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Association of the Polycystic Ovary Syndrome with Genomic Variants Related to Insulin Resistance, Type 2 Diabetes Mellitus, and Obesity

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We have evaluated the possible association of polycystic ovary syndrome (PCOS) with 15 genomic variants previously described to influence insulin resistance, obesity, and/or type 2 diabetes mellitus.

Seventy-two PCOS patients and 42 healthy controls were genotyped for 15 variants in the genes encoding for paraoxonase (three variants), plasma cell differentiation antigen glycoprotein, human sorbin and SH3 domain containing 1, plasminogen activator inhibitor-1, peroxisome proliferator-activated receptor-γ2, protein tyrosine phosphatase 1B (two variants), adiponectin (two variants), IGF1, IGF2, IGF1 receptor, and IGF2 receptor.

Compared with controls, PCOS patients were more frequently homozygous for the –108T variant in paraoxonase (36.6% *vs.* 9.5%; $P = 0.002$) and homozygous for G alleles of the *Apa*I variant in IGF2 (62.9% *vs.* 38.1%; $P = 0.018$). Paraoxonase is a serum antioxidant enzyme and, because –108T alleles result in decreased paraoxonase expression, this increase in oxidative stress might result in insulin resistance. G alleles of the *Apa*I variant in IGF2 may increase IGF2 expression, and IGF2 stimulates adrenal and ovarian androgen secretion.

In conclusion, the paraoxonase –108 C→T variant and the *Apa*I polymorphism in the IGF2 gene are associated with PCOS and might contribute to increased oxidative stress, insulin resistance, and hyperandrogenism in this prevalent disorder. (*J Clin Endocrinol Metab* 89: 2640–2646, 2004)

THE POLYCYSTIC OVARY syndrome (PCOS) is one of the most common endocrine disorders in women of fertile age (1). As defined by endocrine criteria, PCOS is present in approximately 6.5% of women from Spain (2). Although hyperandrogenism and chronic anovulation are the key findings in PCOS patients, insulin resistance (3) and obesity (4) are frequently found in these patients.

The increase in serum insulin levels resulting from insulin resistance facilitates androgen secretion from the ovaries and the adrenals in PCOS patients (3), and obesity worsens the insulin resistance of these women. In conceptual agreement, amelioration of insulin resistance by weight loss (4) or by insulin-lowering drugs (5) improves hyperandrogenism in PCOS women.

Familial aggregation provides evidence supporting a genetic basis for PCOS (6), but the precise genetic mechanisms remain unknown despite significant efforts. Of note, hyperandrogenism and insulin resistance cosegregate in families of PCOS patients (7, 8), suggesting a common genetic origin of these disorders.

Considering the frequent association of PCOS with insulin resistance and obesity, in the present case-control study we have conducted a systematic evaluation of the possible role in the pathogenesis of PCOS of 15 genomic variants located in 11 candidate genes, previously reported to influence the pathogenesis of insulin resistance, type 2 diabetes mellitus, and/or obesity. Specifically, we have studied genomic variants in the following genes: plasma cell differentiation antigen (PC-1) glycoprotein (9), human sorbin and SH3 domain containing 1 (SORBS1) (10), plasminogen activator inhibitor-1 (PAI-1) (11), peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) (12, 13), paraoxonase (PON1) (14, 15), protein tyrosine phosphatase 1B (PTP1B) (16), adiponectin (17, 18), IFG1 (19), IGF2 (20), IGF1 receptor (IGF1R), and IGF2 receptor (IGF2R) (21).

Subjects and Methods

Subjects

Seventy-two PCOS patients [age, 24.6 ± 6.9 yr (mean ± SD; range, 14–42 yr); body mass index (BMI), 29.9 ± 8.6 kg/m^2 (range, 16.3–57.5 kg/m^2)] and 42 healthy nonhyperandrogenic women [age, 31.1 ± 8.0 yr (range, 16–47 yr); BMI, 28.1 ± 7.8 kg/m^2 (range, 16.2–44.9 kg/m^2)] were studied. PCOS was defined by oligo-ovulation, clinical and/or biochemical hyperandrogenism, and exclusion of hyperprolactinemia (serum prolactin <24 ng/ml), nonclassic congenital adrenal hyperplasia [ACTH-stimulated 17-hydroxyprogesterone levels <10 ng/ml (22)], and androgen-secreting tumors (23). In these patients, evidence for oligo-ovulation was provided by chronic oligomenorrhea, by luteal phase progesterone less than 4 ng/ml, or by basal body temperature charts.

The control group was composed of lean female volunteers and consecutive patients referred to one of the authors (H.F.E.-M.) for dietary treatment of obesity. The controls were carefully evaluated to avoid any selection bias. None of the controls, either lean or obese, had signs or

Abbreviations: BMI, Body mass index; IGF1R, IGF1 receptor; PAI-1, plasminogen activator inhibitor-1; PC-1, plasma cell differentiation antigen glycoprotein; PCOS, polycystic ovary syndrome; PON1, paraoxonase; PPAR-γ2, peroxisome proliferator-activated receptor-γ2; PTP1B, protein tyrosine phosphatase 1B; SORBS1, human sorbin and SH3 domain containing 1.

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symptoms of hyperandrogenism, menstrual dysfunction, or history of infertility before or after clinical and biochemical evaluation. All the controls presented with fasting glucose concentrations less than 110 mg/dl, and all had blood pressure less than 140/90 mm Hg.

The patients and controls had not taken hormonal medications, including contraceptive pills and antiobesity drugs, for the last 6 months. All the subjects were Caucasian. The ethics committee of the Hospital Ramón y Cajal approved the study, and informed consent was obtained from each patient and control or from the legal representatives in minors.

Protocol

Studies were performed between d 5 and 10 of the menstrual cycle or during amenorrhea after excluding pregnancy by proper testing. Hirsutism was quantified by a modified Ferriman-Gallwey score (24). Between 0800 and 0900 h 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 total testosterone, dehydroepiandrosterone sulfate, sex hormone-binding globulin, glucose, and insulin. Samples were immediately centrifuged, and serum was separated and frozen at 20 C until assayed.

The technical characteristics of the assays used for hormone measurements have been reported elsewhere (2, 25, 26). The free testosterone concentration was calculated from total testosterone and sex hormone-binding globulin concentrations, assuming a serum albumin concentration of 43 g/liter and taking a value of 1×10^9 liters/mol for the association constant of sex hormone-binding globulin for total testosterone and a value of 3.6×10^4 liters/mol for that of albumin for total testosterone (27). Insulin resistance in the fasting state was estimated from glucose and insulin levels using the fasting insulin resistance index [glucose (mmol/liter) × insulin (mU/liter)/25 (28)].

DNA extraction and genotype analyses

Genomic DNA from peripheral blood mononuclear cells was extracted using commercial DNA purification kits (Wizard genomic DNA purification kit, Promega, Madison, WI, and Nucleon BAC C3, Amersham Pharmacia, Buckinghamshire, UK). After DNA extraction, patients and controls were genotyped as follows: Genotyping of a dinucleotide repeat on IGF1 (19) and of a trinucleotide repeat on IGF1R gene (29) were performed by PCR using fluorescent dye-labeled forward primers, followed by use of an ABI310 automated sequencer (Applied Biosystems, Foster City, CA). Primer sequences and allele sizes were described previously (19, 29). The PCR fragments were sized with an internal size standard using the GeneScan analysis software (Applied Biosystems). The dinucleotide repeat polymorphism in IGF1 resulted in six different alleles, sized 188, 190, 192, 194, 196, or 198 bp. This method was also used for genotyping of the ACAA-insertion/deletion polymorphism at the 3′ nontranslated region (3′-UTR) of IGF2R gene, which results in alleles sized 140 or 144 bp (30).

Several variants were analyzed by PCR restriction fragment length polymorphism as previously described: *Apa*I polymorphism in the 3′-UTR of the IGF2 (31); variant Lys121Gln in exon 4 of PC-1 gene (9); polymorphism Thr228Ala in exon 7 of SORBS1 (10); variant Pro12Ala in exon 2 of PPAR-γ2 gene (32); variants 981 C→T in exon 8 (33) and 1484 insG (16) in the 3′-UTR of PTP1B; polymorphism –675 4G/5G in the 5′ regulatory region of PAI-1 gene (34); and polymorphisms –108 C→T (35), Leu55Met, and Gln192Arg (36) in the PON1 gene.

Genotyping of polymorphisms 45 T→G and 276 G→T in the adiponectin gene (17) was performed by PCR restriction fragment length polymorphism using endonucleases *Ava*I and *Bsm*I, respectively. Primers were designed from contig NT005962 (www.ncbi.nlm.nih.gov) for amplifying a 439-bp fragment (from nucleotide 2,301,053 to nucleotide 2,301,491) that includes both polymorphisms.

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. Analysis of covariance was used to compare patients and controls, allowing correction for the difference in age between both groups.

To evaluate the association between discontinuous variables we used the χ^2 test and Fisher's exact test as appropriate. *A priori* power analysis of the differences in frequencies between PCOS patients and controls was conducted. Our sample size permitted the detection of effect sizes for the difference between frequencies (w) of 0.26 for the χ^2 test with one degree of freedom, and 0.29 for the χ^2 test with two degrees of freedom, used here. By convention, effects sizes for the differences between frequencies are considered very small or trivial when less than 0.10, small from 0.10–0.30, moderate from 0.30–0.50, and large when greater than 0.50 (37). Consequently, our sample size permitted the detection of small differences between the differences in PCOS patients and controls. On the contrary, very small and minor differences between frequencies in both groups of subjects may not have been detected in our study because of the relatively small sample size. Therefore, our study does not have the power to detect associations comparable to those already published for at least some variants (*i.e.* PPAR-γ2).

Logistic regression was used to analyze the role of the genomic variants studied here as predictive factors for PCOS in our model. The backward likelihood-ratio test was used as the method for variable selection (38). Finally, the influence of the different genotypes on clinical and biochemical variables related to hyperandrogenism and to insulin resistance was analyzed by one-way ANOVA followed by the least-significant differences test for *post hoc* comparisons. Analyses were performed using SPSS 10 for the Macintosh (SPSS Inc., Chicago, IL) with the exception of power analysis, which was performed using the G*Power software (39). $P < 0.05$ was considered statistically significant.

Results

The comparison of clinical, biochemical, and hormonal variables between PCOS patients and controls is shown in Table 1. Compared with controls, PCOS patients presented with increased hirsutism scores, total and calculated free testosterone levels, androstenedione, and fasting insulin levels; an increased fasting insulin resistance index; and decreased sex hormone-binding globulin concentrations.

TABLE 1. Clinical and biochemical variables in PCOS patients and healthy controls

	PCOS (n = 72)	Controls (n = 42)	*P*
Hirsutism score	13.2 ± 6.6	1.6 ± 1.4	<0.001
Total testosterone (ng/dl)	70 ± 31	42 ± 10	<0.001
Free testosterone (ng/dl)	1.3 ± 0.9	0.6 ± 0.3	<0.001
SHBG (μg/dl)	314 ± 174	545 ± 242	<0.001
Dehydroepiandrosterone sulfate (ng/ml)	2686 ± 1408	1833 ± 897	0.058
Basal androstenedione (ng/ml)	3.9 ± 1.4	2.6 ± 0.9	<0.001
Fasting glucose (mg/dl)	86 ± 9	87 ± 10	0.385
Fasting insulin (μU/ml)	16 ± 10	11 ± 7	<0.010
Fasting insulin resistance index (mmol/mU·liter^2)	3.2 ± 2.2	2.2 ± 1.4	<0.010

Data are means ± SD. The mean values were compared by analysis of covariance to correct for the difference in age between patients and controls. Normality was ensured by logarithmic transformation as needed. To convert to SI units, multiply total testosterone by 0.03467 (result in nmol/liter), free testosterone by 34.67 (result in pmol/liter), sex hormone-binding globulin by 0.111 (result in nmol/liter), dehydroepiandrosterone sulfate by 0.002714 (result in μmol/liter), glucose by 0.0555 (result in mmol/liter), and insulin by 6.945 (result in pmol/liter).

The distribution of the different genotypes according to the 15 variants studied here, in PCOS patients compared with controls, are shown in Table 2. Only the –108 C→T polymorphism in PON1 and the *Apa*I variant in IGF2 were distributed differently in PCOS patients compared with controls. However, and although the distribution of the remaining variants was not statistically different between PCOS patients and controls, it should be noted that the relatively limited sample size of our study precludes ruling out very small and minor differences in the distribution of these variants between PCOS patients and control, especially in the distribution of PPAR-γ2 and PAI-1 genotypes which showed *P* values close to 0.1.

In agreement with the different distribution of the PON1 and IGF2 genotypes cited above, PCOS patients were more frequently homozygous for the –108T variant in PON1 (PCOS, 36.6% *vs.* controls, 9.5%; $\chi^2 = 9.9$; $P = 0.002$) and homozygous for G alleles of the *Apa*I IGF2 variant (PCOS, 62.9% *vs.* controls, 38.1%; $\chi^2 = 6.5$; $P = 0.018$), compared with healthy controls. Of note, the association of PCOS with homozygosity for the –108T variant in PON1 retained statistical significance even after applying an *a priori* Bonferroni correction to the level of significance, which was reset from $P < 0.05$ to $P < 0.0034$ considering the 15 variants tested in this study.

To evaluate the contribution of the genomic variants studied here to PCOS, a logistic regression model was used. The dependent variable of the model was coded 1 for PCOS patients and 0 for healthy controls. All the genomic variables studied here were introduced as independent variables.

The model only retained homozygosity for the –108T variant in PON1 (odds ratio = 7.09; 95% CI = 2.08–23.81; $P =$

TABLE 2. Frequencies of the genotypes according to the 15 variants included in the study in PCOS patients compared with healthy controls

Gene	Genotype	Healthy women (n = 42)	PCOS patients (n = 72)	χ^2	*P* value
PON1	−108 TT	0.095	0.366	10.996	0.004
	−108 TC	0.619	0.366		
	−108 CC	0.286	0.268		
PON1	Leu55Leu	0.333	0.362	1.370	0.504
	Leu55Met	0.452	0.348		
	Met55Met	0.214	0.290		
PON1	Gln192Gln	0.571	0.580	0.946	0.623
	Gln192Arg	0.286	0.333		
	Arg192Arg	0.143	0.087		
IGF2	*Apa*I AA	0.095	0.114	8.320	0.016
	*Apa*I GA	0.524	0.257		
	*Apa*I GG	0.381	0.629		
IGF2R	Homozygous 140-bp allele	0.071	0.072	0.553	0.758
	Heterozygous 140/144-bp alleles	0.476	0.406		
	Homozygous 144-bp allele	0.452	0.522		
IGF1[a]	Homozygous 192-bp allele	0.452	0.394	0.368	0.832
	Heterozygous 192-bp allele	0.405	0.451		
	Noncarrier 192-bp allele	0.143	0.155		
IGF1R	Homozygous 90-bp allele	0.143	0.088	0.935	0.626
	Heterozygous 90/93-bp alleles	0.571	0.574		
	Homozygous 93-bp allele	0.286	0.338		
SORBS1	Thr228Thr	0.857	0.871	0.046	1.000
	Thr228Ala	0.143	0.129		
Adiponectin	T45T	0.690	0.667	0.114	0.945
	T45G	0.286	0.300		
	G45G	0.024	0.033		
Adiponectin	G276G	0.429	0.387	0.610	0.737
	G276T	0.476	0.468		
	T276T	0.095	0.145		
PPARγ-2	Pro12Pro	0.786	0.900	4.240	0.120[b]
	Pro12Ala	0.214	0.086		
	Ala12Ala	0.000	0.014		
PTP1B	Wild-type/wild-type	0.929	0.898	0.277	0.732
	Wild-type/Ins1484G	0.071	0.102		
PTP1B	C981C	0.833	0.881	0.474	0.565
	C981T	0.167	0.119		
PC-1	Lys121Lys	0.619	0.714	1.958	0.376
	Lys121Gln	0.381	0.271		
	Gln121Gln	0.000	0.014		
PAI-1	−675 4G/4G	0.190	0.254	3.442	0.179[b]
	−675 4G/5G	0.405	0.507		
	−675 5G/5G	0.405	0.239		

Data are frequencies.

[a] The IGF1 polymorphism was coded depending on the presence or absence of the common 192-bp allele (19).

[b] Because of the small sample size, these nonsignificant results lack the statistical power to definitely rule out the small differences found between the frequencies of these genomic variants in PCOS patients and controls.

0.002) and homozygosity for G alleles of the *Apa*I variant in IGF2 (odds ratio = 3.10; 95% CI = 1.25–7.64; $P = 0.014$) for the prediction of PCOS (Nagelkerke's $R^2 = 0.214$).

Finally, we studied the influence of the genomic variants on clinical and biochemical markers of hyperandrogenism, BMI, and insulin resistance, including PCOS patients and healthy controls as a whole. As expected from its association with PCOS, and compared with carriers of –108C alleles, subjects homozygous for –108T alleles of the –108 C→T polymorphism in PON1 presented with increased hirsutism scores (12.8 ± 8.6 *vs.* 8.1 ± 7.1; $P = 0.005$) and total testosterone (73 ± 37 *vs.* 55 ± 25 ng/dl; $P = 0.003$), free testosterone (1.5 ± 1.2 *vs.* 0.9 ± 0.5 ng/dl; $P = 0.001$), and androstenedione (4.3 ± 1.4 *vs.* 3.1 ± 1.3 ng/ml; $P = 0.001$) concentrations.

Of the variants not associated with PCOS, only the PON1 Leu55Met, IGF1R, and SORBS1 polymorphisms resulted in differences in some of the clinical and biochemical variables studied here.

Compared with carriers of the common 55L allele in PON1, subjects homozygous for 55M alleles presented with increased BMI (31.9 ± 9.5 *vs.* 28.3 ± 7.7 kg/m^2; $P = 0.045$), fasting insulin (17 ± 9 *vs.* 13 ± 9 μU/ml; $P = 0.033$), and glucose concentrations (90 ± 10 *vs.* 85 ± 9 mg/dl; $P = 0.029$) and increased fasting insulin resistance index (3.5 ± 2.1 *vs.* 2.6 ± 1.9; $P = 0.022$). Subjects homozygous for 90-bp alleles of IGF1R presented with increased fasting glucose levels (93 ± 8 *vs.* 86 ± 10 mg/dl; $P = 0.015$), increased fasting insulin resistance index (3.81 ± 1.70 *vs.* 2.69 ± 2.01; $P = 0.030$), and an almost significant increase in fasting insulin concentrations (18 ± 8 *vs.* 14 ± 9 μU/ml; $P = 0.05$) compared with carriers of 93-bp alleles. Also, carriers of Ala228 alleles of SORBS1 presented with increased BMI compared with subjects homozygous for 228T alleles (34.5 ± 7.9 *vs.* 28.4 ± 8.1 kg/m^2; $P = 0.008$). Finally, no other variant included in the study influenced any phenotypic trait characteristic of PCOS, obesity, or insulin resistance (data not shown).

Discussion

Initial studies regarding the genetics of PCOS suggested a model in which a few genes played a major effect on its inheritance (40). However, the number of genomic variants associated with PCOS is growing rapidly, suggesting that PCOS may result from the interaction of multiple genomic variants with environmental factors such as obesity and a sedentary lifestyle.

Certain genomic variants associated with components of the metabolic syndrome might have provided a survival advantage during the process of natural selection (41). Hyperandrogenism may have also favored survival during evolution, as proposed by Witchel *et al.* (42) for carriers of 21-hydroxylase deficiency. Considering the frequent association of PCOS with components of the metabolic syndrome, such as insulin resistance (3) and obesity (4), genomic variants associated with the metabolic syndrome should be considered candidate genes to explain PCOS inheritance, even more so when hyperandrogenemia cosegregates with insulin resistance within families of PCOS probands (7, 8), irrespective of the presence or absence of menstrual irregularity (7).

Of the 15 variants studied here, we have been able to demonstrate the association of PCOS with the –108 C→T variant in PON1 and with the *Apa*I variant in IGF2. Moreover, the association of PCOS with homozygosity for T alleles of the –108 C→T variant in PON1 persisted even after correcting for multiple testing, further suggesting that this association did not result merely from chance.

Regarding the association of homozygosity for –108T alleles of PON1 with PCOS, our present results are in conceptual agreement with previous reports, considering that PCOS is associated with insulin resistance (3), and homozygosity for –108T alleles is more frequent in nondiabetic subjects showing abnormal fasting glucose concentrations, and therefore suspected to have insulin resistance, compared with subjects with normal serum glucose concentrations (15).

The PON1 gene is expressed mainly in the liver and encodes for serum paraoxonase, which is an antioxidant high-density lipoprotein-associated enzyme. Liver PON1 mRNA expression is influenced by genetic and environmental factors, and both androgens and proinflammatory mediators decrease liver PON1 expression (43). Interestingly, both androgen excess and proinflammatory genotypes contribute to the pathogenesis of PCOS (44–46). The –108 C→T polymorphism is responsible of approximately 23% of PON1 expression levels in some cell systems, in which –108TT constructs showed reduced PON1 expression compared with –108CC constructs (35). Therefore, we speculate that homozygosity for –108T alleles, hyperandrogenism, and proinflammatory genotypes might contribute to reduced PON1 expression, resulting in a higher oxidative stress in these women.

Because oxidative stress may impair insulin action (47), reduced serum paraoxonase activity may contribute to insulin resistance. This hypothesis is supported by the finding of reduced serum paraoxonase activity in insulin-resistant disorders such as type 2 diabetes mellitus (48, 49) and cardiovascular atherosclerotic disease (50, 51). If confirmed in future studies, the association of homozygosity for –108T alleles of PON1 with PCOS might contribute to explain the insulin resistance and the increased risk for atherosclerosis associated with this syndrome (52).

In our series, the Leu55Met and Gln192Arg polymorphisms in PON1 were not associated with PCOS, but subjects homozygous for Met55 alleles presented with a higher BMI and increased indexes of insulin resistance, as previously suggested by others (14, 53).

G alleles of the *Apa*I polymorphism in the IGF2 gene increase IGF2 mRNA in leukocytes compared with A alleles (54) and possibly result in increased liver IGF2 expression and secretion (55). IGF2 stimulates adrenal (56, 57) and ovarian (58) androgen secretion and, together with IGF1 and IGF binding proteins, has been suggested to play a role in the pathogenesis of PCOS (56, 58, 59). Therefore, increased IGF2 levels resulting from G alleles of the *Apa*I polymorphism in the IGF2 gene might contribute to hyperandrogenism and may explain the association with PCOS.

Moreover, our findings regarding the *Apa*I polymorphism in the IGF2 gene are in conceptual agreement with previous reports in different populations. In a large series of middle-aged males, BMI was increased in subjects homozygous for the common G allele compared with those homozygous for A alleles of the *Apa*I polymorphism in the IGF2 gene (55), and

obesity is a common finding in PCOS women (4). However, we have not found a direct influence of the *Apa*I polymorphism in the IGF2 gene on BMI, but we included only women in our study.

Other genomic variants, which were not associated with PCOS, influenced phenotypic traits associated with obesity and insulin resistance. In addition to the effects of the Leu55Met polymorphism in PON1 on BMI and indexes of insulin resistance described above, carriers of Ala228 alleles of SORBS1 presented with increased BMI when compared with subjects homozygous for Thr228 alleles, in conceptual agreement with a large study conducted in Europe (60). In the latter, the Thr228Ala polymorphism in SORBS1 was equally distributed among obese and lean subjects, but subjects homozygous for Ala228 alleles were found only in obese patients (60).

In our series, women homozygous for 90-bp alleles of IGF1R had increased indexes of insulin resistance compared with carriers of 93-bp alleles. In conceptual agreement, the IGF1R gene has been proposed as a candidate for insulin resistance-associated traits, although conflicting reports have been observed depending on the population studied (61).

On the contrary, we have not been able to confirm previous reports regarding the influence of other genomic variants on phenotypic traits associated with the metabolic syndrome. However, because of the relatively small sample size of our study, these negative findings lack the statistical power needed to rule out a minor role for these genomic variants on PCOS or on other insulin resistance-associated traits. Therefore, our present data must not be considered as definite evidence against the involvement of these variants in PCOS and in insulin resistance. This consideration is especially important for variants such as the Pro12Ala polymorphism in the PPAR-γ2 gene and the −675 4G/5G polymorphism in PAI-1, which showed small but considerable differences in the frequencies in PCOS patients compared with controls between 0.10 and 0.17, with *P* values that were close to 0.1.

The differences in the distribution of these variants might have reached statistical significance if analyzed in larger series, explaining the conflicting results with previous studies by others; Ala12 alleles of the PPAR-γ2 gene have been shown to favor weight gain in obese adults (62) and in obese hyperandrogenic girls and adolescents (32) and also to preserve insulin sensitivity in Caucasian men (12) and in Caucasian women presenting with PCOS (13). However, the later study did not include healthy women (13), and therefore no differences between PCOS patients and controls in the allele frequencies of the Pro12Ala variant in the PPAR-γ2 gene has been reported to date. Also, an increased frequency of 4G alleles of the −675 4G/5G polymorphism in PAI-1 has been reported in PCOS patients (63).

On the contrary, the differences between the frequencies in PCOS patients and controls of the other variants not associated with PCOS in our study were less than 0.10. These differences should be considered very small (37) had the differences between frequencies reached statistical significance if a larger sample size was used, and therefore unlikely to play an important role for the pathogenesis of PCOS.

The IGF2R polymorphism was not associated with PCOS in our series, despite the evidence for linkage found in non-diabetic Mexican-Americans between insulin-resistant phenotypes and the D6S264 marker close to the IGF2R gene (21). We did not find any association of polymorphisms in the adiponectin gene with PCOS, in contrast with the increased risk for type 2 diabetes in subjects homozygous for 45G in the Japanese (17). And also, none of the polymorphisms in the PTP1B gene was associated with PCOS or influenced insulin resistance indexes, in contrast to the higher values of insulin resistance measured by the homeostasis model assessment observed in men carrying the 1484ins allele (16), or the reduction of the risk for type 2 diabetes in the Oji-Cree subjects carrying 981T alleles (33).

We have also not found any association of the Lys121Gln variant in PC-1 with PCOS. PC-1 inhibits tyrosine kinase activity of the insulin receptor, and increases in the PC-1 content in fibroblasts from normal glucose-tolerant subjects are related to decreased insulin action *in vivo* and *in vitro* (64). Subsequently, Gln121 alleles of PC-1 have been proposed to increase insulin resistance (9, 65), although conflicting results have been found in different populations (66). Finally, the IGF-1 variant was not associated with PCOS or insulin resistance-associated traits in our study, even considering that noncarriers of 192-bp alleles have an increased risk for type 2 diabetes mellitus, and myocardial infarction, in the Dutch population (19).

In summary, our results suggest that genomic variants in the genes encoding PON1 and IGF2 are associated with PCOS. Also, some of these variants (and others in the SORBS1 and IGF1R genes) influence clinical and biochemical variables related to hyperandrogenism, obesity, and insulin resistance.

Considering that to date a large number of genomic variants has been found to be associated with PCOS, and that many of these associations have not been replicated when studied in different populations, the emerging picture is that of a multigenic etiology for this disorder, in which nongenetic factors also have a strong influence on its development.

The pathogenesis of PCOS may be influenced by complex interactions between predisposing and protective genomic variants with environmental factors, such as diet and exercise. And because the latter are subject of considerable ethnic, geographic, and even familial variability, the genomic variants resulting in PCOS may also be different depending on these factors. Additional studies in large populations of PCOS patients, in which these environmental factors are clearly defined, will undoubtedly help in the identification of the genes involved in the pathogenesis of this prevalent disorder.

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