrogen levels in women with normal serum androgen levels (8, 9). We profiled the expression of 5α-reductase and aromatase messenger RNA (mRNA) in DPCs from the lower abdominal region of the skin and in the peripheral blood of women with idiopathic hirsutism and healthy controls. Our results provide insight into the regulation of androgen action in human hair follicles by local androgen modification at the target cell level.

MATERIALS AND METHODS

Patients

Forty-five women with idiopathic hirsutism and 24 control women matched for race and ethnicity, ranging in age from 16 to 35 years, were selected for the study. The criterion for case selection was having IH plus giving informed consent for skin biopsies. None of the control women had ovarian dysfunction, hirsutism, hyperandrogenemia, or had been taking any drugs known to interfere with androgen, estrogen, or gonadotropin serum levels for at least 6 months before the study. The purpose of the protocol was explained to all participants, and informed consent was obtained before the study initiation. This study was approved by the ethics committee and the institutional review board of Erciyes University Medical School.

The diagnosis of IH was based on the presence of hirsutism (modified Ferriman-Gallwey score >8), regular ovulatory menstrual cycles, normal ovarian morphology, and a normal serum androgen profile, including total and free testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEAS). We also measured serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17α-hydroxyprogesterone (17-OHP), and thyroid hormone in all patients. Normal androgen levels were defined according to the reference values of the commercial kits: serum free testosterone <3.18 pg/mL, androstenedione <3 ng/mL, and DHEAS <3,330 ng/mL.

Thyroid dysfunction, hyperprolactinemia, nonclassic adrenal hyperplasia, polycystic ovary syndrome (PCOS), and adrenal/ovarian tumors were excluded by appropriate blood tests and ultrasonography of the ovaries and adrenal glands. None of the women had a history of taking medication known to interfere with carbohydrate metabolism or to cause hirsutism. Ovulation was confirmed by the day-24 serum progesterone levels (>5 mmol/L) in the patients and healthy controls. The patients and the healthy women...
Preparation of Hair Follicle Subunits
Punch biopsies were taken under local anesthesia from the midline subumbilical area of healthy volunteers and hirsute patients. Intact anagen hair follicles were isolated from the subcutaneous fat according to standard procedures (10) and were studied in the follicular phase (days 2 to 9) of their cycles. Serum samples for assessing hormone levels were drawn after an overnight fast in the morning. The sex hormone–binding globulin (SHBG) level was measured by immunoradiometric assay (Orion Diagnostica, Finland), using commercial kits. Serum FSH (ACS 180; Bayer), LH (ACS 180; Bayer), and estradiol (ACS 180; Bayer) levels were determined by an automated chemiluminescence system; free and total testosterone (DSL-4900; Diagnostic Systems Laboratories, Inc.), DHEAS (Immunootech), and androstenedione (Immunootech) levels were measured by radioimmunoassay (RIA); the sex hormone–binding globulin (SHBG) level was measured by immunoradiometric assay (Orion Diagnostica, Finland), using commercial kits.

Quantitative Real-Time RT-PCR Protocol
Total RNA was extracted from patient and control dermal papillae with Mobio (BiOstic Blood Total RNA Isolation Kit) and was extracted from peripheral blood mononuclear cell (PBMCs) with Qiagen (RNeasy midi kit, Qiagen), according to the manufacturers’ instructions.

The RNA was dissolved in diethylpyrocarbonate-treated, double-distilled water and was stored at −80°C. We used the First Strand cDNA Synthesis Kit (Fermentas) to reverse transcribe 2 μg of total RNA, according to the manufacturer’s instructions. The RNA integrity was verified by examining the real-time reverse-transcriptase polymerase chain reaction (RT-PCR) product of β-actin mRNA. The resultant complementary DNA (cDNA) from each woman was submitted to PCR by use of primer pairs for SRD5A1, SRD5A2, and CYP19A (target genes) and β-actin (reference gene). The primer sequences are listed in Table 1.

Quantitative PCR reactions were performed in duplicate with 5 μL of diluted cDNA template in a 20-μL reaction containing Precision Mastermix (PrimerDesign), 300 nM primers, and 200 nM probe using the Rotor Gene 6000 Real-Time PCR Machine (Corbett). We used the TaqMan system and internal positive and negative controls for each gene and designed primers and probes with Primer Express software (PrimerDesign Ltd) (Table 2). The PCR conditions were initial denaturation for 10 minutes at 95°C, 45 amplification cycles (15 seconds at 95°C, 30 seconds at 50°C, and 15 seconds at 72°C), and final denaturation for 10 minutes at 72°C. Product specificity was examined by melt curve analysis and agarose gel electrophoresis after each real-time PCR run. For analysis of quantitative results, the relative quantification method was performed by software provided by Corbett.

Statistical Analysis
All analyses were performed using the Statistical Package for the Social Sciences (SPSS, Inc.). Continuous variables with normal distribution (age, body mass index) were analyzed as means ± standard deviation and ranges; continuous variables without normal distribution were analyzed as median values and interquartile range. As continuous variables were without normal distribution, we analyzed differences by Mann-Whitney U test. Correlations among Ferriman-Gallwey score in the subgroup of hirsute women were determined by using Spearman’s test. P < .05 was considered statistically significant. For categorical variables, differences were analyzed by means of the chi-square test and Fisher’s exact test when appropriate.

RESULTS
Fifteen patients and four controls did not meet our selection criteria and were disqualified from the study. The isolation of dermal papillae and/or RNA extraction was unsuccessful for technical reasons in two cases, so the final study included of 28 patients and 20 controls. The patients and controls did not differ in mean age (21.7 ± 5.94 and 20.8 ± 5.94 years, respectively), and the patients and controls did not differ in mean body mass index (BMI) (21.7 ± 5.94 and 23.0 ± 4.12 kg/m², respectively). The patients and controls did not differ in mean Ferriman-Gallwey (mFG) score (17.1 ± 4.36 and 15.7 ± 4.10, respectively). We performed a genetic association study with mRNA expression data for four candidate genes and expected sizes of amplified fragments for expression analysis.

Gene | Sequence (5′→3′) | Tm | Size of fragment |
--- | --- | --- | --- |
CYP19A | GAGGGACAGGAAGAGGAAG | 71.9 | 108 bp |
SRD5A1 | GTGATGCTGATGACTGGTAA | 71.4 | 120 bp |
SRD5A2 | GGCAATACCAAATAATGAGTAGTG | 70.3 | 109 bp |

Note: BMI = body mass index; IH = idiopathic hirsutism; mFG = modified Ferriman-Gallwey score; SD = standard deviation.
22.6 ± 0.4 years, respectively) or body mass index (26.6 ± 5.75 and 24.5 ± 2.5 kg/m², respectively) values, whereas the modified Ferriman-Gallwey score was statistically significantly (P<.001) higher in the patients with IH (17.1 ± 4.3) than in the healthy women (1.9 ± 0.6) (Table 2). The hormonal profile of the patient group is shown in Table 3.

The mRNA expression levels of SRD5A1, SRD5A2, and CYP19A were statistically significantly lower in tissue and PMBCs from patients when compared with controls (Table 4). Within the patient group, the mRNA expression levels of SRD5A1, SRD5A2, and CYP19A genes were statistically significantly higher in tissue than in PMBCs (P<.05) (Table 4). There was no statistically significant correlation between serum hormone values and SRD5A1, SRD5A2, or CYP1A1 gene expression in patient tissues or PBMCs.

**DISCUSSION**

Androgens are important regulators of human hair growth, but the mechanism of androgen action is not completely understood. Assuming that the specific response of hair follicles to androgens depends on androgen metabolism in the target cells, differences in intracellular androgen metabolism may be responsible for the different androgen sensitivities of hair follicles at different body sites. The dermal papilla contains androgen receptors and the enzymes 17β-hydroxysteroid dehydrogenase (17β-HSD) and 5α-reductase isotypes type 1 and 2. Both of these isotypes convert testosterone to the more active androgen 5α-dihydrotestosterone, which can in turn be metabolized to 5α-androstenedione by the action of 17β-HSD and further converted to 5α-androstenedione by the enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD). Additionally, testosterone can be metabolized to estradiol via a sequence of reactions involving the enzyme aromatase.

A well-known hypothesis suggests that the activity of 5α-reductase enzyme is increased in skin with hirsutism. Previously, Serafini and Lobo (11) suggested that 5α-reductase skin activity is increased by an androgen-independent mechanism that may be genetically determined. Our study was designed to assess the expression of 5α-reductase type 1 and type 2 mRNA in blood and dermal papilla cells from the lower midline abdominal skin in women with hirsutism. Our results showed decreased expression of SRD5A1 and SRD5A2 in the peripheral blood and dermal papillae cells of patients versus controls. It has to be emphasized that gene expression and enzyme activity are not equivalent.

Previously, Sultan et al. (12) and Oliveira et al. (13) used RT-PCR to investigate SRD5A1 and SRD5A2 expression in plucked scalp hairs from hirsute patients (13 idiopathic and 20 with PCOS) and controls (10 men and 15 nonhirsute women). Their study did not detect SRD5A2 expression in hair samples and found no differences in SRD5A1 expression between any of the groups. With these results, they argued that SRD5A1 gene expression is not responsible for differences in hair growth in normal men, normal women, or hirsute patients, with the caveat that more detailed studies including other follicular compartments would be necessary (13).

There are several differences in study design that can explain the contrast between our results and those of Oliveira et al. (13). They analyzed SRD5A expression in plucked anagen hairs but not in the connective tissue sheath, the lower bulb, the dermal papilla cells, and the sebaceous gland; we isolated the dermal papilla. The scalp hair follicle cells that they studied are generally not considered to be a main target for hirsutism, which is why we focused on subumbilical hair. In addition, we used a more sensitive molecular method to assess gene expression, real-time PCR rather than reverse-transcriptase PCR. Our study also included a larger number of IH patients (28 vs. 13). Alternatively, androgens may have different effects on gene transcription, translation, or posttranslational processes in various body sites or even within same follicle.

### Table 3

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Patients with IH (± SD) (n = 28)</th>
<th>Control group (± SD) (n = 20)</th>
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<tbody>
<tr>
<td>FSH (mIU/mL)</td>
<td>5.4 ± 2.22</td>
<td>4.9 ± 1.5</td>
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<tr>
<td>LH (mIU/mL)</td>
<td>3.1 ± 1.52</td>
<td>3.88 ± 2.4</td>
</tr>
<tr>
<td>E₂ (pg/mL)</td>
<td>48.8 ± 24.68</td>
<td>46.2 ± 19.7</td>
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<tr>
<td>Total T (ng/dL)</td>
<td>29.2 ± 15.51</td>
<td>32.4 ± 10.1</td>
</tr>
<tr>
<td>FT (pg/mL)</td>
<td>1.5 ± 0.52</td>
<td>1.24 ± 0.82</td>
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<tr>
<td>DHEAS (ng/mL)</td>
<td>2,106.2 ± 1,103.4</td>
<td>2,600.9 ± 500.7</td>
</tr>
<tr>
<td>17-OHP (ng/mg)</td>
<td>0.5 ± 0.7</td>
<td>0.74 ± 0.3</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>13.8 ± 8.7</td>
<td>22.1 ± 14.5</td>
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<tr>
<td>A (ng/mL)</td>
<td>1.5 ± 0.7</td>
<td>1.9 ± 0.2</td>
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*Note: 17-OHP = 17α-hydroxyprogesterone; A = androstenedione; DHEAS = dehydroepiandrosterone sulfate; E₂ = estradiol; FSH = follicle-stimulating hormone; FT = free testosterone; IH = idiopathic hirsutism; LH = luteinizing hormone; SD = standard deviation; SHBG = sex hormone–binding globulin; T = testosterone.

**Caglayan. Aromatase 5α-reductase hirsutism. Fertil Steril 2011.**

### Table 4

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<tbody>
<tr>
<td>Tissue expression</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>246.84</td>
<td>3,358.27</td>
<td>1,953.12</td>
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<tr>
<td>Patient</td>
<td>7.523</td>
<td>4.06</td>
<td>6.16</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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<tr>
<td>PBMC expression</td>
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<tr>
<td>Control</td>
<td>84.29</td>
<td>956.06</td>
<td>452.7</td>
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<tr>
<td>Patient</td>
<td>1.51</td>
<td>1.78</td>
<td>2.41</td>
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<tr>
<td>P value</td>
<td>&lt;.001</td>
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Skalba et al. (14) investigated mRNA expression of SRD5A1 and SRD5A2 in dermal papillae from the lower abdominal skin in 42 patients with PCOS and IH. They found higher levels of SRD5A1 mRNA than SRD5A2 mRNA in both groups, and also they found a positive correlation between both SRD5A gene isotypes and concentrations of free serum testosterone. Based on these results, they argued that testosterone increases the mRNA level of both SRD5A gene isotypes mRNA level in dermal papillae from the lower abdominal regions in patients with hirsutism. Our results did not replicate the positive correlation between the free serum testosterone and the SRD5A expression and decreased SRD5A gene. Both our study and Skalba et al. (14) study need further validation of mRNA expression results.

It is believed that the presence and activity of androgen receptors, growth hormone, insulin-like growth factor, insulin, thyroid hormones, and estrogens play an important role in the development of hirsutism, especially in its idiopathic form (15–17). An altered regulation of hair growth—indeed independent of androgens but linked to an increased production of local hormones or mediators—has been hypothesized. Recently, two studies have shown that insulin resistance, a well-known hallmark of PCOS, may also be present in women with idiopathic hirsutism (5, 18). However, at least in most patients, this association seems to be linked to the presence of polycystic ovaries (increased ovarian size) (19); therefore, these reports may just confirm that a mild insulin resistance is present in women with ovulatory PCOS (19, 20).

Another aim of our study was to assess the expression of aromatase mRNA in peripheral blood and dermal papilla cells from the lower midline abdominal skin in women with hirsutism. We found statistically significant differences in aromatase gene expression in the tissue and PBMCs between the patient and the control groups. Unluhizarci et al. (5) previously found that the estradiol/free testosterone ratio, which is a marker of aromatase activity, is lower in patients with IH when compared with healthy women. One aim of our study was to evaluate aromatase activity at the tissue level, and we demonstrated decreased aromatase gene expression activity in the skin and blood of women with IH. It is well known that the pilosebaceous unit may synthesize androgens de novo from cholesterol or by locally converting circulating weak androgen to more potent one (5, 20).

More studies with a larger number of patients should be performed to investigate the role of decreased 5α-reductase and aromatase gene expression and 5α-reductase enzyme activity in women with IH. By establishing the pathogenetic mechanisms underlying IH, new therapeutic strategies may play an important role in offering more effective therapies. Improved treatment of hirsutism awaits advancements in the understanding of the interaction between androgens and other determinants of hair-follicle development.

Acknowledgments: The authors thank Victoria Clark for her contribution in editing the manuscript.

REFERENCES