Genetic Variation in 11β-Hydroxysteroid Dehydrogenase Type 1 Predicts Adrenal Hyperandrogenism among Lean Women with Polycystic Ovary Syndrome

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Context: Elevated adrenal androgen levels are common in polycystic ovary syndrome (PCOS), but the underlying pathogenetic mechanism is poorly understood. In the rare cortisone reductase deficiency, impaired regeneration of active cortisol from inert cortisone by 11β-hydroxysteroid dehydrogenase (11β-HSD1) results in compensatory activation of ACTH secretion and adrenal hyperandrogenism. 11β-HSD1 deficiency may protect against obesity and its metabolic consequences because of impaired regeneration of cortisol in adipose tissue.

Objective: Our objective was to investigate a functional polymorphism in *HSD11B1* (T→G in the third intron rs12086634, which associates with lower 11β-HSD1 activity) in PCOS with and without obesity.

Design and Setting: We conducted a case-control study in lean and obese PCOS patients and controls at an academic hospital.

Participants: Participants included 102 Caucasian PCOS patients and 98 controls comparable for age, weight, and race.

Main Outcome Measures: We assessed genotype distribution and influence of genotypes on clinical, hormonal, and metabolic parameters.

Results: The G allele was significantly related to PCOS status ($P = 0.041$), and this association was mainly attributable to lean ($P = 0.025$), rather than obese ($P = 0.424$), PCOS patients. The G allele was associated with lower 0800–0830 h plasma cortisol ($P < 0.001$) and higher cortisol response to ACTH$_{1-24}$ ($P < 0.001$) in all women with PCOS and with higher dehydroepiandrosterone sulfate levels ($P < 0.001$), greater suppression of dehydroepiandrosterone sulfate by dexamethasone ($P < 0.001$), and lower fasting plasma low-density lipoprotein cholesterol ($P = 0.002$) levels in lean PCOS women.

Conclusions: Genetic variation in 11β-HSD1 contributes to enhanced cortisol clearance and compensatory adrenal hyperandrogenism in lean patients with PCOS but may be protective against obesity and some features of the metabolic syndrome. (*J Clin Endocrinol Metab* **91: 2295–2302, 2006**)

THE ENZYME 11β-HYDROXYSTEROID dehydrogenase type 1 (11β-HSD1) converts the inactive steroid cortisone into the active glucocorticoid cortisol, principally in liver and adipose tissue. This provides a mechanism for tissue-specific control of corticosteroid receptor activation which is independent of circulating cortisol concentrations (1, 2). 11β-HSD1 seems to be important in metabolic disease, because in mice, increasing enzyme activity in adipose tissue enhances local glucocorticoid levels and produces a metabolic syndrome (3), whereas decreasing enzyme activity protects against obesity and the metabolic syndrome (4, 5). In humans, 11β-HSD1 expression is increased in adipose tissue in obesity (6), whereas inhibition of 11β-HSD1 enhances insulin sensitivity and provides a new approach to treat type 2 diabetes (7–9).

Polymorphisms in the *HSD11B1* gene, which encodes 11β-HSD1, have been associated with type 2 diabetes (10) and hypertension (11). A T→G polymorphism in the third intron (rs12086634) is protective against diabetes in Pima Indians (10). This polymorphism reduces *HSD11B1* gene transcription *in vitro* (12), which is consistent with reduced cortisol generation within cells. However, when regeneration of cortisol in peripheral tissues is impaired, the overall metabolic clearance rate for cortisol is increased. Although any tendency for plasma cortisol concentrations to fall is compensated for by reduced negative feedback suppression of ACTH and hence increased adrenal cortisol secretion, this compensation occurs at the expense of increased ACTH-dependent adrenal androgen secretion. These mechanisms appear to operate in the rare syndrome of cortisone reductase deficiency, characterized by an inability to convert cortisone to cortisol (12, 13). This syndrome has been associated with the T→G polymorphism in the third intron (rs12086634), and female patients affected by cortisol reductase deficiency present with hyperandrogenism and a phenotype resembling polycystic ovary syndrome (PCOS) (hirsutism, irregular menses, and polycystic ovaries) (12, 13).

In patients with PCOS, lower ratios of cortisol/cortisone metabolites in urine have been reported (14), suggesting a reduced 11β-HSD1 activity, albeit that this may be confounded by coexistent obesity.

These findings highlight the rs12086634 T→G *HSD11B1* polymorphism as a candidate to explain the adrenal androgen excess in PCOS. However, any reduction in 11β-HSD1 might paradoxically protect against obesity and associated metabolic dysfunction. We therefore hypothesized that the T→G *HSD11B1* genotype would be enriched only in the subgroup of PCOS patients with adrenal androgen excess in whom hyperandrogenism is not associated with obesity and the metabolic syndrome; the association of these features is frequent, but not universal, in this heterogeneous condition (15–17). To test this hypothesis we characterized the T→G polymorphism in the third intron of *HSD11B1* gene (rs12086634) and its association with hormonal and metabolic phenotype in women with PCOS who were either lean or obese.

Subjects and Methods

Subjects

We investigated 102 unmedicated Caucasian women with PCOS, aged 18–45 yr, and 98 controls comparable for age, weight, and race. PCOS women had polycystic ovarian morphology at ultrasound and at least one of the following: chronic oligo-anovulation, with luteal serum progesterone less than 2 ng/ml (18), 100%; hirsutism with a Ferriman-Gallwey score of at least 8 (19), 68%; and elevated serum testosterone levels more than 2 SD above our reference mean values (20), 51%, according to the Rotterdam consensus (21). Hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, and androgen-secreting tumors were excluded by specific laboratory analysis (22). Controls had normal ovaries by ultrasound, no signs of hyperandrogenism, and regular and ovulatory menstrual cycles. Ovulation was documented by the presence of progesterone levels above 8 ng/ml during the luteal phase of the menstrual cycle, tested during the recruitment period (18). To better analyze the impact of obesity on T→G *HSD11B1* genotype, we included only lean or obese subjects, excluding overweight women. The degree of obesity was established depending on the body mass index (BMI), and women were classified as lean if BMI was not more than 25 kg/m^2 and obese if BMI was at least 30 kg/m^2 (23). The protocol was approved by the local ethics committee, and written informed consent was obtained from each patient and control.

Assessment program

Standard anthropometric data (height, weight, and waist circumference) and an L4–L5 computerized tomography scan of body fat distribution (to estimate total, visceral, and sc adipose tissue areas) were obtained from each subject. Moreover, basal blood samples for hormonal [total testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEA-S), SHBG, and cortisol] and metabolic [glucose, insulin, total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides] determinations were collected at 0800–0830 h after overnight fast. All participants underwent also a fasting 75-g oral glucose tolerance test, an ACTH$_{1-24}$ stimulation test (250 μg ACTH$_{1-24}$ iv at 0800–0830 h with blood taken at 0 and 60 min), and a dexamethasone suppression test (0.5 mg orally every 6 h for 4 d with blood taken at 0800–0830 h on the fifth day). Samples were immediately chilled on ice and centrifuged; serum was stored at −20 C and plasma at −80 C until assayed. Studies were performed between d 5 and 10 of the menstrual cycle, or during amenorrhea, after excluding pregnancy by appropriate testing.

Biochemical assay

The assays employed for biochemical measurements have been reported elsewhere (22, 24, 25). Low-density lipoprotein (LDL)-cholesterol was calculated by the Friedewald equation (26). Insulin resistance was estimated using the quantitative insulin-sensitivity check index (QUICKI) and the insulin sensitivity index during the oral glucose tolerance test (ISI) (27, 28).

Genetic analysis

Blood samples were collected in tubes containing disodium-EDTA as anticoagulant and stored at 4 C until extraction. DNA was extracted using

QIAGEN silica gel-based spin columns (QiaAmp DNA blood mini kit; QIAGEN, Hilden, Germany). DNA obtained gave a 260/280 absorbance ratio of 1.6–2.0, indicating high-quality DNA. Then samples were loaded in wells made in agarose gel 1% for separation by electrophoresis, to verify the DNA integrity and stored at −20 C until analysis. Genotyping of rs12086634 was performed by allelic discrimination real-time PCR on a TaqMan 7900 HT (Applied Biosystems, Foster City, CA) with probe sequences FAM CAGA**T**GATTTCT and VIC CCCAGA**G**GATTTCT, forward primer 5′-GGAGGAGAATGGGAAAGGTATCAAC-3′, and reverse primer 5′-TCCTCCTGCAAGAGATGGCTATATT-3′. Real-time PCR allelic discrimination was unsuccessful in 19 subjects, who were genotyped by direct sequencing using Big Dye Terminator and an automated capillary sequencer (3730 DNA analyzer; Applied Biosystems), using the forward primer 5′-TGAGCAATCTCTCATTTAAGCCC-3′ and reverse primer 5′-TGTCCCTGTCCCACTTACCAGCC-3′. To evaluate whether real-time PCR and direct sequencing were comparable, we analyzed 10 samples with both techniques and obtained the same results.

Power analysis

The number of cases to be included in the study was calculated taking into account a G allele frequency in rs12086634 of 14% in the control Caucasian population (12) and assuming a frequency of 35% in PCOS (estimated from the frequency of adrenal hyperandrogenism in this disorder) (29); a case-control study of 200 subjects has a power of 90% to detect this difference at a significance level of $P < 0.05$.

Statistical analysis

Data are shown as means ± SD and frequencies. The data were evaluated by means of two-way ANOVA to analyze the overall effect of PCOS and of obesity in the entire population (each adjusted for the other) and to evaluate the interactions between them. The effects within groups were evaluated using the nested design of the two-way ANOVA. Two-way ANOVA with the same design was also applied to analyze the effects of T/G+G/G *HSD11B1* genotype (rs12086634) and of obesity. Univariate and multivariate logistic regressions were used to relate the frequency of T/G+G/G *HSD11B1* genotype (rs12086634) with the presence of PCOS and with the degree of obesity. The odds ratios (OR) evaluated by the logistic regression, together with their 95% confidence intervals (CI), were also reported. No *post hoc* analysis was performed. Statistical analyses were performed by running the SPSS/PC+ version 8 (Chicago, IL) software package. Two-tailed P values < 0.05 were considered statistically significant.

Results

Influence of PCOS and obesity on clinical, hormonal, and metabolic parameters

Table 1 shows clinical and biochemical characteristics in PCOS and controls, according to BMI (lean and obese), whereas Table 2 shows the effect attributable to PCOS status, to obesity, and to their interaction on all the characteristics analyzed.

PCOS women and controls were of similar age and had similar BMI and body fat distribution, glucose tolerance, and LDL-cholesterol and triglycerides levels. However, these parameters were significantly higher in obese than lean women in both PCOS patients and in controls.

PCOS women had higher levels of androgens (testosterone, androstenedione, and DHEA-S) and lower levels of SHBG, independently of body weight. However, the difference between PCOS and controls in SHBG levels was more evident in lean than obese subjects.

PCOS women had an exaggerated response of cortisol to ACTH$_{1-24}$, were more insulin resistant, and had higher insulin responsiveness to the glucose load and lower HDL-cholesterol levels when compared with controls. Insulin resistance and insulin responsiveness to the glucose load were significantly

TABLE 1. Clinical, hormonal, and metabolic characteristics in PCOS women and controls

Variables	Lean			Obese		
	PCOS (n = 38)	Controls (n = 38)	P value[a]	PCOS (n = 64)	Controls (n = 60)	P value[a]
Age (yr)	23.9 ± 4.7	23.9 ± 4.1	0.972	25.9 ± 6.6	26.6 ± 6.7	0.483
BMI (kg/m^2)	22.9 ± 2.1	20.8 ± 1.8	0.280	35.8 ± 5.2	36.4 ± 4.7	0.433
Waist circumference (cm)	74.3 ± 6.1	69.1 ± 5.8	0.490	101.2 ± 12.3	102.5 ± 10.5	0.474
Total abdominal fat (cm^2)	263 ± 98	259 ± 97	0.936	607 ± 155	598 ± 81	0.765
Visceral abdominal fat (cm^2)	37 ± 17	34 ± 16	0.836	108 ± 61	101 ± 39	0.591
sc abdominal fat (cm^2)	226 ± 95	225 ± 92	0.997	499 ± 121	497 ± 64	0.905
Fasting 0800–0830 h plasma						
Total testosterone (ng/dl)	67.5 ± 18.9	45.0 ± 16.4	<0.001	68.3 ± 28.0	48.2 ± 12.8	<0.001
Androstenedione (ng/dl)	349 ± 127	238 ± 79	<0.001	338 ± 142	223 ± 64	<0.001
DHEA-S (μg/ml)	2.31 ± 1.14	1.90 ± 0.60	0.046	2.22 ± 1.08	1.77 ± 0.62	0.011
SHBG (mmol/liter)	35.3 ± 14.1	63.2 ± 25.1	<0.001	23.5 ± 15.3	35.5 ± 19.8	0.001
Cortisol (μg/dl)	12.4 ± 6.3	15.4 ± 4.1	0.029	12.9 ± 5.1	13.0 ± 2.5	0.986
ACTH$_{1-24}$ stimulation test						
% $\Delta_{(60-0)}$ cortisol	170 ± 122	97 ± 67	0.009	166 ± 109	117 ± 33	0.317
% $\Delta_{(60-0)}$ DHEA	157 ± 282	109 ± 103	0.294	134 ± 102	114 ± 116	0.633
% $\Delta_{(60-0)}$ androstenedione	27 ± 37	37 ± 44	0.347	42 ± 40	35 ± 41	0.472
% $\Delta_{(60-0)}$ 17OH-progesterone	120 ± 94	220 ± 246	0.062	183 ± 134	180 ± 203	0.949
Dexamethasone suppression test						
% $\Delta_{(5-b)}$ cortisol	−93 ± 4	−94 ± 3	0.376	−93 ± 4	−93 ± 3	0.838
% $\Delta_{(5-b)}$ DHEA-S	−67 ± 36	−83 ± 7	0.060	−77 ± 11	−82 ± 7	0.287
% $\Delta_{(5-b)}$ androstenedione	−38 ± 29	−35 ± 24	0.806	−36 ± 37	−39 ± 25	0.644
% $\Delta_{(5-b)}$ 17OH-progesterone	−23 ± 69	−38 ± 43	0.323	−26 ± 48	−38 ± 40	0.416
Oral glucose tolerance test						
Glucose$_{AUC}$ (mg/dl·min)	17,624 ± 3,108	17,948 ± 2,716	0.798	21,565 ± 5,714	23,404 ± 5,343	0.068
Insulin$_{AUC}$ (μIU/ml·min)	9,373 ± 9,067	6,248 ± 3,056	0.324	19,154 ± 16,725	12,664 ± 8,902	0.005
QUICKI	0.366 ± 0.042	0.374 ± 0.031	0.391	0.317 ± 0.028	0.339 ± 0.059	0.004
ISI	8.43 ± 4.86	13.75 ± 9.02	<0.001	3.61 ± 2.36	5.98 ± 5.50	0.018
LDL-cholesterol (mg/dl)	76.4 ± 24.8	77.0 ± 16.8	0.915	111.6 ± 32.9	116.0 ± 22.3	0.358
HDL-cholesterol (mg/dl)	56.2 ± 12.9	60.5 ± 14.6	0.144	47.2 ± 11.7	54.2 ± 10.7	0.002
Triglycerides (mg/dl)	68.7 ± 25.7	60.7 ± 25.2	0.421	102.4 ± 49.1	119.5 ± 47.3	0.062

Data are means ± SD. To convert to SI units, multiply total testosterone by 0.0347 (result in nmol/liter), androstenedione by 0.0349 (result in nmol/liter), DHEA-S by 2.714 (result in μmol/liter), cortisol by 27.59 (result in nmol/liter), glucose by 0.0555 (result in mmol/liter), insulin by 6.945 (result in pmol/liter), LDL- and HDL-cholesterol by 0.0259 (result in mmol/liter), and triglycerides by 0.0113 (result in mmol/liter). % $\Delta_{(60-0)}$, Percent change in hormone levels (60-min value minus 0-min value) in response to ACTH$_{1-24}$; % $\Delta_{(5-b)}$, percent change in hormone levels (d-5 value minus baseline value) in response to dexamethasone; AUC, area under the curve of the oral glucose tolerance test, calculated by the trapezoidal rule.

[a] Effect of PCOS within lean and obese subjects evaluated by means of nesting design of two-way ANOVA.

higher, whereas HDL-cholesterol levels were significantly lower in obese than lean women in both PCOS and controls.

No effects of PCOS or obesity on basal cortisol levels, on the responsiveness of androgens to ACTH$_{1-24}$, and on the suppression of adrenal steroids by dexamethasone were observed.

HSD11B1 genotype (rs12086634) in PCOS women compared with controls

The allelic frequency of the T→G polymorphism (rs1208664) in the third intron of *HSD11B1* was 14% in the entire population. Sixty-seven percent (n = 68) of PCOS and 80% (n = 78) of controls were T/T; 33% (n = 34) of PCOS and 18% (n = 18) of controls were T/G; and 0% of PCOS and 2% (n = 2) of controls were G/G. Therefore, the G allele was significantly related to PCOS status, and this association was mainly attributable to lean, rather than obese, PCOS patients (Table 3).

Influence of the T/G+G/G HSD11B1 genotype (rs12086634) on clinical, hormonal, and metabolic characteristics within PCOS and controls

Table 4 and Figs. 1 and 2 show clinical and biochemical characteristics in PCOS women, according to the *HSD11B1* genotype (rs12086634) (T/T and T/G+G/G), whereas Table 5 shows the effect attributable to T/G+G/G *HSD11B1* genotype (rs12086634), to obesity, and to their interaction on all the characteristics analyzed within the PCOS group.

Among women with PCOS, the G allele of rs12086634 was associated with higher DHEA-S, lower 0800–0830 h plasma cortisol, higher cortisol response to ACTH$_{1-24}$, greater suppression of DHEA-S and androstenedione by dexamethasone, and lower fasting plasma LDL-cholesterol levels (Tables 4 and 5). When we considered the effect of obesity, we found that the associations of the G allele with cortisol and its response to ACTH$_{1-24}$ were significant in all PCOS women, independently of body weight, whereas associations with elevated DHEA-S concentrations and greater DHEA-S suppression after dexamethasone administration were significant in lean rather than obese PCOS women (Fig. 1 and Table 5). The *HSD11B1* rs1208664 genotype did not predict body fat distribution, insulin sensitivity, or glucose tolerance, although the G allele was associated with lower fasting plasma LDL-cholesterol in PCOS, particularly in lean PCOS women (Fig. 2 and Table 5). Among control subjects, *HSD11B1* rs1208664 genotype had no influence on any of the clinical, metabolic, and hormonal variables (data not shown).

TABLE 2. Estimated effect of PCOS status, obesity, and their interaction on clinical, hormonal, and metabolic characteristics in the entire population

Variables	PCOS status		Obesity		PCOS status × obesity	
	Effect (95% CI)	P value	Effect (95% CI)	P value	Effect (95% CI)	P value
Age (yr)	−0.40 (−2.09 to 1.30)	0.646	2.29 (0.59 to 3.99)	0.009	−0.70 (−4.10 to 2.70)	0.686
BMI (kg/m^2)	0.75 (−0.43 to 1.93)	0.210	14.20 (13.02 to 15.37)	<0.001	−2.66 (−5.01 to 0.30)	0.057
Waist circumference (cm)	1.94 (−1.21 to 5.09)	0.226	30.11 (26.96 to 33.26)	<0.001	−6.50 (−12.80 to 0.199)	0.063
Total abdominal fat (cm^2)	6.59 (−46.47 to 59.65)	0.806	341.28 (288.22 to 394.34)	<0.001	6.34 (−99.79 to 112.46)	0.906
Visceral abdominal fat (cm^2)	4.92 (−14.86 to 24.71)	0.623	68.42 (48.63 to 88.21)	<0.001	3.26 (−36.31 to 42.84)	0.871
sc abdominal fat (cm^2)	1.67 (−41.82 to 45.15)	0.940	272.86 (229.37 to 316.35)	<0.001	3.07 (−83.90 to 90.05)	0.944
Fasting 0800–0830 h plasma						
Total testosterone (ng/dl)	21.25 (15.37 to 27.14)	<0.001	1.99 (−3.89 to 7.88)	0.505	−2.35 (−14.12 to 9.42)	0.694
Androstenedione (ng/dl)	112.8 (78.7 to 146.9)	<0.001	−13.1 (−47.2 to 21.0)	0.449	4.0 (−64.2 to 72.2)	0.908
DHEA-S (μg/ml)	0.43 (0.16 to 0.70)	0.002	−0.11 (−0.38 to 0.16)	0.422	0.05 (−0.49 to 0.60)	0.848
SHBG (mmol/liter)	−19.9 (−25.5 to −14.3)	<0.001	−19.8 (−25.4 to −14.1)	<0.001	15.9 (4.6 to 27.1)	0.006
Cortisol (μg/dl)	−1.53 (−4.31 to 1.24)	0.276	−0.87 (−3.65 to 1.90)	0.534	2.98 (−2.57 to 8.53)	0.290
ACTH$_{1-24}$ stimulation test						
% $\Delta_{(60-0)}$ cortisol	61.1 (5.6 to 116.5)	0.031	8.3 (−47.1 to 63.8)	0.767	−23.9 (−134.9 to 87.0)	0.670
% $\Delta_{(60-0)}$ DHEA	34.1 (−27.1 to 95.3)	0.273	−8.8 (−70.0 to 52.5)	0.777	−28.1 (−150.5 to 94.4)	0.651
% $\Delta_{(60-0)}$ androstenedione	−1.4 (−15.5 to 12.7)	0.847	6.8 (−7.3 to 20.9)	0.341	16.8 (−11.3 to 45.0)	0.239
% $\Delta_{(60-0)}$ 17OH-progesterone	−48.5 (−107.3 to 10.2)	0.105	11.7 (−47.0 to 70.5)	0.694	102.2 (−15.3 to 219.8)	0.088
Dexamethasone suppression test						
% $\Delta_{(5-b)}$ cortisol	0.8 (−1.5 to 3.0)	0.513	0.6 (−1.6 to 2.9)	0.585	−0.7 (−5.3 to 3.8)	0.756
% $\Delta_{(5-b)}$ DHEA-S	11.2 (−3.4 to 18.9)	0.055	−4.3 (−12.1 to 3.5)	0.274	−11.4 (−27.0 to 4.1)	0.147
% $\Delta_{(5-b)}$ androstenedione	0.9 (−11.5 to 13.2)	0.890	−1.1 (−13.5 to 11.2)	0.855	6.2 (−18.5 to 31.0)	0.620
% $\Delta_{(5-b)}$ 17OH-progesterone	13.5 (−7.3 to 34.4)	0.202	−0.9 (−21.8 to 19.9)	0.932	−3.5 (−45.2 to 38.2)	0.868
Oral glucose tolerance test						
Glucose$_{AUC}$ (mg/dl·min)	−1082 (−2627 to 464)	0.169	4698 (3153 to 6244)	<0.001	−1515 (−4606 to 1575)	0.334
Insulin$_{AUC}$ (μIU/ml·min)	4807 (964 to 8651)	0.015	8099 (4256 to 11942)	<0.001	3365 (−4322 to 11051)	0.389
QUICKI	−0.015 (−0.027 to −0.003)	0.015	−0.042 (−0.054 to −0.030)	<0.001	−0.014 (−0.038 to 0.011)	0.272
ISI	−3.84 (−5.45 to −2.24)	<0.001	−6.30 (−7.91 to −4.69)	<0.001	2.94 (−0.27 to 6.16)	0.072
LDL-cholesterol (mg/dl)	−2.56 (−10.42 to 5.30)	0.522	37.12 (29.25 to 44.98)	<0.001	−3.78 (−19.49 to 11.96)	0.637
HDL-cholesterol (mg/dl)	−5.64 (−9.26 to −2.02)	0.002	−7.64 (−11.26 to −4.02)	<0.001	−2.74 (−9.98 to 4.51)	0.457
Triglycerides (mg/dl)	−4.56 (−16.90 to 7.79)	0.468	46.20 (33.85 to 58.54)	<0.001	−25.15 (−49.85 to 0.454)	0.066

The 95% CI are reported in *parentheses*. P values shown are for two-way ANOVA. Abbreviations and conversion factors to SI units are described in the legend to Table 1.

TABLE 3. Frequency of T/G+G/G HSD11B1 genotype (rs12086634) in PCOS women and controls

| | PCOS (%) | Controls (%) | PCOS vs. controls | | All cases (%) | Lean vs. obese |
			OR	P value		
Lean	15/38 (40)	6/38 (16)	3.48 (1.17–10.3)	0.025[a]	21/76 (28)	P = 0.848[b]
Obese	19/64 (30)	14/60 (24)	1.39 (0.62–3.10)	0.424[a]	33/124 (27)	
All cases	34/102 (33)	20/98 (20)	1.95 (1.03–3.70)	0.041[b]		

A borderline significant effect of the interaction between PCOS status and obesity on T/G+G/G HSD11B1 genotype (rs12086634) was observed (P = 0.056).
[a] Univariate logistic regression.
[b] Multivariate logistic regression.

Discussion

This case-control study shows that a polymorphism that predicts lower 11β-HSD1-dependent peripheral regeneration of cortisol is 1) related to PCOS status, 2) associated with lower morning cortisol values and increased adrenal cortisol response to ACTH (consistent with compensatory activation of the hypothalamic-pituitary adrenal axis in response to an enhanced metabolic clearance rate for cortisol) in PCOS, and 3) associated with increased adrenal hyperandrogenism and with a protective serum lipid profile (lower LDL-cholesterol levels) in lean PCOS. These data linking genotype with both intermediate phenotype and disease prevalence strongly support a role for the HSD11B1 gene in the pathogenesis of PCOS, at least in a subgroup of patients.

The association of the HSD11B1 genotype with PCOS was mainly attributable to lean, rather than obese, PCOS patients; lean patients are relatively enriched in this study cohort by the selection criteria for lean and obese groups. The lack of overweight subjects (BMI, 26–29 kg/m²) distinguishes this study from that of San Millán et al. (30), performed in another population of Caucasian women, in which no association between HSD11B1 genotype and PCOS was found. In addition, ovarian morphology was not used in the diagnostic criteria by San Millán et al. (30), suggesting that different populations of PCOS and control subjects were included in the two studies and reinforcing that HSD11B1 polymorphisms may be relevant only in some subgroups of this heterogeneous condition. Also, data from White (31) did not show association between HSD11B1 genotype and PCOS. However, these data were from a multiethnic population, and the criteria used by the author to diagnose PCOS (only presence of more than 10 cysts detected by magnetic resonance imaging in one or both ovaries) are inadequate, because they are not in accordance with the diagnostic criteria recommended either by the National Institutes of Health (32) or the Rotterdam Consensus (21).

The finding of an association of HSD11B1 genotype with adrenal hyperandrogenism only in lean PCOS women suggests that in obese PCOS women, adrenal hyperandrogenism must have a different pathogenetic mechanism. This might involve abnormal adrenal steroidogenesis as a consequence

TABLE 4. Clinical, hormonal, and metabolic characteristics in lean and obese PCOS women according to the HSD11B1 genotype (rs12086634)

| Variables | Lean PCOS | | | Obese PCOS | | |
	T/T (n = 23)	T/G+G/G (n = 15)	P value[a]	T/T (n = 45)	T/G+G/G (n = 19)	P value[a]
Age (yr)	24.1 ± 4.7	23.7 ± 4.8	0.843	26.6 ± 6.9	24.1 ± 5.7	0.132
BMI (kg/m²)	22.2 ± 2.2	24.0 ± 1.4	0.214	35.4 ± 3.8	36.7 ± 7.6	0.261
Waist circumference (cm)	77.8 ± 6.9	72.2 ± 4.4	0.115	103.4 ± 14.7	100.2 ± 11.3	0.272
Total abdominal fat (cm²)	229 ± 105	316 ± 63	0.215	602 ± 125	621 ± 211	0.653
Visceral abdominal fat (cm²)	35 ± 13	42 ± 23	0.786	106 ± 50	111 ± 83	0.755
sc abdominal fat (cm²)	195 ± 101	274 ± 62	0.158	496 ± 94	510 ± 171	0.681
Fasting 0800–0830 h plasma						
Total testosterone (ng/dl)	71.1 ± 19.9	61.9 ± 16.1	0.276	68.9 ± 28.1	66.8 ± 28.5	0.757
Androstenedione (ng/dl)	361 ± 122	330 ± 136	0.491	323 ± 128	371 ± 168	0.200
SHBG (mmol/liter)	36.9 ± 15.3	33.1 ± 12.5	0.460	23.3 ± 15.7	24.0 ± 14.5	0.868
$ACTH_{1-24}$ stimulation test						
% $\Delta_{(60-0)}$ DHEA	175 ± 339	125 ± 138	0.455	136 ± 90	130 ± 129	0.920
% $\Delta_{(60-0)}$ androstenedione	31 ± 40	20 ± 32	0.379	39 ± 38	48 ± 42	0.418
% $\Delta_{(60-0)}$ 17OH-progesterone	113 ± 97	131 ± 92	0.667	185 ± 143	178 ± 113	0.834
Dexamethasone suppression test						
% $\Delta_{(5-b)}$ cortisol	−94 ± 3	−92 ± 6	0.140	−93 ± 2	−92 ± 7	0.184
% $\Delta_{(5-b)}$ androstenedione	−32 ± 29	−47 ± 27	0.245	−30 ± 40	−48 ± 27	0.064
% $\Delta_{(5-b)}$ 17OH-progesterone	−16 ± 79	−34 ± 55	0.375	−21 ± 51	−36 ± 39	0.336
Oral glucose tolerance test						
$Glucose_{AUC}$ (mg/dl·min)	17,154 ± 3,154	18,363 ± 2,996	0.474	21,192 ± 4,504	22,487 ± 8,068	0.362
$Insulin_{AUC}$ (μIU/ml·min)	9,062 ± 7,510	9,922 ± 11,655	0.864	18,763 ± 17,674	20,025 ± 14,836	0.759
QUICKI	0.366 ± 0.048	0.366 ± 0.032	0.984	0.318 ± 0.029	0.314 ± 0.027	0.630
ISI	8.67 ± 5.65	8.07 ± 3.44	0.604	3.66 ± 2.36	3.46 ± 2.41	0.832
HDL-cholesterol (mg/dl)	55.6 ± 12.1	57.1 ± 14.5	0.708	44.2 ± 13.3	48.5 ± 10.9	0.206
Triglycerides (mg/dl)	79.3 ± 25.9	52.6 ± 15.3	0.046	100.6 ± 47.2	106.5 ± 54.5	0.604

Data are means ± SD. Abbreviations and conversion factors to SI units are described in the legend to Table 1.
[a] Effect of the HSD11B1 genotype (rs12086634) within lean and obese subjects evaluated by means of nesting design of two-way ANOVA.

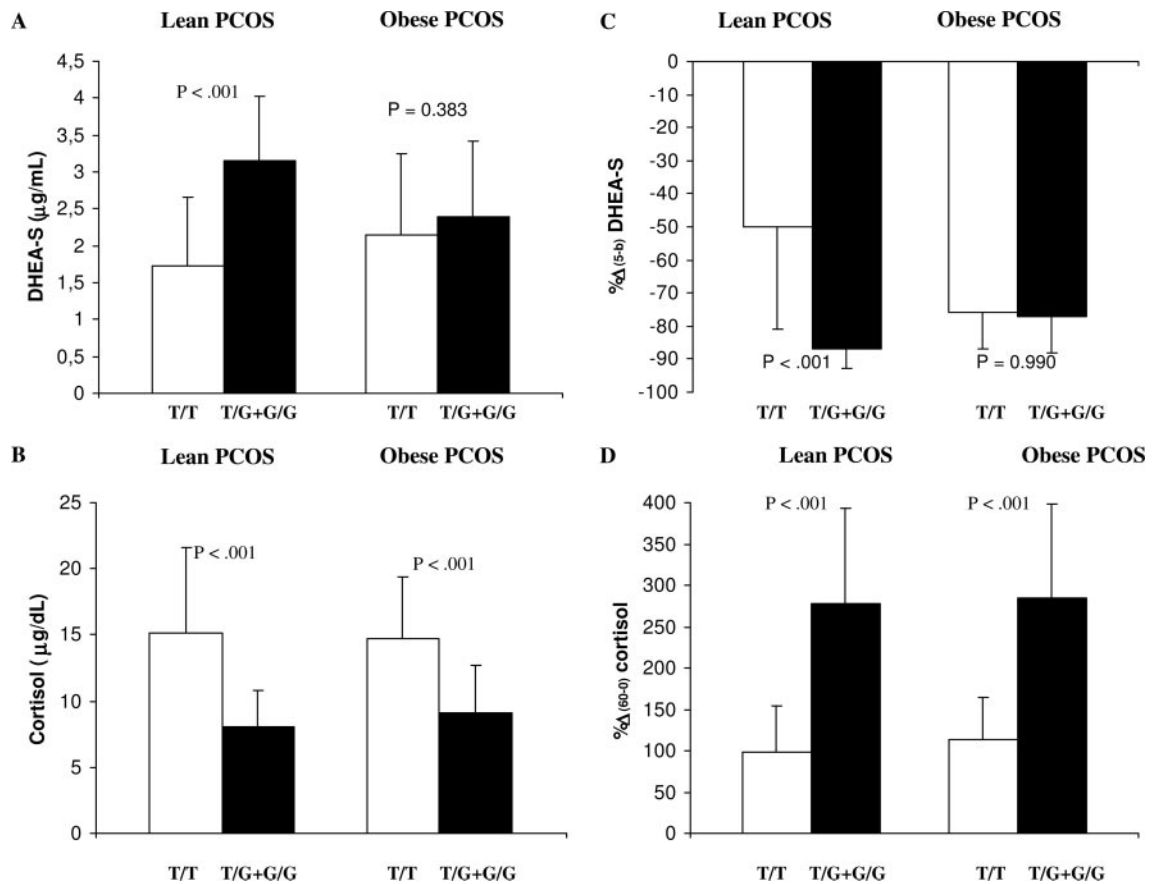FIG. 1. Fasting plasma DHEA-S (A) and cortisol levels (B), percent change in DHEA-S levels in response to dexamethasone (% $\Delta_{(5-b)}$ DHEA-S) (C), and percent change in cortisol levels in response to ACTH$_{1-24}$ (% $\Delta_{(60-0)}$ cortisol) (D) in lean and obese PCOS women according to the *HSD11B1* genotype (rs12086634) (T/T and T/G+G/G). *P* values refer to the effect of the *HSD11B1* genotype (rs12086634) within lean and obese PCOS women. Abbreviations and conversion factors to SI units are described in the legend to Table 1.

of hyperinsulinemia (33) or may also be related to increased cortisol clearance, because obese patients have increased metabolic clearance of cortisol by 5α-reductase (34).
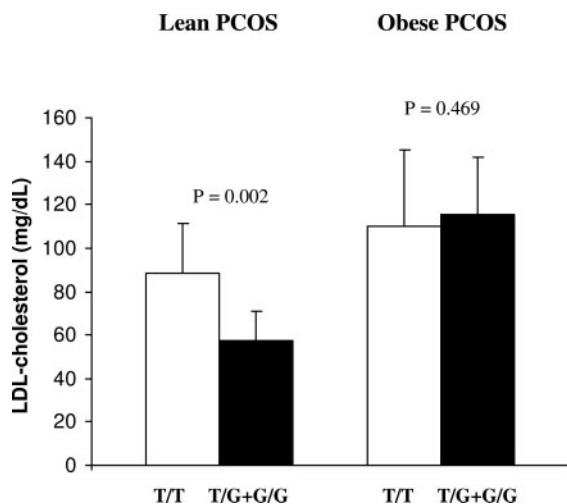

FIG. 2. Fasting plasma LDL-cholesterol levels in lean and obese PCOS women according to the *HSD11B1* genotype (rs12086634) (T/T and T/G+G/G). *P* values refer to the effect of the *HSD11B1* genotype (rs12086634) within lean and obese PCOS women. Abbreviations and conversion factors to SI units are described in the legend to Table 1.

Among PCOS women, the apparent lower prevalence of rs1208664 T→G in the obese than lean patients also suggests that this genotype either reduces the chances of becoming obese or reduces the chance of developing PCOS in the presence of obesity. This could be attributed to lower intracellular cortisol levels, particularly in adipose tissue and liver, which are protective against features of the metabolic syndrome (1, 2). This is supported by the atheroprotective lipid profile observed in lean PCOS women carrying the G allele.

However, contrary to our expectations, we did not find any association of the *HSD11B1* genotype with measures of insulin sensitivity. This result is in accordance with the study performed by San Millán *et al.* (30) but in apparent contrast with a previous report obtained in a large population of Pima Indians (10). This discrepancy might be attributed to ethnic and environmental differences between the cohorts or to the different methods used to asses insulin sensitivity. The principal association in the Pima Indians was with insulin sensitivity in a euglycemic hyperinsulinemic clamp rather than with fasting insulin levels and insulin responses to a glucose load, the methods used by us and by San Millán *et al.* (30).

The lack of association of *HSD11B1* genotype with biochemical phenotype in control subjects may reflect the lack of statistical power or the presence of modifying environmental or genetic factors.

TABLE 5. Estimated effect of T/G+G/G *HSD11B1* genotype (rs12086634) and obesity and their interaction on clinical, hormonal, and metabolic characteristics in the population of PCOS

Variables	T/G+G/G		Obesity		T/G+G/G × obesity	
	Effect (95% CI)	P value	Effect (95% CI)	P value	Effect (95% CI)	P value
Age (yr)	−1.43 (−3.97 to 1.11)	0.266	1.48 (−1.06 to 4.02)	0.250	−2.08 (−7.15 to 3.00)	0.418
BMI (kg/m²)	1.55 (−0.28 to 3.38)	0.095	12.95 (11.12 to 14.78)	<0.001	−0.45 (−4.11 to 3.20)	0.807
Waist circumference (cm)	−4.38 (−8.88 to 0.12)	0.056	26.82 (22.31 to 31.32)	<0.001	−2.47 (−11.47 to 6.54)	0.588
Total abdominal fat (cm²)	52.82 (−27.95 to 133.60)	0.196	338.51 (257.73 to 419.28)	<0.001	−67.94 (−229.50 to 93.61)	0.404
Visceral abdominal fat (cm²)	6.11 (−24.75 to 36.98)	0.694	70.23 (39.36 to 101.09)	<0.001	−2.23 (−63.96 to 59.50)	0.943
sc abdominal fat (cm²)	46.71 (−18.20 to 111.63)	0.156	268.28 (203.37 to 333.19)	<0.001	−65.71 (−195.54 to 64.12)	0.316
Fasting 0800–0830 h plasma						
Total testosterone (ng/dl)	−5.63 (−16.34 to 5.09)	0.300	1.34 (−9.37 to 12.06)	0.804	6.99 (−14.44 to 28.42)	0.519
Androstenedione (ng/dl)	8.6 (−49.7 to 66.9)	0.770	1.6 (−56.8 to 59.9)	0.958	79.7 (−36.9 to 196.3)	0.178
DHEA-S (µg/ml)	0.83 (0.40 to 1.27)	<0.001	−0.17 (−0.61 to 0.27)	0.444	−1.18 (−2.06 to −0.30)	0.009
SHBG (mmol/liter)	−1.5 (−7.9 to 4.9)	0.640	−11.3 (−17.8 to −4.9)	0.001	4.4 (−8.4 to 17.3)	0.498
Cortisol (µg/dl)	−6.38 (−8.41 to −4.34)	<0.001	0.27 (−1.76 to 2.31)	0.792	1.46 (−2.61 to 5.52)	0.479
ACTH$_{1-24}$ stimulation test						
% Δ$_{(60-0)}$ cortisol	175.1 (141.4 to 208.9)	<0.001	10.4 (−23.4 to 44.2)	0.543	−9.6 (−77.2 to 58.0)	0.778
% Δ$_{(60-0)}$ DHEA	−27.4 (−111.0 to 56.2)	0.517	−16.9 (−100.6 to 66.7)	0.688	44.3 (−123.0 to 211.5)	0.601
% Δ$_{(60-0)}$ androstenedione	−1.3 (−18.0 to 15.3)	0.876	18.0 (1.4 to 34.7)	0.034	20.1 (−13.2 to 53.5)	0.233
% Δ$_{(60-0)}$ 17OH-progesterone	5.3 (−47.9 to 58.5)	0.844	59.3 (6.0 to 112.5)	0.029	−25.0 (−131.5 to 81.5)	0.642
Dexamethasone suppression test						
% Δ$_{(5-b)}$ cortisol	1.89 (−0.01 to 3.77)	0.058	0.36 (−1.52 to 2.23)	0.707	−0.62 (−4.38 to 3.14)	0.744
% Δ$_{(5-b)}$ DHEA-S	−18.5 (−30.4 to −6.6)	0.003	−7.7 (−19.6 to 4.2)	0.197	36.8 (13.1 to 60.6)	0.003
% Δ$_{(5-b)}$ androstenedione	−16.2 (−31.8 to −0.7)	0.041	0.3 (−15.3 to 15.8)	0.972	−3.5 (−34.6 to 27.6)	0.822
% Δ$_{(5-b)}$ 17OH-progesterone	−17.0 (−43.1 to 9.1)	0.200	−3.6 (−29.8 to 22.5)	0.782	3.7 (−48.5 to 55.9)	0.889
Oral glucose tolerance test						
Glucose$_{AUC}$ (mg/dl·min)	1252 (−928 to 3433)	0.257	4081 (1900 to 6261)	<0.001	86 (−4276 to 4447)	0.969
Insulin$_{AUC}$ (µIU/ml·min)	1061 (−5374 to 7496)	0.744	9902 (3467 to 16337)	0.003	403 (−12468 to 13273)	0.951
QUICKI	−0.002 (−0.017 to 0.012)	0.748	−0.050 (−0.064 to −0.035)	<0.001	−0.004 (−0.034 to 0.025)	0.771
ISI	−0.41 (−1.91 to 1.10)	0.593	−4.81 (−6.31 to −3.30)	<0.001	0.40 (−2.61 to 3.41)	0.791
LDL-cholesterol (mg/dl)	−12.55 (−24.90 to −0.20)	0.047	39.65 (27.30 to 52.00)	<0.001	36.62 (11.92 to 61.32)	0.004
HDL-cholesterol (mg/dl)	−1.37 (−6.58 to 3.85)	0.604	−10.03 (−15.25 to −4.82)	<0.001	−5.78 (−16.21 to 4.65)	0.274
Triglycerides (mg/dl)	−10.37 (−28.12 to 7.39)	0.249	37.63 (19.88 to 55.39)	<0.001	32.59 (−2.92 to 68.10)	0.072

The 95% CI are reported in *parentheses*. *P* values shown are for two-way ANOVA. Abbreviations and conversion factors to SI units are described in the legend to Table 1.

In conclusion, genetic variation in 11β-HSD1 may underlie adrenal hyperandrogenism in lean patients with PCOS but may protect against obesity and associated metabolic dysfunction. These observations lend additional support to the concept that the pathogenesis of PCOS is different among the different phenotypes of the syndrome.

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