The −597 G→A and −174 G→C Polymorphisms in the Promoter of the IL-6 Gene Are Associated with Hyperandrogenism

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To evaluate whether genetic variability at the IL-6 gene (IL-6) is associated with hyperandrogenism, we studied four common polymorphisms in the IL-6 promoter (−597G→A, −572G→C, −373A, Tn, −174G→C) in 85 hyperandrogenic patients and 25 healthy women. We found 5 different haplotypes when considering the 3 biallelic polymorphisms at positions −597, −572, and −174 of IL-6 (relative frequencies in parentheses): GGG (0.505), AGC (0.377), GCC (0.059), GCG (0.055), and GCC (0.005). The frequencies of the GGG haplotype were 0.559 in patients and 0.320 in controls, whereas those of the AGC haplotype were 0.318 in patients and 0.580 in controls (χ² = 12.145; P < 0.02). The −597G→A and −174G→C polymorphisms were in linkage disequilibrium (χ² = 152.220; P < 0.00001), and were associated with patient or control status. −597G and −174G alleles were more frequent in patients in homozygosity or considering subjects homozygous and heterozygous for G alleles as a whole (P < 0.05 for all analyses).

In healthy women G alleles at −597 and −174 were associated with statistically significant higher circulating levels of IL-6 and basal cortisol, 11-deoxycortisol, and 17-hydroxyprogesterone and a tendency (P < 0.10) for higher total T concentrations compared with −597A and −174C alleles. On the contrary, neither the −572G→C nor the −373A, Tn polymorphism was related to hyperandrogenism or influenced any clinical or biochemical variable.

In conclusion, our present results suggest that the −597G→A and −174G→C polymorphisms in IL-6 are involved in the pathogenesis of hyperandrogenic disorders. (J Clin Endocrinol Metab 87: 1134–1141, 2002)

HYPERANDROGENISM and the polycystic ovary syndrome are common disorders in women of reproductive age, with prevalences ranging from 5–10% around the world (1, 2). There is increasing evidence that these disorders have a genetic basis and appear to be complex diseases in terms of inheritance (3, 4).

Common genetic disorders might result from adaptive changes that, from an evolutionary perspective, favor short-term survival of these individuals. However, such changes may lead to disease with prolonged life expectancy or when these subjects are exposed to the present lifestyle in Western countries.

Hyperandrogenism may result from these adaptive changes. As suggested by Witchel et al. (5) for congenital adrenal hyperplasia, which is one of the most common inherited disorders, with carrier frequencies of approximately 10% in all populations studied, and a relatively common cause of hyperandrogenism in children and adults, the rapid maturation of the reproductive axis found in these subjects together with the increase in assertive behavior resulting from increased androgen secretion might be advantageous during times of environmental stress (5–7). Also, the relative infertility of these women could increase the interval between pregnancies, decreasing the birth rate and favoring maternal and infant survival (5). The same mechanisms might apply for other forms of hyperandrogenism.

Adaptive changes during evolution may also be involved in the pathogenesis of type 2 diabetes mellitus, insulin resistance, and obesity (8), disorders that are also frequently associated with the polycystic ovary syndrome and hyperandrogenism (9–12). By preserving glucose for brain metabolism, insulin resistance could provide a survival advantage against starvation during evolution, overcoming the possible inconveniences of atherosclerosis and glucose intolerance that may appear with prolonged life expectancy of affected subjects, especially when these subjects are exposed to high carbohydrate and saturated fat contents, low fiber diets, and sedentary habits (8).

Among the genes suggested to favor survival through insulin resistance, those of the inflammatory cytokines such as IL-6 and TNFα have been studied (8). Aside from defense against infection, a high cytokine responder genotype might favor survival during periods of food shortage (8).

Insulin resistance, hyperlipidemia, and type 2 diabetes mellitus are associated with genomic variants in the genes encoding IL-6, TNFα, and TNF type 2 receptor (13–16). Moreover, the serum levels of these cytokines and of the soluble fraction of TNF type 2 receptor are increased in obesity and insulin-resistant states (17–19), reflecting the increased secretion of both cytokines at the adipose tissue in these disorders (17, 20).

We reported recently that a G→A single nucleotide polymorphism at position −308 from the start of translation site of the TNFα gene is associated with increased androgen secretion in hyperandrogenic and healthy women independent of obesity and insulin resistance (21), suggesting that
inflammatory cytokines may play a role in the pathogenesis of hyperandrogenism.

To evaluate the possible influence of IL-6 in the pathogenesis of hyperandrogenism, we have studied serum IL-6 concentrations and four common polymorphisms in the regulatory region of IL-6 in a series of 85 hyperandrogenic women and 25 healthy controls.

Subjects and Methods

Subjects

The hyperandrogenic group was prospectively recruited and included women complaining of hirsutism and/or hyperandrogenic anovulation. The control group was composed of lean female volunteers and consecutive patients attending the clinical practice of one of the authors (H.F.E.-M.) for treatment of obesity. None of the controls had signs or symptoms of hyperandrogenism, menstrual dysfunction, or history of infertility.

To facilitate blinded matching for obesity between patients and controls, and because recent studies have shown that the genetic influence of inflammatory cytokines in the pathogenesis of obesity and obesity-associated hypertension is especially important in nonmorbid obesity [body mass index, 27–35 kg/m² (22)], only women presenting with a body mass index of 35 kg/m² or less were included in the study.

Eighty-five hyperandrogenic patients (age, 22.8 ± 6.6 yr; body mass index, 24.7 ± 4.0 kg/m² mean ± sn) were studied. Hirsutism, as defined by a modified Ferriman-Gallwey score (23) of 8 or more, was present in 82 of the patients with a score of 15.8 ± 5.5. Polycystic ovary syndrome, defined by clinical and/or biochemical hyperandrogenism, oligomenorrhea, and exclusion of other etiologies (24), was present in 30 patients, including 3 nonhirsute patients. Forty patients had hirsutism, increased serum androgen levels, and regular menses, and 15 patients presented with idiopathic hirsutism, defined by hirsutism, normal androgen levels, and regular menstrual cycles.

None of the patients had features of Cushings disease or drug-induced hirsutism. Hyperprolactinemia and congenital adrenal hyperplasia were ruled out because all of the patients presented with basal serum PRL levels below 24 μg/liter, ACTH-stimulated 17-hydroxyprogesterone levels below 30 nmol/liter (25), and ACTH-stimulated 17-hydroxyprogesterone levels below 24 nmol/liter (mean ± 2 s of the control group).

The control group included 25 women (age, 30.6 ± 8.2 yr; body mass index, 26.2 ± 4.9 kg/m²). All of the controls and patients had blood pressure below 140/90 mm Hg, and fasting glucose levels below 110 mg/dl. Data from some patients and controls, regarding different as-

DNA extraction and genotype analysis

Genomic DNA was extracted from leukocytes obtained from whole blood samples, using commercial DNA purification kits (Wizard Genomic DNA purification kit, Promega Corp., Madison, WI, and Nu
cleon BAC C3, Amersham Pharmacia Biotech, Little Chalfont, UK). A PCR system with sequence-specific primers was used for direct haplo-
typing of three biallelic polymorphisms in the promoter of IL-6 (−597G→A, −572G→C, and −174G→C). The method and sequences of the PCR primers have been previously described (30). Using a 12-
reaction PCR system with sequence-specific primers, mismatches at the 3’-end of forward and reverse primers of each PCR allowed us to unequivocally establish both haplotypes for the three biallelic sites in each subject.

PCR amplifications were carried out in 96-well plates using a Gene Amp 9700 PCR System (PE Applied Biosystems, Foster City, CA) under conditions previously described (30). PCR products were submitted to electrophoresis in 2.5% agarose gels containing ethidium bromide in 1 × TBE. Gels were photographed under UV light and scored for the presence or absence of an allele-specific band, provided a PCR control band was present.

Terry et al. (30) recently suggested that IL-6 transcription in individuals presenting with G alleles at −597, −572, and −174 positions (GGG haplotype) is different depending on the −573A,−373AnTn run polymorphism. For that reason we studied the −573A,−373AnTn run polymorphism in a subset of subjects by direct sequencing of the allele-specific PCR products using an ABI 310 automated sequencer (PE Applied Biosystems) and 5’-GCT GGC ATG GAG TCA GAG-3’ as primer sequence (30).

Statistical analysis

Results are expressed as the mean ± s unless otherwise stated. The Kolmogorov-Smirnov statistic, with a Lilliefors significance level for

test or one-way ANOVA followed by the least significant difference test for post-hoc multiple mean comparisons was used to compared the central tendencies of the different groups. Two-way ANOVA was also used, as described below. To evaluate the association between discontinuous variables we used the x²

test, whereas correlation analysis was used to explore the relationship between continuous variables. P < 0.05 was considered statistically significant.

Results

Biochemical profiles of patients and controls

The comparison of the hormone profiles of patients and controls is summarized in Table 1. No differences were observed in serum IL-6 levels. Patients presented with increased total T, free T, dehydroepiandrosterone sulfate, basal 11-deoxycortisol levels, and basal and ACTH-stimulated Δ4-
androstenedione and 17-hydroxyprogesterone concentrations. Serum SHBG levels were decreased in patients compared with controls, whereas no differences were observed in E2, LH, FSH, basal cortisol, and ACTH-stimulated cortisol and 11-deoxycortisol levels among these groups. Patients presented with higher fasting insulin levels and tended to have higher FIRI and lower fasting glucose compared with controls.

Study protocol and hormone profiles

Studies were performed between d 5 and 10 of the menstrual cycle, or during amenorrhea, after excluding pregnancy by proper testing. Between 0800–0900 hr after a 12-h overnight fast, an indwelling iv line was placed in a forearm vein, and after 15–30 min, basal blood samples were obtained for the measurement of IL-6, total T, Δ4-androstenedione, 17-hydroxyprogesterone, dehydroepiandrosterone sulfate, LH, FSH, E2, SHBG, glucose, and insulin. Immediately after obtaining basal samples, a 250-μg iv bolus of ACTH (1–24) (Syna
cthen, Ciba-Geigy, Basle, Switzerland) was injected, and blood samples were obtained at 0 and 60 min for the measurement of cortisol, 11-deoxycortisol, 17-hydroxyprogesterone, and Δ4-androstenedione. Samples were immediately centrifuged, and serum was separated and frozen at −20°C until assayed.

Serum IL-6 levels were measured by ELISA (Cytoscreen Human Interleukin-6 UltraSensitive Immunoassay Kit, Biosource International, Camarillo, CA) with a lower limit of detection of 0.104 pg/ml and mean intra- and interassay coefficients of variation of 6.4% and 7.8%, respec-
tively. The technical characteristics of the assays employed for hormone measurements have been reported previously (26–28). The free T con-
centration was calculated from total T and SHBG concentrations, as-
suming a serum albumin concentration of 43 g/liter and taking a value of 1 × 10⁶ liters/mol for the association constant of SHBG for total T and a value of 3.6 × 10⁶ liters/mol for that of albumin for total T (29). Insulin resistance in the fasting state was estimated from glucose and insulin levels using the fasting insulin resistance index [FIRI = glucose (mmol/liter) × insulin (mU/liter)/25] (17).

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Also, we sequenced several non-GGG haplotypes confirming the constant associations with the −373A>T haplotype described by Terry et al. (30): AGC with A5T12, GCG with A10T16 and GGC with A3T11. This result suggests that the association of GGG haplotypes with hyperandrogenism and of AGC haplotypes with controls actually depends on the individual biallelic polymorphisms at positions −597 and −174, rather than being determined by the different haplotypes.

When studied separately, the −597G→A and −174G→C polymorphisms were in linkage disequilibrium ($\chi^2 = 152.20; P < 0.0001$; only 6.4% of alleles had a G at −597 and a C at position −174, and there were no alleles showing an A at position −597 and a G at position −174) and were associated with patient or control status. Allele frequencies for the −597G→A polymorphism were different in patients and in controls (Fig. 2; $\chi^2 = 11.398; P < 0.003$). G alleles were more frequent in patients either considering subjects homozygous for G alleles separately, or considering subjects homozygous and heterozygous for G alleles as a whole ($\chi^2 = 9.972; P < 0.003$ and $\chi^2 = 4.712; P < 0.05$, respectively). Homozygosity for G alleles was especially frequent in patients presenting with the polycystic ovary syndrome and in patients with idiopathic hirsutism (polycystic ovary syndrome, 60.0%; hyperandrogenemic hirsutism, 35.0%; idiopathic hirsutism, 53.3%; controls, 12.0%; $\chi^2 = 14.774; P < 0.003$).

As expected from the linkage disequilibrium with the −597G→A polymorphism, similar results were observed when studying allele frequencies of the −174G→C polymorphism. Frequencies were different in patients and controls (Fig. 2; $\chi^2 = 9.035; P < 0.02$). G alleles were more frequent in patients either considering subjects homozygous
for G alleles separately or considering subjects homozygous and heterozygous for G alleles as a whole ($\chi^2 = 7.457; P < 0.01$ and $\chi^2 = 4.153; P < 0.05$, respectively). Homozygosity for G alleles was especially frequent in patients presenting with the polycystic ovary syndrome and in patients with idiopathic hirsutism (polycystic ovary syndrome, 43.3%; hyperandrogenemic hirsutism, 27.5%; idiopathic hirsutism, 46.7%; controls, 8.0%; $\chi^2 = 10.405; P < 0.02$). Obviously, controls showed the A allele at $-597$ and the C allele at $-174$ more frequently than patients (Fig. 2).

On the contrary, the $-572$G→C polymorphism was equally distributed in patients and healthy controls; C alleles at position $-572$ were carried by 10.6% of patients and 12.0% controls ($\chi^2 = 0.428; P = 0.807$).

**Influence of the $-597$G→A and $-174$G→C polymorphisms in the promoter of IL-6 on clinical and biochemical variables**

The influence of the $-597$G→A and $-174$G→C polymorphisms on clinical and biochemical characteristics related to hyperandrogenism and insulin resistance was tested in the control group of 25 healthy women to avoid a possible predetermination of the results by the increased frequency of G alleles in patients (i.e., because G alleles are more frequent in patients and patients have clinical and biochemical characteristics related to disease, including patients in these analyses predetermine an association of G alleles with disease traits).

The presence of $-597$G and $-174$G alleles was related to increased circulating IL-6, basal cortisol, 11-deoxycortisol, and 17-hydroxyprogesterone levels, and a near-significant tendency to higher total T levels (Table 2). No differences were found in other clinical and biochemical variables, including body mass index and insulin resistance (Table 2).

**TABLE 2. Influence of the $-597$G→A and $-174$G→C polymorphisms on clinical and biochemical variables in the control group of 25 healthy women**

<table>
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<tbody>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>25.1 ± 8.2</td>
<td>25.8 ± 4.8</td>
<td>27.7 ± 3.7</td>
<td>29.2 ± 6.0</td>
<td>25.4 ± 5.3</td>
<td>27.0 ± 4.0</td>
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<tr>
<td>Age (yr)</td>
<td>24.0 ± 6.9</td>
<td>31.9 ± 7.7</td>
<td>30.6 ± 9.3</td>
<td>29.0 ± 8.5</td>
<td>31.9 ± 7.7</td>
<td>30.3 ± 8.6</td>
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<tr>
<td>Hirsutism score</td>
<td>0.5 ± 0.7</td>
<td>1.6 ± 1.7</td>
<td>2.4 ± 1.5</td>
<td>0.5 ± 0.7</td>
<td>1.6 ± 1.8</td>
<td>2.4 ± 1.4</td>
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<td>Fasting insulin (pmol/liter)</td>
<td>64 ± 66</td>
<td>62 ± 31</td>
<td>72 ± 22</td>
<td>94 ± 58</td>
<td>59 ± 33</td>
<td>69 ± 24</td>
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<tr>
<td>Fasting glucose (mmol/liter)</td>
<td>4.1 ± 0.4</td>
<td>4.8 ± 0.6</td>
<td>4.9 ± 0.4</td>
<td>3.9 ± 0.3</td>
<td>4.8 ± 0.5</td>
<td>4.8 ± 0.7</td>
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<tr>
<td>Fasting insulin resistance index (mmol/MU/liter$^{-2}$)</td>
<td>1.46 ± 1.58</td>
<td>1.72 ± 0.87</td>
<td>2.08 ± 0.71</td>
<td>2.14 ± 1.47</td>
<td>1.67 ± 0.95</td>
<td>1.92 ± 0.79</td>
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<tr>
<td>IL-6 (pg/ml)</td>
<td>1.38 ± 0.56</td>
<td>0.98 ± 0.4</td>
<td>0.49 ± 0.13</td>
<td>1.39 ± 0.79</td>
<td>1.00 ± 0.43</td>
<td>0.56 ± 0.22</td>
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<tr>
<td>Total T (nmol/liter)</td>
<td>1.9 ± 0.2</td>
<td>1.4 ± 0.5</td>
<td>1.2 ± 0.4</td>
<td>1.9 ± 0.3</td>
<td>1.5 ± 0.5</td>
<td>1.2 ± 0.4</td>
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<tr>
<td>Free T (nmol/liter)</td>
<td>21 ± 3</td>
<td>16 ± 7</td>
<td>19 ± 8</td>
<td>22 ± 1</td>
<td>16 ± 7</td>
<td>18 ± 8</td>
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<td>SHBG (nmol/liter)</td>
<td>71 ± 15</td>
<td>75 ± 24</td>
<td>43 ± 9</td>
<td>66 ± 16</td>
<td>73 ± 22</td>
<td>51 ± 26</td>
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<tr>
<td>Dehydroepiandrosterone sulfate (nmol/liter)</td>
<td>8.0 ± 2.0</td>
<td>5.1 ± 2.5</td>
<td>5.2 ± 2.9</td>
<td>9.2 ± 0.4</td>
<td>5.1 ± 2.5</td>
<td>5.1 ± 2.7</td>
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<tr>
<td>Basal Δ4-androstendione (nmol/liter)</td>
<td>11.9 ± 3.1</td>
<td>8.7 ± 3.5</td>
<td>8.4 ± 3.1</td>
<td>12.9 ± 3.5</td>
<td>8.7 ± 3.5</td>
<td>8.7 ± 2.8</td>
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<tr>
<td>ACTH-stimulated Δ4-androstendione (nmol/liter)</td>
<td>13.3 ± 2.8</td>
<td>11.9 ± 3.8</td>
<td>11.9 ± 4.2</td>
<td>13.6 ± 3.8</td>
<td>11.5 ± 3.8</td>
<td>12.2 ± 4.2</td>
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<tr>
<td>Basal 17-hydroxyprogesterone (nmol/liter)</td>
<td>3.9 ± 1.2</td>
<td>2.1 ± 1.2</td>
<td>1.5 ± 0.6</td>
<td>4.5 ± 1.5</td>
<td>2.1 ± 1.2</td>
<td>1.8 ± 0.6</td>
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<tr>
<td>ACTH-stimulated 17-hydroxyprogesterone (nmol/liter)</td>
<td>9.7 ± 3.6</td>
<td>7.3 ± 3.9</td>
<td>7.6 ± 3.0</td>
<td>7.8 ± 1.2</td>
<td>7.5 ± 4.2</td>
<td>7.8 ± 3.0</td>
</tr>
<tr>
<td>Basal cortisol (nmol/liter)</td>
<td>579 ± 276</td>
<td>414 ± 110</td>
<td>276 ± 83</td>
<td>690 ± 276</td>
<td>414 ± 110</td>
<td>303 ± 110</td>
</tr>
<tr>
<td>ACTH-stimulated cortisol (nmol/liter)</td>
<td>910 ± 359</td>
<td>828 ± 163</td>
<td>773 ± 128</td>
<td>1076 ± 359</td>
<td>800 ± 110</td>
<td>855 ± 221</td>
</tr>
<tr>
<td>Basal 11-deoxycortisol (nmol/liter)</td>
<td>9.2 ± 4.9</td>
<td>5.5 ± 2.0</td>
<td>4.3 ± 2.0</td>
<td>11.8 ± 2.0</td>
<td>5.2 ± 1.7</td>
<td>4.9 ± 2.6</td>
</tr>
<tr>
<td>ACTH-stimulated 11-deoxycortisol (nmol/liter)</td>
<td>14.2 ± 4.3</td>
<td>12.1 ± 6.1</td>
<td>13.3 ± 5.5</td>
<td>16.5 ± 0.3</td>
<td>10.7 ± 4.0</td>
<td>15.0 ± 7.5</td>
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<tr>
<td>LH (IU/liter)</td>
<td>1.5 ± 0.4</td>
<td>7.1 ± 4.4</td>
<td>3.2 ± 1.9</td>
<td>1.7 ± 0.3</td>
<td>6.6 ± 4.5</td>
<td>3.2 ± 1.9</td>
</tr>
<tr>
<td>FSH (IU/liter)</td>
<td>3.3 ± 0.1</td>
<td>9.8 ± 11.9</td>
<td>5.8 ± 2.1</td>
<td>3.2 ± 0.1</td>
<td>9.3 ± 11.5</td>
<td>5.8 ± 2.1</td>
</tr>
</tbody>
</table>

Results are the means ± SD.
The −597G and −174G alleles did not influence any clinical and biochemical parameter when studying the patients separately (data not shown).

**Impact of obesity on serum IL-6 and hormone concentrations and lack of relationship of obesity with IL-6 polymorphisms**

The comparisons included 75 lean subjects and 35 obese individuals. As stated above, obesity was defined by a body mass index higher than 27 kg/m². For these analyses patients and controls were considered as a whole. Lean subjects were younger (lean, 22.7 ± 5.7 yr; obese, 28.5 ± 9.9 yr; \( P < 0.003 \)) and presented with lower body mass index (lean, 22.7 ± 2.4 kg/m²; obese, 30.2 ± 2.2 kg/m²; \( P < 0.001 \)), fasting insulin (lean, 72 ± 44 pmol/liter; obese, 106 ± 44 pmol/liter; \( P < 0.001 \)), glucose (lean, 4.4 ± 0.5 mmol/liter; obese, 4.8 ± 0.5 mmol/liter; \( P < 0.004 \)), FIRI (lean, 1.85 ± 1.20 mmol/mU-liter⁻²; obese, 2.92 ± 1.24 mmol/mU-liter⁻²; \( P < 0.001 \)), and serum IL-6 (lean, 0.75 ± 0.36 pg/ml; obese, 1.05 ± 0.56 pg/ml; \( P < 0.007 \)) compared with obese subjects. Also, total T levels (lean, 2.1 ± 0.7 nmol/liter; obese, 1.7 ± 0.8 nmol/liter; \( P < 0.02 \)), SHBG levels (lean, 49 ± 25 nmol/liter; obese, 38 ± 24 nmol/liter; \( P < 0.03 \)), and hirsutism scores (lean, 14.2 ± 7.0; obese, 10.2 ± 7.8; \( P < 0.02 \)) were higher in lean subjects compared with obese individuals. No difference in any other clinical or biochemical variable was found (data not shown).

None of the IL-6 polymorphisms studied here was associated with obesity (−597G→A: \( \chi^2 = 0.106; P = 0.948 \); −572G→C: \( \chi^2 = 1.475; P = 0.478 \); −174G→C: \( \chi^2 = 0.744; P = 0.689 \); −373A/35T: \( \chi^2 = 0.408; P = 0.815 \)).

However, because of the profound effect of obesity on serum IL-6 concentrations, we decided to study the influence of IL-6 polymorphisms on serum IL-6 levels depending on the presence or absence of obesity. Two-way ANOVA, including serum IL-6 levels as a dependent variable and obesity and IL-6 polymorphisms as independent variables, showed that in obese subjects serum IL-6 levels were increased in subjects homozygous or heterozygous for G alleles at positions −597 and −174 compared with subjects homozygous for A and C alleles at these positions of IL-6 (Fig. 3). This difference was not found in lean subjects (Fig. 3).

**Discussion**

From an evolutionary perspective, subjects with a special ability to reproduce early in life, gain weight when food is scarce and available, and respond to injury with a brisk inflammatory response have a survival advantage. However, this advantage might be lost when subjects are exposed to environmental changes, such as the present lifestyle in Western countries where access to food is not restricted and life expectancy is long for these individuals to develop complications from these earlier beneficial adaptive characteristics.

Recently, Fernandez-Real and Ricart (8) hypothesized that certain cytokine genotype and phenotype combinations that facilitate inflammation and insulin resistance and are related to common endocrine disorders such as obesity, type 2 diabetes mellitus, dyslipidemia, atherosclerosis, and arterial hypertension (8, 13, 14, 16, 31, 32) might have been selected during evolution.

Considering the high prevalence of hyperandrogenic disorders in women (1, 2), the fact that hyperandrogenism might represent per se a survival advantage during evolution (5), and the frequent association of hyperandrogenism with the metabolic disorders cited above (33), we decided to study the possible role of inflammatory cytokines in the pathogenesis of hyperandrogenism.

Our present results suggest that two common polymorphisms in the regulatory region of IL-6, which are in linkage disequilibrium, are related to hyperandrogenism. On the one hand, hyperandrogenism was associated with the presence of G alleles at the −597G→A and −174G→C polymorphisms, or conversely, subjects homozygous for A and C alleles at these positions were protected against the development of androgen excess. Further, subjects carrying G alleles at −597 and −174 positions of IL-6 presented with higher serum IL-6 and 17-hydroxypregesterone levels and relatively hyperactive adrenal axis, as suggested by the increased serum cortisol and 11-deoxycorticisol levels. Moreover, there was a near-significant tendency (\( P < 0.1 \)) for increased serum total T levels in these subjects, suggesting a
with increased serum IL-6 levels compared with subjects/H11002jects, carriers of G alleles at position (44). Furthermore, in a series of 102 healthy Caucasian sub-
androsterone (37).
secretion of mineralocorticoids, cortisol, and dehydroepi-
adrenal function are well known (36), resulting in increased lipid metabolism (35). The stimulatory effects of IL-6 on pituitary-adrenal axis, stimulation of vasopressin and GH secretion, suppression of the thyroid axis, and modulation of lipids metabolism (35). The stimulatory effects of IL-6 on adrenal function are well known (36), resulting in increased secretion of mineralocorticoids, cortisol, and dehydroepi-
androsterone (37).

Recently, IL-6 has been proposed to play a role in the pathogenesis of obesity, insulin resistance, and atherosclerosis (38). Approximately 15–35% of circulating IL-6 is se-
creted by adipose tissue (39), explaining the increased serum IL-6 levels associated with obesity and the subsequent decrease in circulating IL-6 observed in obese subjects after weight loss (17). Also, serum IL-6 levels correlate with insulin resistance and blood pressure in healthy men even after controlling for body mass index (32).

IL-6 is also expressed at the ovary, but little is known regarding its role, if any, in the regulation of ovarian function. Both granulosa and theca cells from the rabbit produce IL-6 in vitro, modulating gonadotropin-induced progester-
one secretion (40). Similar results have been described for human granulosa cells (41, 42). Also, IL-6 appears to mediate the angiogenic process associated with follicle development (43). To our knowledge, the possible role of IL-6 in the reg-
ulation of ovarian androgen secretion has not been studied. However, because IL-6 stimulates androgen synthesis in the adrenal gland (37), which shares most steroidogenic en-
zymes with theca cells, the possibility exists that IL-6 might also stimulate androgen synthesis in the ovary. This hypothesis might explain at least partly the association of polymorphisms in the promoter of IL-6 with hyperandrogenism, a condition characterized by increased androgen secretion at the adrenal and/or the ovary.

Because of the rapid plasma clearance of IL-6, its circu-
lating levels are mainly regulated at the level of expression (30). Polymorphisms in the regulatory region of IL-6 mod-
ulate transcription and expression and may determine cir-
culating IL-6 levels (30). The influence of the −174G→C polymorphism on the expression and serum levels of IL-6 has been recently studied (44). Transient transfection assays into HeLa cells showed a much higher expression of a −174G construct compared with a −174C construct, both unstimu-
lated and stimulated with lipopolysaccharide or IL-1 (44). Both constructs contained the A/C/T/C sequence usually asso-
ciated with the C allele to avoid any confounding effects of a different A/C/T/C allele between the two reporter constructs (44). Furthermore, in a series of 102 healthy Caucasian sub-
jects, carriers of G alleles at position −174 of IL-6 presented with increased serum IL-6 levels compared with subjects homozygous for C alleles at this position (44) in agreement with our present results.

More recently, Terry et al. (30) suggested that the four polymorphisms in the promoter of IL-6 studied here influ-
cenced the regulation of IL-6 transcription not by a simple additive mechanism, but through complex interactions de-
termined by the haplotype. Although they could not demon-
strate any difference in transfection assays using HeLa cells, in ECV304 cells transfected with different constructs, and after treatment with IL-1, the GG(A/G/T)G haplotype resulted in increased luminescence compared with GG(A/G/T/G), GG(A/G/T/G), GC(A/G/T/G), AG(A/G/T/G), and AG(A/G/T/G) constructs (30).

Our present results suggest that the −597G→A and −174G→C polymorphisms in IL-6, which are in linkage dis-
equilibrium, are associated with hyperandrogenism and polycystic ovary syndrome and influence serum IL-6 and hormone concentrations. As a result, subjects homozygous for A and C alleles at these positions appear to be protected against androgen excess. On the contrary, we could not de-
tect any influence of the −572G→C and −373A/C poly-
morphisms on any clinical or biochemical variable studied here, but we have only studied the −373A/C, polymorphism in 40 subjects heterozygous for the GGG haplotype.

Furthermore, our results also provide indirect evidence for several functional roles of the −597G→A and −174G→C polymorphisms in the promoter of IL-6. In our series, and especially in obese subjects, G alleles were associated with higher serum IL-6 levels, suggesting that these polymor-
phisms increase IL-6 secretion in vivo, an action that appears to be amplified by obesity.

Moreover, in healthy women, carriers of G alleles showed increased basal cortisol and 11-deoxy cortisol levels, suggest-
ing adrenal hyperactivity that may result from increased IL-6 secretion (35). As stated above adrenal hyperactivity is fre-
cently found in the polycystic ovary syndrome (11) and in hyperandrogenic patients (34). Further, G alleles might in-
fluence hyperandrogenism, as healthy carriers of G alleles tended to have higher serum total T concentrations and had higher 17-hydroxy progesterone levels. 17-Hydroxyprogester-
one is produced in the adrenal and the ovary, and its serum levels are usually increased in hyperandrogenic patients, including women presenting with the polycystic ovary syn-
drome (45, 46). Our previous data demonstrating a decrease in 17-hydroxyprogesterone levels in hyperandrogenic women after administration of a long-acting GnRH analog suggest an ovarian origin of 17-hydroxyprogesterone in these patients (47).

The fact that these functional roles for IL-6 polymorphisms arise from the study of healthy controls rules out significant ascertainment bias, further suggesting that inflammatory cy-
tokines may be involved in the regulation of adrenal and, possibly, ovarian function. Although these results should be interpreted with caution because of the multiple clinical and biochemical variables compared in this analysis, the highly significant differences found in some of these variables merit further consideration because of the small number of subjects included in the comparisons.

However, when restricting the analysis to our series of hyperandrogenic patients, separately from the controls, we
did not find any difference in clinical and biochemical variables depending on the IL-6 genotype. This result suggests that although IL-6 polymorphisms may contribute to hyperandrogenism, other etiological factors are involved. As with other complex metabolic disorders, hyperandrogenism possibly results from the interaction of several environmental factors with multiple genetic variants, suggesting a polygenic model of inheritance.

Hyperandrogenism might be considered a low grade chronic inflammatory disorder, a hypothesis supported by the very recent finding of increased C-reactive protein, nonspecific inflammatory marker, in women presenting with polycystic ovary syndrome (48). Moreover, genomic variants in IL-6 and in the TNFα gene (21) might contribute to this inflammatory response considering that serum IL-6 and C-reactive protein levels have been shown to be correlated in previous studies (17, 32).

Serum IL-6 levels and the IL-6 polymorphisms studied here were not related to insulin resistance, and only a weak correlation with body mass index was found. We observed no differences in fasting insulin concentrations or in insulin resistance, depending on the −597G→A and −174G→C polymorphisms in IL-6 promoter. Our results disagree with those of a previous study performed in a small series of 32 subjects by Fernández-Real et al. (13), who found higher insulin levels and a lower insulin sensitivity index in carriers of G alleles for the −174G→C polymorphism.

Also, we have not found the strong correlation between serum IL-6 concentrations and the FIRI described by Bastard et al. (17). These researchers included healthy lean and obese subjects and type 2 diabetic subjects in their study, whereas the subjects included in our study were only moderately obese and did not have diabetes. By covering a wide range in the degree of insulin resistance and obesity, the study by Bastard et al. (17) was possibly more appropriate than our present experimental design to uncover the correlation between serum IL-6 and the FIRI. Nevertheless, a very recent study by Fernández-Real et al. (32), performed in a large series of 228 healthy subjects, ruled out any significant relationship between insulin sensitivity and serum IL-6 concentrations in healthy women, in conceptual agreement with our present results.

In conclusion, we report a novel association of hyperandrogenism with the −597G→A and −174G→C polymorphisms in the promoter of IL-6. This association is independent of insulin resistance and is modulated by obesity. Our data suggest that these genotypes might be associated with increased IL-6 expression and secretion, resulting in IL-6-mediated stimulation of the adrenal gland and, possibly, of ovarian steroidogenesis.

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