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Glucocorticoid Resistance in Premature Adrenarche and PCOS: From Childhood to Adulthood

Aristotle Panayiotopoulos,[1] Amrit Bhangoo,[2] Divya Khurana,[3] Svetlana Ten,[4] Josef Michl,[5] and Steven Ghanny[6]

[1]Pediatric Endocrinology, Staten Island University Hospital, Staten Island, New York 11364; [2]Pediatric Endocrinology, CHOC Children's' Hospital, Orange, California 92618; [3]Pediatric Endocrinology, Texas Tech University, Lubbock, Texas 79430; [4]Pediatric Endocrinology, Richmond University Medical Center, Staten Island, New York 10310; [5]Departments of Pathology, Cell and Molecular Biology, SUNY Downstate Medical Center, Brooklyn, New York 11235; and [6]Pediatric Endocrinology, Hackensack University Medical Center, Hackensack, New Jersey 07601

ORCiD number: 0000-0002-1443-4489 (A. Bhangoo).

Context: We hypothesize that impaired glucocorticoid sensitivity (GC sensitivity) plays a role in the development of premature adrenarche (PA) and polycystic ovarian syndrome (PCOS) by increasing androgen synthesis.

Objective: To study glucocorticoid sensitivity in vitro in subjects with PA and PCOS.

Patients and Methods: Fourteen subjects (10 girls, 4 boys, 6.9 ± 0.6 years) with PA; 27 subjects with PCOS (17 ± 2.5 years) and 31 healthy controls were enrolled in the study. All subjects and controls underwent GC sensitivity analysis in vitro using a fluorescein labeled-dexamethasone (F-DEX) assay. A GC sensitivity index (GCSI) was calculated as area under the curve of the F-DEX assay results. Subjects were classified as GC resistant if the GCSI ≤ 264 and GC sensitive if the GCSI ≥ 386.

Results: In the PA group, 8 of 14 subjects were resistant with GCSI of 179.7 ± 39.9, 4 were within the normal range with GCSI of 299.6 ± 27.9, and 2 had increased GC sensitivity with GCSI of 423.5 ± 47.9. In the PCOS group, 18 of 27 subjects were GC-resistant with GCSI of 180.9 ± 58.2, 8 were within the normal range with GCSI of 310.7 ± 26.4, and 1 had increased GCSI of 395.4. In the PCOS GC-resistant subgroup, cortisol was higher compared with PCOS with normal GCSI ($P < 0.05$). In the combined PCOS plus female control group, GCSI correlated negatively with cortisol and testosterone ($P < 0.05$).

Conclusion: GC resistance was found in more than 50% of patients with PCOS and PA. The findings strongly suggest that GC resistance is associated with states of PA and PCOS.

Key Words: premature adrenarche, PCOS, glucocorticoid receptor, glucocorticoid resistance, glucocorticoid sensitivity index

Abbreviations: bB, binding buffer; CAH, congenital adrenal hyperplasia; cCM, complete Culture Medium; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; FBS, fetal bovine serum; F-DEX, fluorescein-labeled dexamethasone; huGCR, human glucocorticoid receptor; GC, glucocorticoid; GCSI, glucocorticoid sensitivity index; H6PDH, hexose-6-phosphate dehydrogenase; PA, premature adrenarche; PCOS, polycystic ovarian syndrome; PBMC, peripheral blood mononuclear cells.

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Adrenarche is the maturation of the adrenal glands typically occurring at 8 to 9 years of age [1]. Premature adrenarche (PA) presents when there is an increase in androgens from the adrenal gland before the age of 8 years in girls and 9 years in boys. PA is usually associated with tall stature for a patient's age and advancement of skeletal maturation [2]. Although PA is commonly encountered in clinical practice, it is a phenomenon that is still not well understood. PA has been recognized also as a forerunner of polycystic ovarian syndrome (PCOS), metabolic syndrome in men and women, and a rare sign of glucocorticoid (GC) resistance [3-5].

During adrenarche, there is a physiological release of the androgen precursors dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) by the zona reticularis. DHEA is then converted to androstenedione and testosterone in the gonads and peripheral tissues, leading to clinical signs such as development of pubic hair, axillary hair, adult body odor, oily hair, and acne [4, 6, 7]. DHEA elevation by itself can exaggerate GC resistance because DHEA decreases nuclear human GC receptor (huGCR) levels in the hippocampus [8]. DHEA has also been shown to decrease 11β-hydroxyl steroid dehydrogenase 1 activity and inhibit the expression of hexose-6-phosphate dehydrogenase (H6PDH), while inducing 11β-HSD2 activity [9, 10]. This would divert overall cortisol production to cortisone resulting in hypothalamic-pituitary-adrenal axis activation and excessive ACTH driven adrenal hyperandrogenism and hypercortisolism, similar to a state of GC resistance [11].

During the initial investigation of PA, it is important to rule out central precocious puberty, congenital adrenal hyperplasia (CAH) [12], virilizing tumors, Cushing as well as McCune Albright syndromes, cases of activating LH receptor and exogenous hormone exposures [13], inactivating PAPS synthase type 2 mutation [14], nonclassical forms of 11β-hydroxylase deficiency [15], 3β-hydroxysteroid dehydrogenase type 2 [16-18], apparent cortisone reductase deficiency, and inactivating mutations in H6PDH [19]. By eliminating the existence any of these conditions in a patient, the diagnosis of idiopathic PA can be made without further investigations [2].

PA presents more in females than in males in a ratio of 10:1. Daughters of women with PCOS have a 30% prevalence of PA [5]. This finding indicates that PA is not a normal variant of pubertal development as previously thought, but may present a clinical marker for future disorders such as PCOS, metabolic syndrome, and impaired sensitivity to GCs [3].

GCs, a group of steroid hormones, have important roles in development and physiological functions throughout human life. Glucocorticoids coupled to huGCR becomes bound to glucocorticoid response elements on DNA, where it initiates the transcription of numerous target genes [20]. Mutations of the huGCR leading to GC resistance have been described in cases of PA [21], PCOS [22, 23], and infertility [24-26]. Resistance to cortisol leads to a compensatory increased secretion of corticotropin-releasing hormone and ACTH, cortisol, androgen, as well as androgen precursors such as androstenedione, DHEA, and DHEAS, resulting in the androgen excess conditions of PA and PCOS. The importance of ACTH in the induction of adrenarche was confirmed in cases of familial glucocorticoid deficiency and ACTH receptor mutations [27]. The lack of ACTH action led to diminished androgen secretion and absence of adrenarche [27]. Lower DHEA and DHEAS levels were found in patients with adrenal and subclinical Cushing syndrome, supporting previous suggestions that ACTH is a major determinant of their secretion [28].

We have hypothesized that GC sensitivity is altered in subgroups of PA and PCOS patients. To test this hypothesis, we evaluated GC sensitivity in 14 children with PA and 27 patients with PCOS and compared the results with those of normal controls. A fluorescein labeled-dexamethasone (F-DEX)-mononuclear cell-binding assay in vitro was used to analyze GC sensitivity.

1. Subjects, Materials, and Methods

A. Subjects

Fourteen children with PA, which included 10 girls and 4 boys with a mean age of 6.9 ± 0.6 years, were enrolled in the study (Table 1). The inclusion criteria for the study

were the findings of elevated DHEA or DHEAS and/or 17 hydroxypregnenolone levels before the age of 8 years with presence of body odor, axillary hair, and/or pubic hair development. Exclusion criteria were precocious puberty, nonclassical CAH, and exogenous hormonal exposure.

Twenty-seven patients with PCOS as per the National Institutes of Health criteria [29] were enrolled in the study with a mean age of 17 ± 2.5 years (Table 2). Inclusion criteria were irregular periods with clinical signs of clinical hyperandrogenism including acne, hirsutism, and/or male pattern alopecia. All patients with PCOS had elevated testosterone levels. LH, FSH, and estradiol levels were measured for the exclusion of hypogonadotropic hypogonadism and ovarian failure [30]. Exclusion criteria were nonclassical CAH and exogenous hormone exposure.

A total 31 of controls were recruited. The controls had no history of PA and/or PCOS or any other disorders involving the glucocorticoid pathway. Of the 20 controls for the group of PCOS patients, a steroid profile was obtained in 9 females (16.1 ± 2.5 years), whereas in the remaining 5 females and 6 males (28.8 ± 2.1 years), a steroid profile could not be obtained. An ACTH test was done in 11 females from the PCOS group and in 4 controls. Of 11 controls for the PA group (8 males and 3 females, 8.3 ± 3.0 years), a steroid profile was obtained in 8 (5 boys and 3 girls). An ACTH test was done in 10 children from the PA patient group

The study was a case-control study approved by the institutional review board at Maimonides Medical Center and SUNY Downstate Medical Center (Protocol Number

Table 1. Characteristic of the Subjects in the PA Group

	PA	Control (n = 11)	Normal Value
Age (yr)	6.9 ± 0.6	8.3 ± 3	
DHEA ng/dL	299 ± 259	191 ± 138	<111
DHEAS µg/dL	135 ± 96	106 ± 69	15–113
Cortisol µg/dL	16.6 ± 5.7[a]	8.4 ± 2.4	3–25
60-minute post ACTH stimulated cortisol levels µg/dL	28.3 ± 5.4	-	
17 OHP ng/dL	95.7 ± 80[a]	21 ± 14	<72
17Δ 5P ng/dL	266 ± 142*	93 ± 74	<91
Testosterone ng/mL	11.2 ± 9.1	6 ± 7	<20
Androstenedione ng/dL	39.6 ± 18	41 ± 18	6–115
ACTH pg/mL	53 ± 62*	12 ± 6	6–48

Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; PA, premature adrenarche; 17 OHP, 17 Hydroxyprogesterone.
[a]Statistical significance ($P < 0.05$) of the respective hormone measurements in PA patients vs controls.

Table 2. Characteristics of Subjects in PCOS Group

	PCOS	Control (n = 9)[a]	Normal Value
Age (yr)	17 ± 2.5	16.1 ± 2.5	
DHEA ng/dL	685.2 ± 449	455 ± 407	113–1360
DHEAS µg/dL	241 ± 119[b]	115 ± 67	18–391
Cortisol µg/dL	13 ± 7	12 ± 5	3–25
17 OHP ng/dL	109 ± 81	106 ± 82	<285
17Δ 5P ng/dL	265 ± 280	558 ± 898	<756
Testosterone ng/mL	75 ± 30*	27.9 ± 9	2–45
Androstenedione ng/dL	222 ± 123*	94 ± 28	22–225
ACTH pg/mL	25 ± 21	25 ± 20	6–48

Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; PCOS, polycystic ovarian syndrome.
[a]Steroid profile was available on 9 of 20 controls in this group.
[b]Statistical significance ($P < 0.05$) of the respective hormone measurements in PCOS patients vs controls.

10-183). Informed assent and consent were obtained from each subject and her or his parents or guardian.

B. Methods

In all PCOS and PA patients, baseline hormonal and biochemical profiles were assessed by established clinical laboratory tests including those for DHEA, DHEAS, cortisol, ACTH, 17 hydroxyprogesterone, 17 hydroxypregnenolone, androstenedione, and testosterone (Tables 1 and 2). GC sensitivity was assessed in all patients and controls using a F-DEX-mononuclear cell-binding assay. The number of huGCR was measured by flow cytometry in 10 PCOS patients and 17 controls.

B-1. huGCR binding protocol: F-DEX-mononuclear cell-binding assay using peripheral blood mononuclear cells. Blood was drawn into heparinized tubes via venipuncture from patients, while they were in the clinic. The blood was diluted 2-fold with normal saline and layered over a Ficoll 400 -Hypaque-solution (density 1.078 g/ml) (Pharmacia -GE HealthCare) as described [31]. The interphase enriched for peripheral blood mononuclear cells (PBMCs) was isolated and the cells were washed with normal saline. The final cell pellet was resuspended in 8 mL of complete Culture Medium (cCM) composed of DMEM (Gibco Laboratories, Grand Island, NY) supplemented with glucose (4.5 mg/L) (high glucose), 110 mg/L Na-pyruvate, 4.0 mM L glutamine, 25 mM HEPES, 10% fetal bovine serum (FBS; HyClone Charcoal/Dextran Treated FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin (all from Gibco Labs) and incubated at 37°C in a shaking water bath to remove endogenous cortisol. Thirty minutes later, the cells were centrifuged and resuspended in fresh cCM, a procedure that was repeated twice. After the last wash, the cells were resuspended in 5 mL of fresh cCM and counted using Trypan blue solution to establish the cells' viability and number. Cell viability was routinely found to be >93%. The PBMCs from each subject were then separated into 2 parts, 1 of which was immediately transferred into binding buffer (bB; 145 mM NaCl, 4 mM KCL, 1 mM Na2HPO4, 0.8 mM CaCl2, 25 mM HEPES, 22 mM glucose) to be processed for the detection of GC-specific cell surface receptors. From the second part of the freshly isolated cells, the monocytes were purified by negative sorting as described in the following section to allow the direct observation of F-DEX binding to the cells.

B-2. Monocyte separation and F-DEX-binding assay. After obtaining PBMC as described, a part of the cell suspension was centrifuged at 300*g* for 10 minutes, the spent supernatant was removed, and the PBMCs were resuspended in ice cold PBS pH7.2 supplemented with 0.5% FBS and 2 nM EDTA (IncBuffer), counted and 30 µL containing 10^7 cells/tube were distributed into several microcentrifuge tubes. To each tube were added immediately 10 µL of FcγR Blocking Reagent (Miltenyi Biotec, Cambridge, MA); the mixture was incubated for 5 minutes on ice, at which time 10 µL of Biotin-Antibody Cocktail (Miltenyi Biotec Pan Monocyte Isolation Kit) were added to each tube [32]. After careful mixing, the incubation was continued on ice for another 15 minutes, when 30 µL of IncBuffer were added to each tube followed by addition of 20 µL of anti-Biotin Magnetic Micro Beads (Miltenyi Biotec) and careful mixing. After another 15 minutes of incubation, fresh IncBuffer (2 mL/tube) was added and the tubes were centrifuged at 300*g* for 10 minutes in a precooled centrifuge. The supernatant was then completely removed and the 1×10^7 cells in each tube were resuspended in 500 µL of fresh ice-cold IncBuffer. The resuspended cells were then placed over a magnetic field for 20 to 30 minutes and the magnetic beads with the magnetized cells attached moved and adhered to the side of the Eppendorf tube directly positioned to the magnetic field. At this point, any liquid along the walls of the tubes that were bare of beads as well as in the bottom of the tubes was carefully suctioned out and

transferred into a set of fresh microcentrifuge tubes which were then centrifuged in the cold for 10 minutes at 250*g*.

The negatively sorted and pelleted monocytes were then resuspended in IncBuffer, counted, diluted to 2×10^5/mL of which 0.1 mL was distributed into each well of a 96-well high-affinity tissue culture plate and allowed to adhere for 1 hour at 37°C followed by incubation in cCM (0.2 mL/well) at 37° in an incubator providing a 5% CO2-95% air mixture. The next day, the cells were washed twice with PBS and once with nonfluorescent Bb. The buffer was then replaced in triplicate wells with 0.1 mL PBS/well containing F-DEX (Invitrogen Life sciences) at concentrations from 400 nm to 6400 nm. To correct for background fluorescence, an additional triplicate set of wells received Bb but without F-DEX. After 1 hour at 37°C, fresh PBS (0.2 mL) was added to each well and the unbound F-DEX was washed off with an additional 2 washes in PBS before the cell-bound F-DEX was detected by reading the fluorescein intensity at OD 488 nm bound to the monocytes in each well in a Microplate Fluorimeter Series 7600 reader (Cambridge Technology, Bedford, MA).

B-3. Cell labeling for flow cytometry to detect number of huGCR receptors. On the same day that cells were collected from the PBMC purification, 4 tubes (labeled 1 through 4) each containing 10^6 cells in 0.1 mL bB were prepared as follows: In tube 1, GCR antibody was added [33]. In tube 2, 100 µL of 1:2 diluted unlabeled dexamethasone (Fisher Scientific) were added. In tube 3, 100 µL of mifepristone (Fisher Scientific) +100 µL of 1:2 diluted unlabeled dexamethasone were added. In tube 4, GCR antibody and 100 µL of F-DEX were added. The tubes were then incubated for 10 to 15 minutes at 37°C when 100 µL of F-DEX (6400 nm) were added to the cells in all tubes [1-4] followed by incubation for another 1 hour at 37°C. The tubes were then centrifuged at 125*g* for 10 minutes, the supernatant was removed from all cell pellets, and the cells were resuspended in 1 mL fresh PBS/tube, which were washed a second time before being pelleted and incubated in 100 µL of 3% buffered (pH 7.2) paraformaldehyde overnight. On the following morning, PBS was added (500 µL/tube) followed by centrifugation at 125*g*. After repeating this washing step twice, the cells were resuspended in 1 mL of fresh PBS and analyzed by flow cytometry on a Beckman Coulter FACS Analyzer.

C. Statistical analysis

The GC sensitivity index (GCSI) was calculated as area under the curve of F-DEX-monocyte binding for control and subjects. Area under the curve was determined by using the linear trapezoidal rule [34]. Student *t* tests were used to compare the GCSI between subjects and controls (Excel v 14.3.8). A result was considered statistically significant if $P < 0.05$.

3. Results

To eliminate the possibility that changes in receptor number was the cause of the noted low GCSI, receptor number studies were done. These studies demonstrated that the number of huGCR when compared between 10 PCOS subjects (16 382 ± 11 011) and 17 controls (11 205 ± 6562) and was found to be similar ($P = 0.20$) as measured by flow cytometry (Fig. 1).

There was 1 outlier in the PCOS group expressing increased numbers of huGCR at 40 430 with a very low GCSI of 67.8. Despite of the increased number of huGCR, the patient was GC resistant. These findings strongly indicate that the differences in GCSI between patients and controls were due to differences in binding of GCs to the receptor and not to the receptor numbers. A normative GCSI was calculated as 325 ± 30.6 (mean ± SD score) from 31 controls. To ascertain the validity of the new assay and its results, a healthy volunteer control and designated as the “control of the day” was included in each

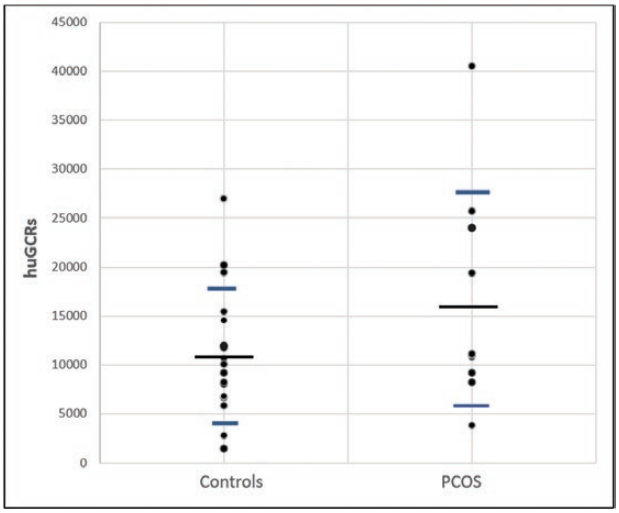

Figure 1. huGCRs number for controls and subjects as established by flow cytometry on PBMC ($P = 0.20$). Ten PCOS subjects (16 382 ± 11 011) and 17 controls (11 205 ± 6562) and was found to be similar ($P = 0.19$) as measured by flow cytometry. huGCR, human glucocorticoid receptor; PCOS, polycystic ovarian syndrome.

test and processed in parallel with the patient sample to standardize all results to the established control curve (Fig. 2). This practice confirmed that the GCSI of 325 ± 30.6 (mean ± SD score) as obtained by the F-DEX-mononuclear cell-binding assay was reproducible when measured on different days with PBMCs from the same or different control donors. A normal GCSI was between 265 and 385. A lower GCSI represents GC resistance. Subjects were classified as GC resistant if the GCSI ≤ 264. The subjects with a GCSI ≥ 386 were considered GC sensitive. There were no differences in the GCSI between children and young adults nor between males and females in the control group. In the PA group of 14 patients and 57.1% (8 patients) were found to be resistant with a low GCSI of 179.7 ± 39.9, 28.6% (4 patients) were within the normal range with a GCSI of 299.6 ± 27.9 (Figs. 3 and 4). The baseline cortisol levels did not correlate with the GCSI in PA. However, the 60-minute simulated post-ACTH stimulated cortisol levels correlated with the GCSI ($R^2 = 0.6$, $P = 0.004$; Fig. 5).

In the 27 patients of the PCOS group, 66.7% or 18 patients were found to be GC resistant with a GCSI of 180.9 ± 58.2, 29.6% or 8 patients were within the normal range with a GCSI of 310.7 ± 26.4, and only 3.7% or 1 patient was found with an increased GCSI of 395.4 (Figs. 6-8). In the PCOS GC-resistant subgroup, cortisol was higher at 17.5 ± 7.2 µg/dL when compared with the PCOS subgroup expressing normal GC sensitivity 9.0 ± 3.2 µg/dL ($P < 0.05$). In an attempt to find a possible relationship between the GCSI established in this study and the levels of cortisol and testosterone measured in the PCOS patients and considering the small number of PCOS patients, the PCOS patient data (blue dots) were combined with the data from the female controls (red triangles) and analyzed for possible correlation. As shown in Figs. 8 and 9, the combined PCOS plus female control group GCSI correlated negatively with testosterone ($R^2 = 0.42$, $P = 0.01$) and cortisol ($R^2 = 0.41$, $P = 0.02$) (Figs. 9 and 10). The results confirm our hypothesis that the more GC-resistant patients have higher levels of cortisol and testosterone.

4. Discussion

PA has been recognized as a forerunner of PCOS and as a rare sign of GC resistance. We hypothesized that impaired GC sensitivity plays a role in the development of both PA and PCOS by increasing androgen synthesis. Consequently, we examined GC sensitivity in subjects with PA and PCOS. The results of the present study show that there is a high

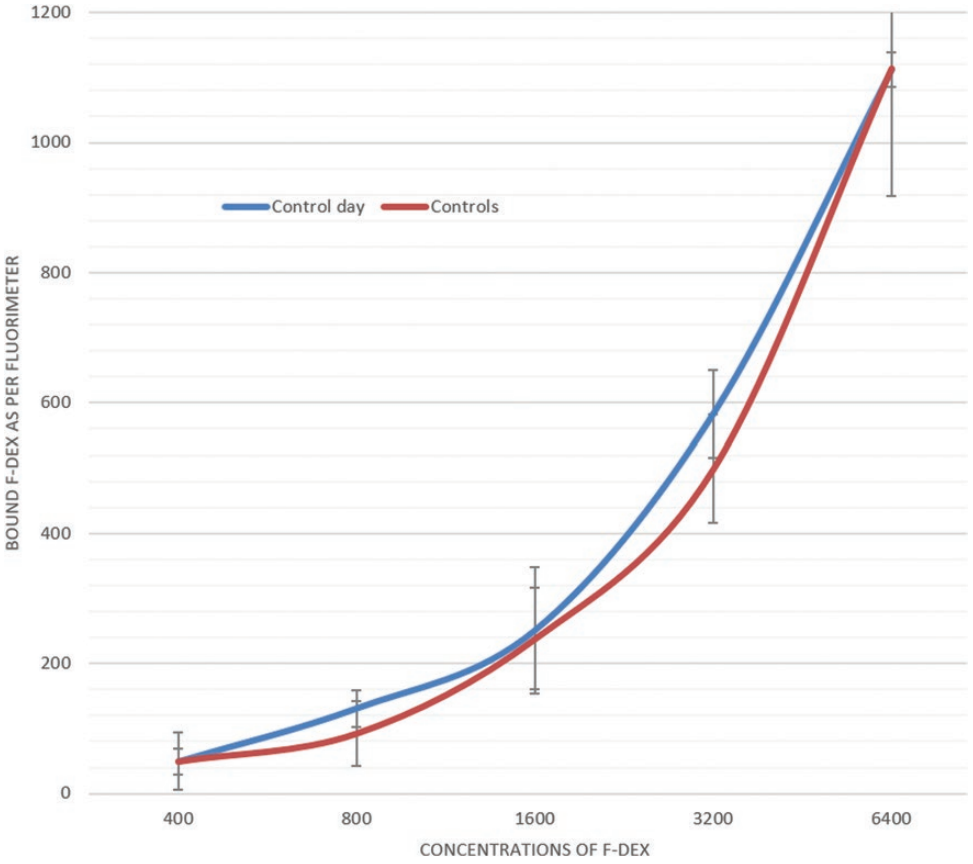

Figure 2. The fluorescein-labeled dexamethasone-monocyte binding assay (F-DEX-MBA). Comparison of the results from the control of the day to the mean values obtained from 31 control subjects used to establish the accuracy of the assay. X-axis, concentrations of F-DEX (400 to 6400 nm) added to triplicate wells. Y-axis, bound F-DEX as per fluorimeter read out at OD 488 nm.

prevalence of GC resistance in conditions of androgen excess. Measurements of F-DEX binding show the presence of GC resistance in more than 50% of PA and PCOS patients when compared with controls (Figs. 3 and 6).

Although most patients had normal baseline cortisol and ACTH levels, some showed fluctuations between normal and elevated cortisol and ACTH. Because these fluctuating levels are noted regularly in clinical practice, we identified a need for an assay that could help in identifying and calculating GC sensitivity with a high degree of reproducibility. Hence, we used a novel F-DEX mononuclear cell-binding assay as described in detail in the Methods section. Other conventional methods such as dexamethasone suppression tests and 24-hour urinary free cortisol levels or salivary cortisol measurements are not enough to make the diagnosis of the milder forms of GC resistance [35]. Although these standard biochemical tests would identify patients with elevated cortisol levels, it would be difficult to discern from these tests the true cause of the elevation in the cortisol level. In contrast, studying F-DEX-mononuclear cell-binding actually identifies abnormalities in the binding of GCs to the huGCR and, hence, is able to identify patients with alteration in GC sensitivity. Previous methodologies described in the literature to measure GC sensitivity in vitro require the use of radioactive agents and are also more time consuming and expensive to perform [26, 36]. These include in vitro dexamethasone suppression of PHA-stimulated lymphocyte proliferation with thymidine incorporation, as well as in vitro dexamethasone suppression of lipopolysaccharide-stimulated cytokine production test combined with multiplexed IL-6/TNF-alpha assay.

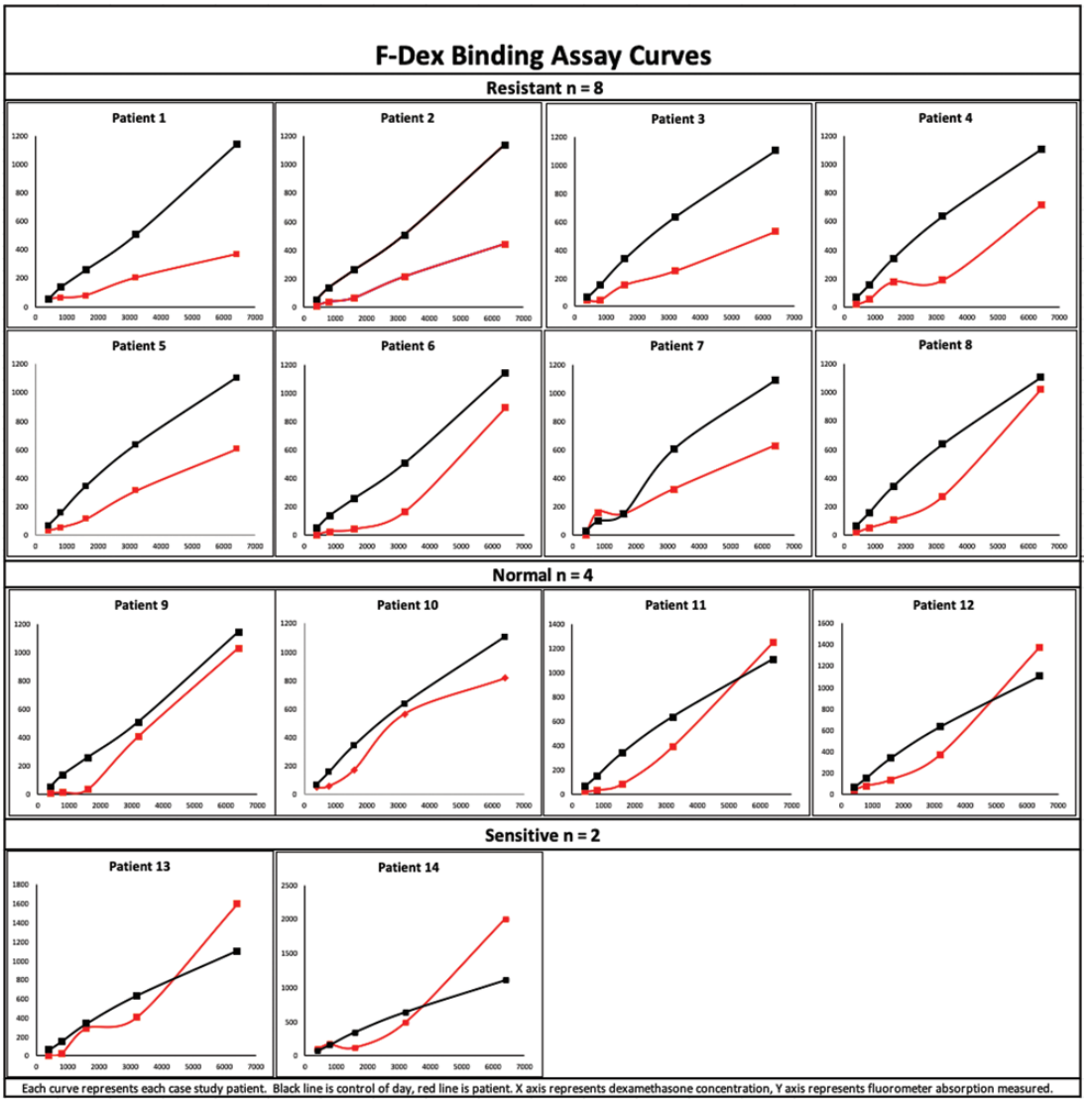

Figure 3. The fluorescein-labeled dexamethasone-monocyte binding assay (F-DEX-MBA) results of the premature adrenarche group (14 patients) represented by the results for each individual patient and control of the day. The patients are grouped according to their GC sensitivity as resistant, normal, and sensitive as defined by our measurements. The red line shows result for the individual patient; the black line shows result obtained on the control of the day. X-axis, concentrations of F-DEX (400-6400 nm) added to triplicate wells. Y-axis, bound F-DEX as per fluorimeter read out at OD 488 nm. GC, glucocorticoid.

Using the F-DEX-MCBA, the data revealed that the number and density of huGCR in adolescent females with PCOS were the same as those in controls, indicating that changes in GC sensitivity were not due to decrease of huGCR numbers. In fact, 1 PCOS patient had increased numbers of huGCR but was GC resistant (Fig. 1). These data support our findings that GC resistance is secondary to impaired glucocorticoid binding but not to a decrease in the number of receptors.

In other conditions such as CAH, huGCR binding was studied in 11 children (8.9 ± 3.5 years) [37]. Mononuclear leukocytes were isolated from peripheral blood and the binding of [3H] dexamethasone to huGCR was examined. The number of huGCRs and the dissociation constant, which is inversely proportional to its binding affinity, were also determined. The authors report that all children had normal numbers of huGCR along with normal binding affinity [37].

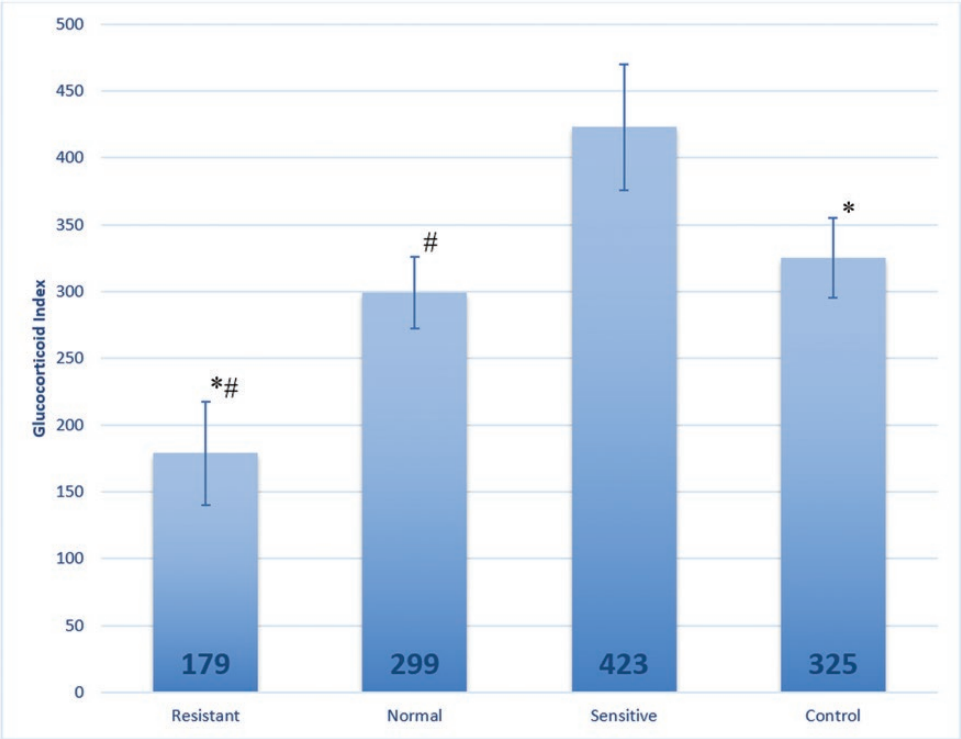

Figure 4. Comparison of GC sensitivity index in the subgroups of premature adrenarche patients with controls. GC, glucocorticoid. *$P < 0.05$ between GC resistant patient subgroup and control group. #$P < 0.05$ between GC resistant and normal premature adrenarche subgroup.

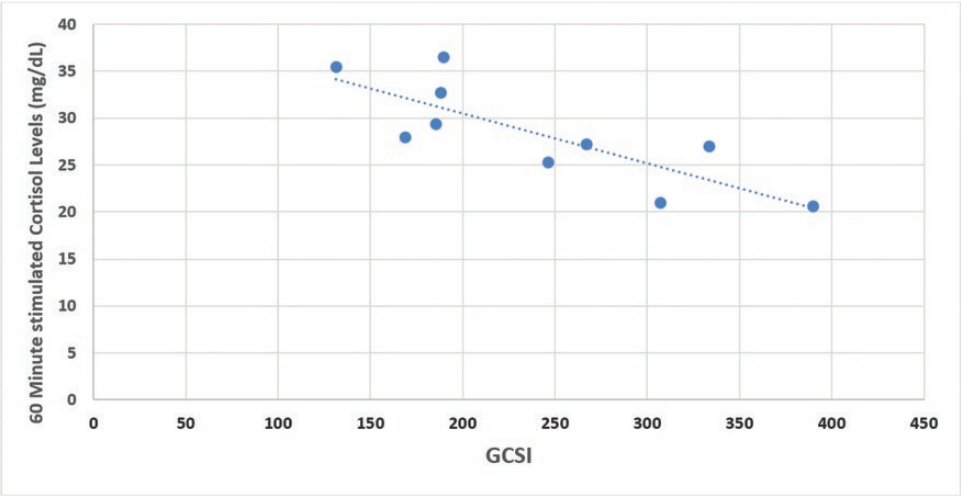

Figure 5. The correlation of the 60-minute simulated post-ACTH stimulated cortisol levels correlated with the GCSI ($R^2 = 0.6$, $P = 0.004$). Data available in 10 of the 14 premature adrenarche subjects. GCSI, glucocorticoid sensitivity index.

Previous publications have reported that women with PCOS were not found to have significant differences in the number of huGCR compared with controls [38, 39]. These reports further confirm our findings of the absence of any significant differences in the number of huGCR between PCOS subjects and controls. When we examined the members of the PA group for GC sensitivity, 57% of the patients were resistant, 28% had normal GCSI, and only 14% were found with increased GCSI.

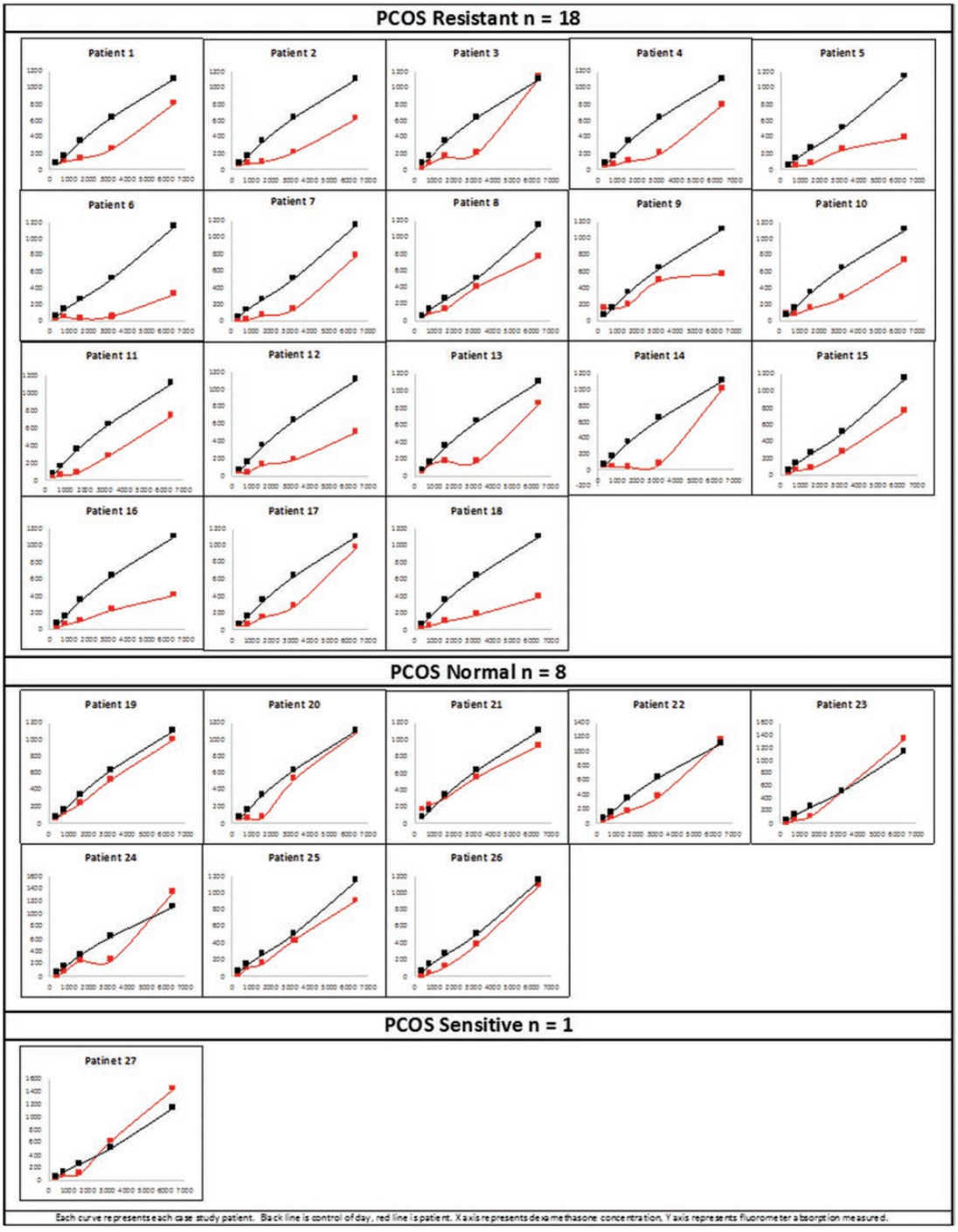

Figure 6. F-DEX-MBA results of PCOS group (27 patients) represented by the results for each individual patient and control of the day. The patients are grouped according to their GC sensitivity as resistant, normal, and sensitive as defined by our measurements. The red line shows the result for the individual patient; the black line shows the result obtained on the control of the day. X-axis, concentrations of F-DEX (400-6400 nm) added to triplicate wells. Y-axis, bound F-DEX as per fluorimeter read out at DO 488 nm. F-DEX, fluorescein-labeled dexamethasone; F-DEX-MBA, fluorescein-labeled dexamethasone-monocyte binding assay; GC, glucocorticoid.

In the PCOS group, 66.7% of the patients were GC resistant, similar to the results seen in PA patients; the GCSI was normal in 29% of PCOS patients, and only 1 patient was found to have increased GC sensitivity. In fact, the representation of GC resistance in PCOS and PA was very similar (Fig. 8).

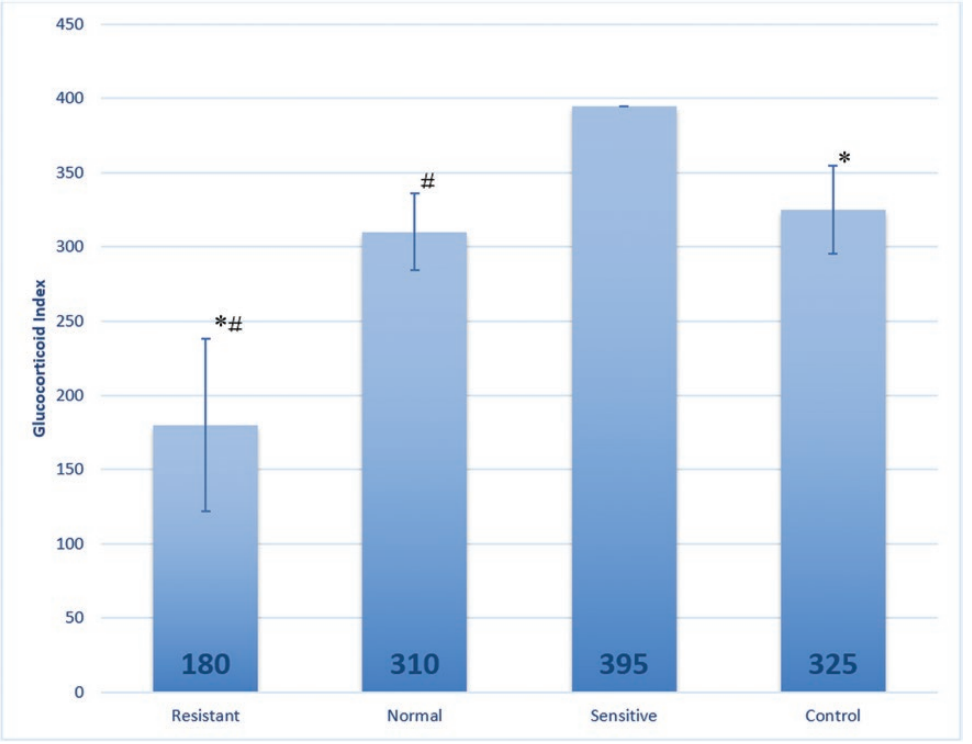

Figure 7. Comparison of GC sensitivity index in PCOS patient subgroups with controls. GC, glucocorticoid; PCOS, polycystic ovarian syndrome. *$P < 0.05$ between GC-resistant patient subgroup and control group. #$P < 0.05$ between GC-resistant and normal PCOS subgroup.

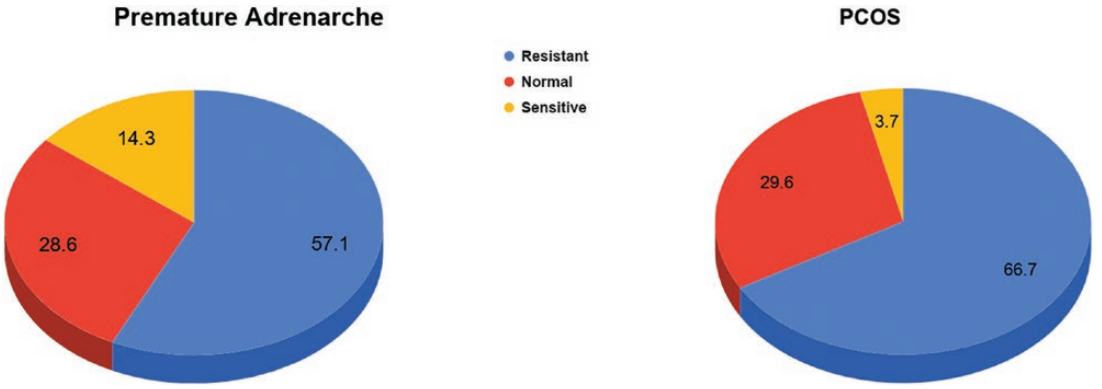

Figure 8. Distribution of glucocorticoid sensitivity index in the premature adrenarche and PCOS subgroups. PCOS, polycystic ovarian syndrome.

GCSI negatively correlated with cortisol and testosterone in PCOS, showing that higher GC-resistant subjects had higher cortisol and testosterone (Figs. 9 and 10). Therefore, we speculate that GC resistance plays a significant role in PCOS patients with hyperandrogenism from early childhood to adolescence. A similar situation may be at work in PA patients.

The evidence of GC resistance in PCOS patients was demonstrated in a recent study [35]. In this study, a subset of PCOS patients had smaller adrenal volumes and higher steroid hormone secretion after dexamethasone compared with the group of PCOS patients that responded to dexamethasone treatment appropriately [35]. Poor suppression of cortisol secretion after dexamethasone is a hormonal marker of GC resistance.

We consider GC resistance to be multifactorial and a metabolic characteristic of the body similar to insulin resistance. GC resistance can be secondary to polymorphism of huGCR

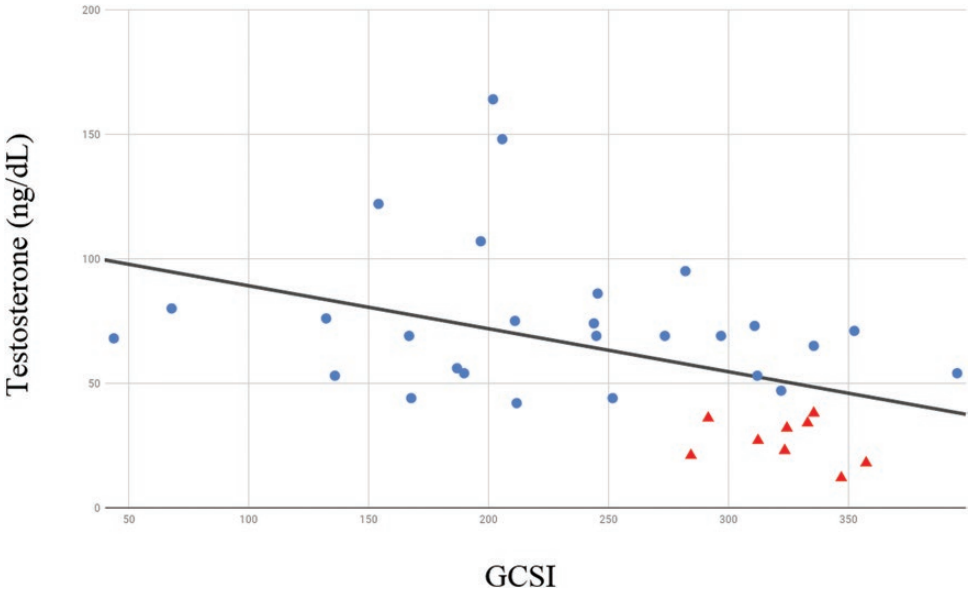

Figure 9. In the combined PCOS plus female control group, GCSI correlated negatively with testosterone (R^2 = 0.42, P = 0.01). The PCOS cohort is shown with blue dots and the controls are shown with red triangles. GCSI, glucocorticoid sensitivity index; PCOS, polycystic ovarian syndrome.

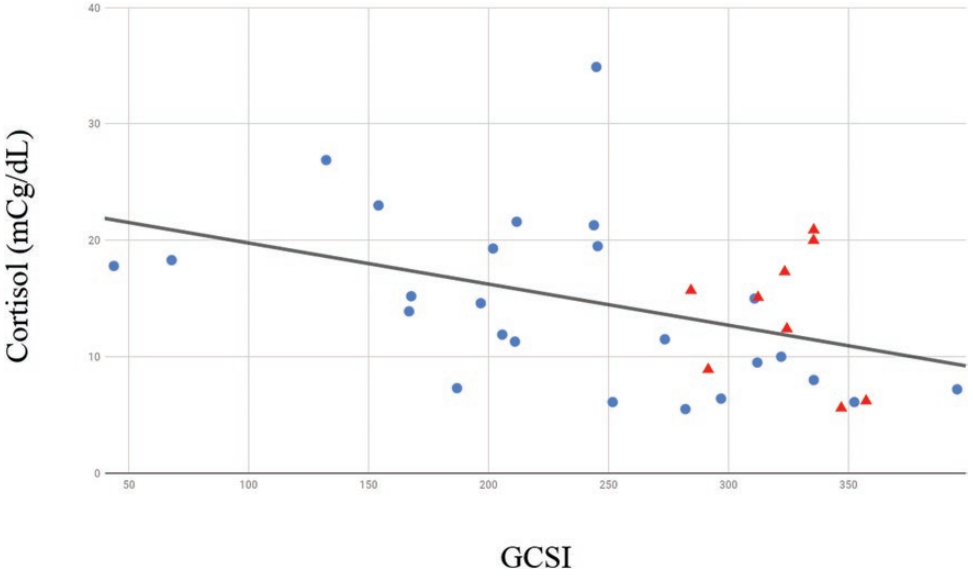

Figure 10. In the combined PCOS plus female control group, GCSI correlated negatively with cortisol (R^2 = 0.41, P = 0.02). The PCOS cohort is shown with blue dots and the controls are shown with red triangles. *Cortisol levels were available on 25 of the 27 PCOS subjects. GCSI, glucocorticoid sensitivity index; PCOS, polycystic ovarian syndrome.

[40-42], chaperone proteins such as FKBP5 [40, 43] and FKBP4, HSPs, coactivators, corepressors, other transcription factors, or epigenetic changes such as serine phosphorylation of huGCR [40, 43, 44]. Interestingly, in our study, some of the patients in both groups had increased GC sensitivity with higher GCSI (14% of PA patients and 3.7% of the PCOS patients). The significance of increased GC sensitivity in these patients is not fully understood and there is a role for further studies to elucidate the clinical picture of hyperandrogenism in PA and PCOS.

In conclusion, we observed the presence of GC resistance in >50% of patients with PA and >60% of PCOS patients. This resistance was shown as a lower GCSI in the F-DEX-mononuclear cell binding assay. To our knowledge this is the first comprehensive study using F-DEX-mononuclear cell binding assay to quantify human GC sensitivity at the cellular level. The findings of our studies are significant because it gives a new insight into PA, which was previously considered a benign condition. Based on the results of the present study, however, it may be more appropriate to reconsider PA as an early indicator of an altered GC sensitivity that could help physicians identify and prevent development of PCOS. Clearly, further investigations are necessary to understand the role and mechanism of GC signaling in cases of PA and PCOS.

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Additional Information

Correspondence: Amrit P.S. Bhangoo, MD, Pediatric Endocrinology, CHOC Children's Hospital, 1201 W. La Veta Ave, Orange, CA 92868. Email: drapsbhangoo@yahoo.com

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