Association of Polymorphisms in the Interleukin 6 Receptor Complex with Obesity and Hyperandrogenism

Héctor F. Escobar-Morreale, Rosa M. Calvo,* Gemma Villuendas,* José Sancho,* and José L. San Millán†*

Abstract

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Objective: Interleukin-6 (IL-6), is an inflammatory cytokine that may influence the pathogenesis of obesity and hyperandrogenism. IL-6 exerts its actions through a heterodimeric receptor consisting of two membrane-bound glycoproteins: an 80-kDa IL-6 binding unit (IL6R-α) and a 130-kDa IL-6 signal transducer (gp130). Genetic variability at these loci might contribute to explain the development of obesity and hyperandrogenism.

Research Methods and Procedures: We have evaluated the possible association of several polymorphisms in the *IL6R-α* and *gp130* genes with obesity and/or hyperandrogenism in a case-control study involving 143 hyperandrogenic patients and 45 healthy women from Spain.

Results: A microsatellite CA-repeat polymorphism in the IL6R-α locus was associated with obesity. The frequency of the common 149-bp allele was markedly increased in obese women compared with controls when considering patients and controls as a whole (0.41 vs. 0.29, $\chi^2 = 17.085$, $p < 0.050$). On the other hand, the uncommon Arg148 allele of the Gly148Arg polymorphism in the *gp130* gene was more frequent in controls compared with hyperandrogenic patients (0.17 vs. 0.08, $\chi^2 = 5.605$, $p = 0.026$). Controls carrying Arg148 alleles had lower 11-deoxycortisol and 17-hydroxyprogesterone concentrations, a lower response of androstenedione to 1–24 adrenocorticotropin, and an almost significant decrease in free testosterone levels, suggesting that Arg148 alleles in the *gp130* gene have a protective effect against androgen excess and adrenal hyperactivity.

Discussion: Polymorphisms in the gp130 and IL6R-α loci influence hyperandrogenism and obesity, respectively. Our present results further suggest that proinflammatory genotypes are involved in the pathogenesis of these common metabolic disorders.

Key words: IL6R-α, gp130, insulin resistance, polycystic ovary syndrome, metabolic syndrome

Introduction

Hyperandrogenism and polycystic ovary syndrome (PCOS)[1] are possibly the most common endocrine disorders in women of fertile age (1). PCOS, defined by endocrine criteria, is present in ~6.5% of women from Spain (2). Familial aggregation of PCOS and hyperandrogenism provide evidence suggesting a genetic basis for these disorders (3). However, their precise genetic mechanisms remain unknown despite significant efforts.

We have recently hypothesized that inflammatory cytokines may be involved in the pathogenesis of hyperandrogenism, as has been proposed for insulin resistance, type 2 diabetes, obesity, and the metabolic syndrome (4). These common genetic disorders might result from adaptive changes that, from an evolutionary perspective, favor short-term survival.

Proinflammatory genotypes might have been selected during evolution because a high cytokine responder phenotype may provide survival advantages. Among others, these inflammatory genotypes and phenotypes may facilitate de-

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*Department of Endocrinology and †Department of Molecular Genetics, Hospital Ramón y Cajal, Madrid, Spain.
Address correspondence to Héctor F. Escobar-Morreale, Department of Endocrinology, Hospital Ramón y Cajal, Carretera de Colmenar km. 9,100, 28034 Madrid, Spain.
E-mail: hector.escobar@uam.es

[1] Nonstandard abbreviations: PCOS, polycystic ovary syndrome; IL, interleukin; TNF, tumor necrosis factor.

fense against injury and infection, and may induce insulin resistance, preserving glucose for brain metabolism, and providing defense against starvation during periods of food shortage. However, these adaptive mechanisms are no longer beneficial when subjects are exposed to the marked increase in caloric intake and the sedentary habits associated with present Western lifestyle, leading to disease.

The rapid maturation of the reproductive axis found in hyperandrogenic 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 hyperandrogenic women could increase the interval between pregnancies, decreasing the birth rate and favoring maternal and infant survival (5).

The recent finding of increased C-reactive protein levels in women with PCOS (8) suggests that chronic inflammation might be involved in the pathogenesis of hyperandrogenic disorders. Moreover, several of our previous studies supported the association of hyperandrogenism with proinflammatory genotypes: 1) the −308A allele in the promoter of tumor necrosis factor (*TNF*)-α gene is associated with increased serum androgens in hyperandrogenic and healthy women independently of obesity and insulin resistance (9); 2) the 196R alleles in exon 6 of the *TNFRSF1B* gene—which encodes type 2 TNF receptor—are associated with hyperandrogenism and PCOS (10); and 3) the −597G and −174G alleles in the promoter of the interleukin-6 (IL-6) gene, which are in linkage disequilibrium, are associated with hyperandrogenism (11).

There is also mounting evidence that obesity, which is frequently found in hyperandrogenic patients (12), is also associated with proinflammatory genotypes and phenotypes (13,14). Accordingly, IL-6 seems to influence the pathophysiology of obesity and the metabolic syndrome (15,16). Of note, we have recently shown that, considering healthy and hyperandrogenic women as a whole, those homozygous for the uncommon −597A or −174C alleles in the promoter of the *IL-6* gene are protected against the increase in serum IL-6 induced by obesity (11).

IL-6 exerts its actions through a heterodimeric receptor consisting of two membrane-bound glycoproteins: an 80-kDa IL-6 binding unit (IL6R-α or gp80) and a 130-kDa IL-6 signal transducer (IL6ST, IL6R-β, or gp130). The latter is a transducer chain shared by other cytokines that is responsible for signal transduction of the α chain-ligand complex through the Janus kinase/signal transducer and activator of transcription pathway (17).

To further delineate the contribution of the IL-6 system to the pathogenesis of obesity and hyperandrogenism, we have studied the possible association of these metabolic disorders with several polymorphisms in the IL6R-α and gp130 loci.

Research Methods and Procedures

Subjects

One hundred forty-three hyperandrogenic patients (age, 24.0 ± 6.5 years; BMI, 28.6 ± 8.0 kg/m^2) and 45 healthy nonhyperandrogenic women (age, 30.8 ± 8.1 years; BMI, 28.1 ± 7.6 kg/m^2) were studied. 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.

Eighty-eight patients presented with PCOS, defined by oligo-ovulation, clinical and/or biochemical hyperandrogenism, and exclusion of hyperprolactinemia (serum prolactin below 24 μg/L); nonclassic congenital adrenal hyperplasia [adrenocorticotropin-stimulated 17-hydroxyprogesterone levels below 30 nM (18)]; and androgen-secreting tumors (19). In these patients, evidence for oligo-ovulation was provided by chronic oligomenorrhea, by luteal phase progesterone below 12.7 nM, or by basal body temperature charts.

Fifty-five patients had hyperandrogenic hirsutism, defined by hirsutism, increased serum androgen levels, regular menstrual cycles, and no definite proof of oligo-ovulation. Because previous studies by others suggest that PCOS and hyperandrogenemia cosegregate in hyperandrogenic families (3) and because the associations between proinflammatory genotypes and hyperandrogenism were not restricted to PCOS in our previous studies (10,11), we decided to study women with PCOS and with hyperandrogenic hirsutism as a whole.

We excluded women presenting with idiopathic hirsutism (regular menstrual cycles and normal serum androgen concentrations), because previous studies of our group suggested a specific genetic etiology (20).

Data from some patients and controls, regarding different aspects of the pathophysiology of hirsutism, have been previously published (9–11,20,21). Particularly, 85 patients and 25 controls were studied for several polymorphisms in the promoter of the *IL-6* gene (11).

The patients and controls had not taken hormonal medications, including contraceptive pills, for the last 6 months. All the subjects were white.

The ethics committee of the Hospital Ramón y Cajal approved the study, and informed consent was obtained from each patient and control.

Study Protocol and Hormone Profiles

Studies were performed between days 5 and 10 of the menstrual cycle, or during amenorrhea, after excluding pregnancy by proper testing. Between 8:00 AM and 9:00 AM,

Table 1. Primers and conditions for polymerase chain reaction, amplification, and size of the amplified fragments

Gene	Primers	Annealing temperature	Mg^{2+} (mM)	Size (bp)
IL6R-α	Forward: HEX-5′-GGC AAC CGA GCA AGA CTC TC-3′	56°C	1.5	149 to 167
	Reverse: 5′-GCG AGG ACA GAA GAT TTG TC-3′			
gp130				
Val8Leu	Forward: 5′-TTT AGA AAC ACA GCT ACA GC-3′	48°C	2.0	155
	Reverse: 5′-GAT AGG GTA TTT CAT GAA CC-3′			
Ser122Ser	Forward: 5′-TAT GGA ATC ACA ATA ATT **C**C-3′*	56°C	1.5	121
	Reverse: 5′-TTT AAA AAC CAA ACA TGT CC-3′			
Gly139Gly, *Lys140Asn*, and *Gly148Arg*	Forward: 5′-TGC CTC CAG AAA AAC CTA AA-3′	56°C	1.5	203
	Reverse: 5′-CAT TCA GAT TTT AAA GTG AAG-3′			
Val392Asp	Forward: 5′-AGT TTC AGA GAT GCA TTA GC-3′	56°C	1.5	149
	Reverse: 5′-CAT TTG CTT CTA TTT CCA C**G**-3′†			

Bold letters were introduced to create a **Bst*N*I restriction site and a †*Mae*III restriction site.

after a 12-h overnight fast, an indwelling intravenous line was placed in a forearm vein, and after 15 to 30 minutes, basal blood samples were obtained for the measurement of gp130, total testosterone, dehydroepiandrosterone-sulfate, luteinizing hormone, follicle-stimulating hormone, estradiol, sex hormone-binding globulin, glucose, and insulin. Immediately after taking basal samples, a 250-μg intravenous bolus of 1–24 adrenocorticotropin (Synacthen; Ciba-Geigy, Basle, Switzerland) was injected, and blood samples were obtained at 0 and 60 minutes for the measurement of cortisol, 11-deoxycortisol, 17-hydroxyprogesterone, and Δ^4-androstenedione. The adrenocorticotropin test was not performed in six control women, but their basal samples were assayed for serum cortisol and Δ^4-androstenedione in addition to the basal determinations described above. Samples were immediately centrifuged, and serum was separated and frozen at −20 °C until assayed.

In 84 patients and 35 controls, serum gp130 levels were measured by enzyme-linked immunosorbent assay kit [Human Soluble IL-6 Receptor β chain (sgp130); Biosource International, Camarillo, CA], with a lower limit of detection of 18.5 pg/mL, and mean intra- and interassay CVs of 4% and 7%, respectively. The technical characteristics of the assays employed for hormone measurements have been reported elsewhere (21–23). The free testosterone concentration was calculated from total testosterone and sex hormone-binding globulin concentrations, assuming a serum albumin concentration of 43 g/L and taking a value of 1×10^9 L/mol for the association constant of sex hormone-binding globulin for total testosterone and a value of 3.6×10^4 L/mol for that of albumin for total testosterone (24). Insulin resistance in the fasting state was estimated from glucose and insulin levels using the fasting insulin resistance index [FIRI = glucose (mM) × insulin (mU/L)/25] (25).

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, Madison, WI; and Nucleon BAC C3; Amersham Pharmacia, Buckinghamshire, UK).

The CA-repeat polymorphism at the IL6R-α locus was amplified by polymerase chain reaction as reported by Tsukamoto et al. (26), but the forward primer was labeled with HEX fluorescent dye (Table 1). Each polymerase chain reaction product was diluted individually and mixed with deionized formamide and an *N*,*N*,*N*′,*N*′-tetramethyl-6-carboxyrhodamine fluorescent molecular weight marker as an internal control (Applied Biosystems, Foster City, CA). Each sample was denatured for 10 minutes at 96 °C and analyzed by capillary electrophoresis on an ABI 310A automated sequencer (Applied Biosystems). The size of the amplified fragments was determined by using the GeneScan software (Applied Biosystems). To obtain further confirmation about the location of this polymorphism, we sequenced the amplified fragment from one subject homozygous for the most common allele, and the sequence enclosing the CA-repeat polymorphism corresponded to that of intron 8 of IL6R-α.

A broad screening for polymorphisms in the gp130 was performed in at least 60 chromosomes (from 15 patients and 15 controls). The following polymorphisms in the *gp130*

gene were studied: 1) two conservative transitions at codons 122 (TCA to TCG change) and 139 (GGG to GGA) (27,28); 2) two nonconservative substitutions converting Lys to Asn (AAG to AAC) and Gly to Arg (GGT to CGT) at codons 140 and 148 (27,28), respectively; and 3) two different nonconservative changes transforming Val to Leu (GTA to CTA) at codon 8, and converting Val to Asp (GTT to GAT) at codon 392 (contig accession NT016864, dbSNP rs1063560 and dbSNP rs10180, respectively; http://www.ncbi.nlm.nih.gov/SNP/).

The gp130 polymorphisms were screened by restriction fragment length polymorphism analysis with the following endonuclease restriction enzymes: *Bst*NI for codon 122, *Mbo*II for codons 139 and 140, *Sau3*AI for codon 148, *Mae*I for codon 8, and *Mae*III for codon 392.

Amplifications were performed in an automated thermocycler (Gene Amp PCR System 2400; Applied Biosystems). Primers, final concentration of Mg^{2+}, and annealing temperatures are summarized in Table 1.

Finally, the −174G/C polymorphism in the promoter of the *IL-6* gene, which we found to be associated with hyperandrogenism in a subset of these patients and controls, was analyzed in the extended series as previously reported (11), to evaluate a possible collaborative effect of this variant with the gp130 polymorphism studied here.

Statistical Analysis

Results are expressed as means ± SD unless otherwise stated. The Kolmogorov-Smirnov statistic was applied to continuous variables. Logarithmic transformation was applied as needed to ensure normal distribution of the variables. Unpaired *t* tests, one-way ANOVA followed by the least significant difference test, and analysis of covariance were used to compare the central tendencies of the different groups as needed. To evaluate the association between discontinuous variables, we used the χ^2 test and Fisher's exact test as appropriate.

Logistic regression was used to analyze the Gly148Arg polymorphism in the *gp130* gene and the −174G/C polymorphism in the promoter of the *IL-6* gene and the interaction of both genomic variants, as predictive factors for hyperandrogenism in our model. The backward likelihood-ratio test was used as the method for variable selection (29). Analyses were performed using SPSS 10 for the Macintosh (SPSS Inc., Chicago, IL). $p < 0.05$ was considered statistically significant.

Results

Clinical, Biochemical, and Hormonal Variables

The comparison of clinical, biochemical, and hormonal variables between hyperandrogenic patients and controls is shown in Table 2. Soluble gp130 concentrations were not different in patients and controls. As expected, hyperandrogenic patients presented with increased hirsutism scores, serum free and total testosterone levels, and dehydroepiandrosterone concentrations. Basal and adrenocorticotropin-stimulated 17-hydroxyprogesterone and androstenedione concentrations and basal 11-deoxycortisol levels were also higher in patients compared with controls, whereas sex hormone-binding globulin and estradiol levels were decreased in patients compared with controls. Fasting insulin levels and the fasting insulin resistance index were increased in patients compared with controls. No differences were observed in fasting glucose, cortisol, and gonadotropin concentrations.

Polymorphisms in the gp130 Gene

Six different polymorphisms were studied in the *gp130* gene as stated above. We did not observe any variant in codons 8, 122, 139, 140, and 392 in the initial screening of 60 chromosomes from 15 patients and 15 controls, and therefore, we did not extend these analyses to include all the experimental subjects.

On the contrary, we found a high degree of heterozygosity in the Gly148Arg polymorphism at codon 148. The frequency of the uncommon Arg148 allele in the whole population was 0.10, and the Arg148 allele was more frequent in controls (0.17) compared with hyperandrogenic patients (0.08; $\chi^2 = 5.605$, $p = 0.026$). Of note, the same results were found when restricting the analysis to the subgroup of PCOS patients (0.17 in controls vs. 0.08 in PCOS patients, $\chi^2 = 4.653$, $p = 0.038$).

One patient and one control were homozygous for Arg148 alleles. Considering subjects homozygous and heterozygous for 148 alleles as a whole, carriers of one or two Arg148 alleles were more frequent in the controls compared with the hyperandrogenic group (0.31 vs. 0.15, $\chi^2 = 5.468$, $p = 0.029$).

Because the frequencies of Arg148 alleles was different in patients and in controls and to avoid ascertainment bias, the influence of having one or two Arg148 alleles on clinical and biochemical variables was studied separately in patients and in controls (Table 3). We found that, compared with controls homozygous for Gly148 alleles, those carrying Arg148 alleles had lower basal 11-deoxycortisol and 17-hydroxyprogesterone concentrations, a decreased response of androstenedione to adrenocorticotropin, and an almost significant decrease in free testosterone levels. No differences were found in the patients with the Gly148Arg genotype.

Logistic regression was used to analyze the possibility of a collaborative effect between the Gly148Arg polymorphism in the *gp130* gene and the −174G/C polymorphism in the promoter of the *IL-6* gene in the pathophysiology of hyperandrogenism. The association of the −174G alleles in the promoter of the *IL-6* gene with hyperandrogenism was confirmed in the extended series presented here. Con-

Table 2. Clinical and biochemical variables in hyperandrogenic patients and controls

	Patients	(*n*)	Controls	(*n*)	*p*
Hirsutism score	14.1 ± 6.4	(143)	1.5 ± 1.4	(45)	<0.001
Soluble gp130 (pg/mL)	225 ± 47	(84)	239 ± 62	(35)	0.483
Total testosterone (nM)	2.5 ± 0.9	(143)	1.4 ± 0.5	(45)	<0.001
Free testosterone (pM)	47 ± 27	(143)	19 ± 8	(45)	<0.001
Sex hormone-binding globulin (nM)	35 ± 19	(143)	59 ± 27	(45)	<0.001
Dehydroepiandrosterone-sulfate (μM)	7.8 ± 3.8	(143)	4.9 ± 2.4	(45)	<0.020
Basal cortisol (nM)	437 ± 164	(143)	403 ± 137	(42)	0.658
ACTH-stimulated cortisol (nM)	858 ± 190	(143)	861 ± 185	(36)	0.477
Basal 11-deoxycortisol (nM)	8.0 ± 4.6	(143)	5.5 ± 2.6	(36)	<0.050
ACTH-stimulated 11-deoxycortisol (nM)	13.3 ± 5.5	(143)	12.1 ± 4.9	(36)	0.678
Basal androstenedione (nM)	13.9 ± 5.0	(143)	9.0 ± 3.1	(45)	<0.001
ACTH-stimulated androstenedione (nM)	17.6 ± 5.5	(143)	12.2 ± 4.2	(36)	<0.001
Basal 17-hydroxyprogesterone (nM)	3.4 ± 2.1	(143)	2.1 ± 1.0	(39)	<0.020
ACTH-stimulated 17-hydroxyprogesterone (nM)	9.8 ± 5.3	(143)	7.6 ± 3.3	(36)	0.068
Luteinizing hormone (IU/L)	5.6 ± 3.4	(143)	5.2 ± 3.6	(45)	0.191
Follicle stimulating hormone (IU/L)	5.6 ± 3.5	(143)	6.5 ± 6.6	(45)	0.977
Estradiol (pM)	166 ± 98	(143)	254 ± 172	(45)	<0.020
Fasting insulin (pM)	103 ± 59	(143)	78 ± 45	(45)	<0.005
Fasting glucose (mM)	4.7 ± 0.6	(143)	4.9 ± 0.5	(45)	0.781
FIRI (mmol × mU)	2.9 ± 1.9	(143)	2.2 ± 1.4	(45)	<0.010

Data are means ± SD. Because patients were younger compared with controls, analysis were corrected for the difference in age by analysis of covariance. Values in parentheses indicate the number of subjects included in each comparison.
FIRI, fasting insulin resistance index.

versely, homozygosity for −174C alleles was more frequent in controls compared with hyperandrogenic patients (26.7% of controls vs. 11.4% of patients, $\chi^2 = 6.156$, $p = 0.018$).

The finding that carriers of Arg148 alleles of the *gp130* gene and that homozygosity for −174C alleles in the promoter of the *IL-6* gene were more frequent in controls compared with hyperandrogenic patients suggested a protective effect of these variants against the development of hyperandrogenism.

The dependent variable of the logistic regression model was coded 1 for hyperandrogenic patients and 0 for healthy controls. Carrying Arg148 alleles of the *gp130* gene, homozygosity for −174C alleles in the promoter of the *IL-6* gene, and the interaction of both were introduced as independent variables.

Independent variables explained only 8.1% (Nagelkerke's $R^2 = 0.081$) of the variation of the dependent variable (29), suggesting a modest contribution of the Gly148Arg polymorphism in the *gp130* gene and the −174G/C polymorphism in the promoter of the *IL-6* gene to the pathophysiology of hyperandrogenism.

Agreeing with their hypothetical protective influence, carrying Arg148 alleles of the *gp130* gene (odds ratio for hyperandrogenism 0.404, $p = 0.026$) and homozygosity for −174C alleles in the promoter of the *IL-6* gene (odds ratio for hyperandrogenism 0.368, $p = 0.022$) predicted significantly absence of hyperandrogenism (i.e., being a healthy control). However, no significant interaction was found between both polymorphisms ($p = 0.247$).

Polymorphism in the IL6R-α Gene

As stated above, we also studied a microsatellite CA-repeat polymorphism in the *IL6R-α* gene. We detected 10 alleles with sizes ranging from 149 to 167 bp in the 184 women (43 controls and 141 patients) who were genotyped for this polymorphism. Their size and frequencies are shown in Table 4. The distribution of alleles was not different in hyperandrogenic patients compared with healthy controls (Table 4).

Because in previous studies we found that serum IL-6 concentrations are increased in obese women (11), we studied a possible influence of the polymorphisms of the IL6R-α polymorphism with obesity (defined by a BMI ≥

Table 3. Influence of the Gly148Arg polymorphism in the *gp130* gene on clinical and biochemical variables, in hyperandrogenic patients and controls separately

	Controls					Patients				
	Gly148Gly	(*n*)	Gly148Arg + Arg148Arg	(*n*)	*p*	Gly148Gly	(*n*)	Gly148Arg + Arg148Arg	(*n*)	*p*
Age (years)	29.5 ± 7.9	(31)	33.8 ± 8.1	(14)	0.094	23.8 ± 6.4	(121)	25.2 ± 6.8	(22)	0.375
Body mass index (kg/m^2)	28.7 ± 8.1	(31)	26.8 ± 6.3	(14)	0.503	28.3 ± 7.8	(121)	30.3 ± 8.9	(22)	0.279
Hirsutism score	1.6 ± 1.4	(31)	1.5 ± 1.6	(14)	0.820	14.1 ± 6.2	(121)	14.4 ± 7.6	(22)	0.817
Soluble gp130 (pg/mL)	243 ± 70	(23)	232 ± 45	(12)	0.627	225 ± 48	(71)	225 ± 40	(13)	0.979
Total testosterone (nM)	1.5 ± 0.5	(31)	1.3 ± 0.4	(14)	0.186	2.4 ± 0.9	(121)	2.6 ± 1.2	(22)	0.313
Free testosterone (pM)	21 ± 9	(31)	16 ± 7	(14)	0.051	46 ± 23	(121)	53 ± 43	(22)	0.469
Sex hormone-binding globulin (nM)	55 ± 27	(31)	67 ± 25	(14)	0.132	35 ± 19	(121)	36 ± 21	(22)	0.927
Dehydroepiandrosterone-sulfate (μM)	5.1 ± 2.4	(31)	4.6 ± 2.5	(14)	0.536	7.9 ± 3.9	(121)	7.3 ± 3.3	(22)	0.461
Basal cortisol (nM)	403 ± 155	(29)	403 ± 94	(13)	0.978	447 ± 166	(121)	384 ± 148	(22)	0.097
ACTH-stimulated cortisol (nM)	869 ± 218	(24)	847 ± 102	(12)	0.827	867 ± 194	(121)	811 ± 164	(22)	0.212
Basal 11-deoxycortisol (nM)	6.1 ± 2.6	(24)	4.3 ± 2.0	(12)	<0.025	8.0 ± 4.5	(121)	8.1 ± 5.5	(22)	0.764
ACTH-stimulated 11-deoxycortisol (nM)	12.4 ± 5.2	(24)	11.3 ± 4.0	(12)	0.575	13.2 ± 5.4	(121)	13.9 ± 6.4	(22)	0.498
Basal androstenedione (nM)	9.5 ± 3.2	(31)	8.0 ± 2.9	(14)	0.131	14.1 ± 5.1	(121)	12.9 ± 4.0	(22)	0.357
ACTH-stimulated androstenedione (nM)	13.3 ± 4.5	(24)	10.5 ± 3.5	(12)	<0.001	17.7 ± 5.6	(121)	17.1 ± 5.0	(22)	0.692
Basal 17-hydroxyprogesterone (nM)	2.3 ± 1.1	(26)	1.6 ± 0.6	(13)	<0.020	3.5 ± 2.2	(121)	2.8 ± 1.7	(22)	0.159
ACTH-stimulated 17-hydroxyprogesterone (nM)	8.2 ± 3.9	(24)	6.7 ± 2.4	(12)	0.172	10.0 ± 5.5	(121)	9.0 ± 3.5	(22)	0.505
Luteinizing hormone (IU/L)	5.6 ± 3.9	(31)	4.5 ± 3.0	(14)	0.434	5.6 ± 3.4	(121)	5.5 ± 3.3	(22)	0.884
Follicle stimulating hormone (IU/L)	6.8 ± 8.1	(31)	5.8 ± 1.7	(14)	0.836	5.7 ± 3.7	(121)	5.5 ± 2.0	(22)	0.435
Estradiol (pM)	255 ± 188	(31)	252 ± 146	(14)	0.707	168 ± 101	(121)	151 ± 82	(22)	0.439
Fasting insulin (pM)	84 ± 47	(31)	64 ± 36	(14)	0.244	103 ± 57	(121)	100 ± 71	(22)	0.368
Fasting glucose (mM)	4.8 ± 0.6	(31)	4.8 ± 0.5	(14)	0.899	4.7 ± 0.6	(121)	4.7 ± 0.4	(22)	0.929
FIRI (mmol × mU/L^2)	2.4 ± 1.5	(31)	1.8 ± 1.0	(14)	0.293	2.9 ± 1.9	(121)	2.8 ± 2.2	(22)	0.436

Data are means ± SD. The mean values were compared by unpaired *t* tests, separately in the control group and in the patients. The homogeneity of the variances was evaluated by Levene's tests, and the results were interpreted accordingly. Values in parentheses indicate the number of subjects included in each comparison.
FIRI, fasting insulin resistance index.

Table 4. Distribution of the different alleles of the IL6R-α dinucleotide polymorphism in the whole population ($n = 184$), in hyperandrogenic patients ($n = 141$) compared with healthy controls ($n = 43$), and in lean women ($n = 76$) compared with obese women ($n = 108$)

IL6R alleles	Whole group	Healthy women	Hyperandrogenic patients	Lean subjects	Obese subjects
149	132 (0.36)	35 (0.41)	97 (0.34)	43 (0.28)	89 (0.41)
151	21 (0.06)	3 (0.04)	18 (0.06)	7 (0.05)	14 (0.07)
153	9 (0.02)	1 (0.01)	8 (0.03)	7 (0.05)	2 (0.01)
155	34 (0.09)	7 (0.08)	27 (0.10)	10 (0.07)	24 (0.11)
157	43 (0.12)	10 (0.12)	33 (0.12)	23 (0.15)	20 (0.09)
159	30 (0.08)	6 (0.07)	24 (0.09)	14 (0.09)	16 (0.07)
161	35 (0.10)	8 (0.09)	27 (0.10)	18 (0.12)	17 (0.08)
163	29 (0.08)	8 (0.09)	21 (0.07)	13 (0.08)	16 (0.07)
165	26 (0.07)	6 (0.07)	20 (0.07)	13 (0.08)	13 (0.06)
167	9 (0.02)	2 (0.02)	7 (0.03)	4 (0.03)	5 (0.02)
Statistics		$\chi^2 = 3.090$, $p = 0.961$		$\chi^2 = 17.085$, $p < 0.050$	

Data are number of alleles. Values in parentheses are the frequencies within each group. Obesity was defined by a BMI $\geq$25 kg/m^2.

25 kg/m^2). The frequency of the common 149-bp allele was markedly increased in obese women compared with lean women when considering patients and controls as a whole (Table 4). In agreement, homozygosity for 149 alleles was much more prevalent in the obese group compared with the lean group (Table 5). Accordingly, subjects homozygous for 149 alleles presented with increased BMI (33.2 $\pm$ 9.2 kg/m^2) compared with carriers of 149 alleles (28.2 $\pm$ 6.9 kg/m^2) and carriers of non-149 alleles (27.5 $\pm$ 8.1 kg/m^2; one-way ANOVA followed by LSD test, $F = 5.049$, $p = 0.007$). On the contrary, IL6R-α genotypes were distributed similarly in hyperandrogenic patients and healthy controls (Table 5).

Discussion

During the past years, significant efforts have been directed toward the elucidation of the genetic mechanisms underlying the pathogenesis of hyperandrogenism and PCOS. Although the initial studies suggested a model in which a few genes played a major role in PCOS inheritance (30), the emerging picture suggests that hyperandrogenism arises from the interaction of multiple genomic variants with environmental factors such as obesity and a sedentary lifestyle.

Certain genomic variants leading to common metabolic disorders might have provided a survival advantage during the process of natural selection (4). Witchel et al. (5) recently proposed that hyperandrogenism in women also provides a survival advantage, and we recently hypothesized that certain proinflammatory genotypes, previously known to influence the pathogenesis of insulin resistance, obesity, and the metabolic syndrome (4), may also be related to the pathogenesis of hyperandrogenism and PCOS (9,11). This hypothesis may also provide an explanation to the well-

Table 5. Association of the IL6R-α genotype with obesity

IL6R-α genotype	Healthy women ($n = 43$)	Hyperandrogenic patients ($n = 141$)	Lean women ($n = 76$)	Obese women ($n = 108$)
149/149 ($n = 24$)	7	17	3	21
149/other ($n = 84$)	21	63	37	47
Other/other ($n = 76$)	15	61	36	40
Statistics		$\chi^2 = 1.135$, $p = 0.567$		$\chi^2 = 9.627$, $p < 0.010$

Data are number of subjects. Subjects were grouped depending on the presence or absence of 149 alleles.

known association of hyperandrogenism and PCOS with insulin resistance (31) and obesity (12) and to the cosegregation of hyperandrogenemia with insulin resistance within families of PCOS probands, irrespective to the presence or absence of menstrual irregularity (32).

We have reported that the −308A allele of the −308 G/A polymorphism in the promoter of the *TNF-α* gene was associated with increased serum androgen levels in a mixed population of hyperandrogenic patients and nonhyperandrogenic controls, although the frequency of this allele was similar in hyperandrogenic and nonhyperandrogenic women (9). More recently, our recent finding of an association between the uncommon 196Arg allele in the *TNFRSF1B* gene—which encodes type 2 TNF receptor—with hyperandrogenism and PCOS (10) further suggest an involvement of the TNF-α axis on hyperandrogenic disorders.

Also, we have studied the possible involvement of IL-6, which is the most endocrine of inflammatory cytokines (33), on the pathogenesis of hyperandrogenism. Two single nucleotide polymorphisms in the promoter of the *IL-6* gene, which are in disequilibrium linkage, were associated with hyperandrogenism. The common G alleles of the −597G/A and −174G/C polymorphisms were more frequent in hyperandrogenic patients compared with controls. Also, in healthy controls, G alleles of the −597G/A and of −174G/C polymorphisms induced a graded increase in serum IL-6, cortisol, 11-deoxycortisol, and 17-hydroxyprogesterone concentrations, and a tendency toward an increase in testosterone concentrations (11). Conversely, these levels were lower in subjects homozygous for −597A and for −174C alleles, suggesting a protective role against increased IL-6 and adrenal steroid secretion for these genomic variants. Of note, this protective effect against the increase in IL-6 levels was more evident in obese subjects (11), who usually have increased serum IL-6 concentrations compared with lean subjects (33).

Our present results regarding the IL-6 receptor complex further expand the notion that proinflammatory genotypes influence the pathogenesis of hyperandrogenism and obesity. On the one hand, the Gly148Arg polymorphism in the *gp130* gene was associated with hyperandrogenism, Gly148 alleles being more frequent in patients and Arg148 alleles more frequent in controls. On the other, controls carrying Arg148 alleles presented with lower basal 11-deoxycortisol and 17-hydroxyprogesterone concentrations, a decreased response of androstenedione to adrenocorticotropin stimulation, and an almost significant decrease in free testosterone levels. However, these hormonal differences should be considered with caution because of the possibility of a type II error, as a large number of clinical and biochemical variables were included in the analysis, and also because of the lack of similar findings in the patients. Also, the results of the logistic regression analysis further suggests that Arg148 alleles play a protective role against androgen excess, as we recently suggested also for −597A and −174C alleles of the *IL-6* gene (11).

However, we have not been able to demonstrate a significant collaborative effect of the Gly148Arg polymorphism in the *gp130* gene and of the −174G/C polymorphism in the promoter of *IL-6* gene, on the development of hyperandrogenism, although this demonstration may require the study of both polymorphisms in a larger series of hyperandrogenism patients and healthy controls.

Furthermore, the microsatellite CA-repeat polymorphism in the *IL6R-α* gene also influenced obesity, irrespective of the presence or absence of hyperandrogenism. The common 149-bp allele was more frequent in obese subjects compared with lean women, especially when present in homozygosis. Of note, IL-6 is secreted by adipose tissue (34), and adipose tissue IL-6 content correlates with insulin resistance (35), suggesting that IL-6 plays an important role on the pathogenesis of the metabolic syndrome (14). In this context, it is not surprising that genomic variation at the IL6R-α locus might also be involved in the pathogenesis of obesity.

However, the functional consequences of the polymorphisms in gp130 and IL6R-α on the transduction of the IL-6 signal are not known at present. Therefore, the association of the Gly148Arg polymorphism in the *gp130* gene with hyperandrogenism and the association of the CA-repeat polymorphism in the *IL6R-α* gene with obesity found in our present study should be considered carefully because a clear pathogenic link is still lacking.

The fact that the common alleles of the polymorphisms studied here are those associated with disease (hyperandrogenism or obesity) permit us to speculate that these genomic variants were selected during evolution because of a previous survival advantage, but now have deleterious consequences because these mechanisms were beneficial only in a hostile environment of food shortage and starvation. This mechanism also contributes to explain the high prevalence of metabolic disorders such as obesity, type 2 diabetes, hypertension, and atherosclerosis reported in most Western areas.

Moreover, based on our present and previous findings in hyperandrogenic women, we can also speculate that the uncommon genomic variants in genes of the IL-6 axis that are associated with healthy controls may protect these individuals who lack these metabolic adaptive mechanisms that nowadays are not beneficial because life expectancy is increased and access to food is not restricted.

Therefore, the pathogenesis of metabolic disorders may be influenced by complex interactions between predisposing and protective genomic variants with environmental factors. Because the latter are different depending on ethnic and geographic factors, the genomic variants resulting in metabolic disorders may also be different depending on

these factors, explaining the frequent discrepancies of studies regarding the genetic basis of complex metabolic disorders.

In summary, we here report novel associations between hyperandrogenism and the Gly148Arg polymorphism in the *gp130* gene and between obesity and the CA-repeat polymorphism in the *IL6R-α* gene, further suggesting that proinflammatory genotypes are involved in the pathogenesis of these common metabolic disorders.

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