Published in final edited form as:
Fertil Steril. 2007 April ; 87(4): 862–869.

GENETIC VARIANTS IN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA INFLUENCE INSULIN RESISTANCE AND TESTOSTERONE LEVELS IN NORMAL WOMEN BUT NOT THOSE WITH POLYCYSTIC OVARY SYNDROME

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Abstract

Polymorphisms in *PPARG* implicated in previous studies of metabolic traits do not appear to influence component phenotypes of PCOS, but do affect androgens and insulin resistance in the general population.

Objective—To investigate the relationship of the *PPARG* Pro12Ala and silent exon 6 (His447His) polymorphisms with the clinical features of polycystic ovary syndrome (PCOS).

Design—PCOS and control subjects were genotyped for Pro12Ala and His447His; associations between genotype, diagnosis, and hormonal/metabolic parameters were assessed.

Setting—Subjects were recruited from the reproductive endocrinology clinic at the University of Alabama at Birmingham; control subjects were recruited from the surrounding community. Genotyping was performed at Cedars-Sinai Medical Center in Los Angeles.

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Presented in part at the 87th Annual Meeting of the Endocrine Society, San Diego, June 4-7, 2005

Supported by: This study was supported in part by the Helping Hand of Los Angeles, and by grants R01-HD29364 and K24-HD01346 from the National Institutes of Health (to RA).

Conflict of Interest: H.J.A., M.P., B.C.T., Y-D.I.C., and M.O.G. have nothing to declare. R.A. has received consulting fees from Procter & Gamble, Merck & Co., and Organon.

Patient(s)—Participants included 285 White women with PCOS and 187 controls.

Intervention(s)—None

Main Outcome Measure(s)—Pro12Ala and His447His genotype, hormonal and metabolic phenotypes.

Result(s)—Pro12Ala and His447His did not influence risk of PCOS or its component phenotypes in PCOS patients. In controls, Pro12Ala did not influence measures of insulin resistance or androgen production; however, carriers of the His447His T allele had significantly decreased free and total testosterone levels and HOMA-IR. Furthermore, haplotypes in controls bearing the His447His T allele were also associated with decreased testosterone.

Conclusion(s)— does not appear to be an important modifier gene in PCOS. In controls, however, the His447His T allele may be in linkage disequilibrium with a functional variant that influences insulin resistance and testosterone production.

Keywords

peroxisome proliferator activated receptor gamma; polycystic ovary syndrome; single nucleotide polymorphism; testosterone; insulin resistance

Polycystic ovary syndrome (PCOS) is a common disorder affecting 6-8% of reproductive aged women (1) and clinically manifests as menstrual irregularities, hyperandrogenism, polycystic ovaries, and often obesity. Several studies demonstrate substantial familial aggregation of PCOS with most showing significantly higher rates of PCOS among first-degree relatives when compared to the general population (2,3). Fifty to seventy percent of PCOS patients exhibit some degree of insulin resistance, a leading risk factor for the development of type 2 diabetes mellitus and cardiovascular disease (4). The increased insulin resistance and compensatory hyperinsulinemia promotes hyperandrogenism in PCOS.

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor expressed in adipose tissue, macrophages, intestines, and ovaries that influences adipocyte differentiation, insulin sensitivity, lipid metabolism, and atherosclerosis (5). As the target of thiazolidinediones (TZDs), a class of pharmaceuticals used to treat type 2 diabetes mellitus by increasing insulin sensitivity, *PPARG* is a logical candidate gene that may influence risk of PCOS or its component phenotypes. Furthermore, a polymorphism in exon 2 of the PPARγ2 isoform, resulting in a Pro12Ala substitution, influences the risk of type 2 diabetes with the rarer Ala allele associated with a lower incidence of diabetes (6). Several small studies have looked at the effects of the Pro12Ala and a silent exon 6 polymorphism (His447His) in association with PCOS, though they have produced conflicting results (7-16). To better understand the role of these polymorphisms in PCOS, we examined the Pro12Ala and silent exon 6 polymorphism in a cohort of 285 women with PCOS and 187 controls, the largest sample size of all published reports of *PPARG* in PCOS to date. We found that neither variant was associated with PCOS risk or hormonal or metabolic traits in women with PCOS; in contrast, His447His appeared to modulate insulin resistance and testosterone levels in control women.

MATERIALS AND METHODS

Patients

A total of 285 consecutive White women with PCOS, aged 13 to 49 years, were recruited from the reproductive endocrinology clinic at the University of Alabama at Birmingham (UAB). The women were taking no medication known to alter hormonal parameters for at least three months prior to evaluation; no pregnant women were studied. Subjects with diabetes were

excluded because the hyperglycemia of diabetes may induce secondary changes in insulin-related traits that reduce their utility for genetic analyses. The UAB and the Cedars-Sinai Medical Center Institutional Review Boards approved this study, and written informed consent was obtained from all subjects.

The diagnosis of PCOS was assigned using the 1990 National Institute of Child Health and Human Development consensus conference criteria, which define PCOS as ovulatory dysfunction plus hirsutism and/or hyperandrogenemia, with exclusion of other disorders (17). PCOS was diagnosed after exclusion of androgen-producing tumors, nonclassic 21-hydroxylase-deficient adrenal hyperplasia (NCAH), hyperprolactinemia, active thyroid disease, or Cushing''s syndrome. Ovulatory dysfunction was defined as menstrual cycles >45 days in length, or a progesterone level <2 ng/mL on days 22–24 of the menstrual cycle, in conjunction with a monophasic basal body temperature chart. Hirsutism was quantified using a modified Ferriman-Gallwey scale (18). Hyperandrogenemia was defined as serum levels of total testosterone, free testosterone, or DHEAS above the 95th percentile of controls, as previously reported (19). Twenty-one hydroxylase-deficient NCAH was excluded by a basal follicular phase 17-hydroxyprogesterone (17-OHP) level <2 ng/mL, or a 17-OHP level after acute ACTH stimulation <10 ng/mL. Normal levels of PRL and TSH excluded hyperprolactinemia and hypothyroidism, respectively; Cushing''s syndrome was excluded by a 24-hour urine free cortisol level <100 μg/day, when screening was clinically indicated.

In addition, 187 healthy White non-hirsute women, aged 14 to 60, with regular menstrual cycles or a history of regular menstrual cycles before menopause, and not taking hormonal medications, were recruited as controls. Controls were recruited by word of mouth and advertisements in the Birmingham, Alabama area, through a call for ”healthy women” without detailing further the nature of the studies. All controls underwent a brief history and physical exam (see below) to ensure that all control women included were non-hirsute and eumenorrheic.

Phenotyping

All subjects underwent a brief physical examination (including height, weight, body mass index (BMI) and waist-hip ratio), hirsutism scoring, and blood sampling for hormonal measurement and DNA extraction. Hormonal measures (total and free testosterone, DHEAS, and sex hormone binding globulin (SHBG)) were obtained between days three and eight (follicular phase) following a spontaneous menstrual cycle or progesterone-induced withdrawal bleed. A modified Ferriman-Gallwey (mFG) scoring system was used to quantify hirsutism. Subjects were deemed hirsute if their mFG score was six or greater (18).

Fasting glucose and insulin values were also obtained in a subset of subjects: 184 PCOS and 105 controls. The homeostasis model assessment (HOMA) was used to calculate indices of insulin resistance and insulin secretion for each patient (20). The computer-based HOMA calculator (available at www.dtu.ox.ac.uk/homa) uses fasting glucose and insulin to generate the index of insulin resistance, HOMA-IR, and the index of beta-cell function, HOMA-%B. An ideal, normal-weight person less than 35 years of age has a HOMA-IR of 1 and HOMA-%B of 100% (21).

Genotyping and Haplotype Determination

PCR primers and TaqMan MGB (Applied Biosystems, Foster City, CA) probes were designed to genotype the *PPARG* variants Pro12Ala (rs1801282) and the silent exon 6 variant (His447His, rs3856806, also known as C161T). Both polymorphisms were genotyped in the 472 subjects using the 5’-exonuclease assay (TaqMan MGB) described previously (22,23). In

total, 435 subjects were successfully genotyped for Pro12Ala and 449 successfully genotyped for His447His.

To determine the C/G (C coding for Pro; G coding for Ala) polymorphism of the Pro12Ala variant, two probes were prepared: a C allele-specific probe, 5'-6FAM-CTATTGACcCAGAAAG-MGBNFQ-3', and a G allele-specific probe, 5′-VIC-CTATTGACgCAGAAAG-MGBNFQ-3′. The 5' 6FAM and VIC are different color fluorescent reporter dyes that are quenched by the minor groove binder non-fluorescent quencher (MGBNFQ) located at the 3′ end. The design of primers for the polymerase chain reaction (PCR) of the flanking region of the C/G Pro12Ala polymorphism was as follows: forward, 5'-TGGGTGAAACTCTGGGAGATTC-3'; reverse, 5'-TGCAGACAGTGTATCAGTGAAGGA-3'.

To determine the C/T polymorphism of the His447His variant of *PPARG*, two other probes were prepared: a C allele-specific probe (containing the complement allele G), 5'-6FAM-TAGCTGCACgTGTTC-MGBNFQ-3', and a T allele-specific probe (containing A), 5′-VIC-TAGCTGCACaTGTTC-MGBNFQ-3′. Similarly, the following flanking primers were designed: forward, 5'-AAAATGACAGACCTCAGACAGATTGT-3'; reverse, 5'-GTCTGTCTCCGTCTTCTTGATCAC-3'.

PCR was carried out using a dual 384 well GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems). Following PCR cycles (initial denaturation at 50 °C for 2 min, followed by 95 °C for 10 min, then 40 cycles of 95 °C for 15 s and 60 °C for 60 s), the fluorescence level of PCR reactions was measured using an ABI PRISM 7900 Sequence Detector (Applied Biosystems), resulting in the identification of three genotypes (major allele homozygotes, heterozygotes, minor allele homozygotes) for each polymorphism.

Haploview 3 was used to determine haplotypes based on Pro12Ala and His447His (24). Haploview constructs haplotypes by using an accelerated expectation maximization algorithm similar to the partition/ligation method (25), which creates highly accurate population frequency estimates of the phased haplotypes based on the maximum likelihood derived from the unphased input genotypes. Haploview was used to calculate linkage disequilibrium (LD, the D' statistic) between the two *PPARG* variants.

Statistical Analysis

The primary phenotype for genetic association analysis was the presence or absence of PCOS, given that such analysis utilizes all available subjects to maximize power. Secondary analyses included the androgen-related traits (total and free testosterone, DHEAS, SHBG, mFG score), insulin-related traits (fasting insulin, fasting glucose, HOMA-IR, HOMA-%B), and adiposity-related traits (body mass index, waist-to-hip ratio) within PCOS and controls separately.

Associations of SNPs and haplotypes with qualitative traits (PCOS and the presence/absence of hirsutism) were evaluated using chi square tests (unadjusted analyses) and multivariate logistic regression (covariate-adjusted analyses). Associations with quantitative traits (androgens, mFG score, and insulin-related traits) were evaluated using analysis of covariance. As appropriate, analyses were adjusted for age and/or body mass index. This was determined for each trait by analyzing whether there was a relationship between age or BMI and the trait of interest. If such a relationship was present, then age and/or BMI was adjusted for by inclusion as an independent variable in the genetic association analysis. Neither age nor BMI was associated with genotype at either SNP.

Quantitative trait values were log- or square root-transformed as appropriate to reduce non-normality. Due to the low numbers of homozygous minor allele genotypes for both SNPs, all

analyses were conducted combining those heterozygous with those homozygous for the rarer allele. Therefore, for all association analyses, the dependent variable was the trait of interest; genotype group (major allele homozygote or minor allele carrier) was entered as an independent variable, plus any covariates if the covariate was individually correlated with the trait. Since testing multiple SNPs and phenotypes could have led to false-positive associations, we corrected for multiple testing by estimating the false-discovery rate (FDR (26)) within each group of subjects and determined that in order to maintain a type 1 error-rate (α) of 0.05, we needed a significance cut-off level of $P<0.01$.

RESULTS

The comparison of hormonal and metabolic parameters of the control and PCOS subjects are given in Tables 1 to 4, presented by genotype for each SNP. In general, women with PCOS were significantly younger, more obese, more hirsute, more hyperandrogenemic, more insulin resistant, and had higher beta cell function (insulin secretion) than control women. While all 285 women with PCOS met the NIH 1990 criteria for the disorder, 228 (80%) had hyperandrogenemia along with their ovulatory dysfunction (with or without hirsutism). Since it may be argued that the diagnosis of PCOS should entail the presence of hyperandrogenemia, we analyzed this subgroup separately.

Genetic association analyses for PCOS risk were adjusted for age and body mass index. The two SNPs are 82.4 kb apart from each other, and have a linkage disequilibrium (D') of 0.65 (95% confidence interval 0.56 to 0.72) in this White cohort. In our study, the frequencies of the Pro12Ala and silent exon 6 (His447His) variations of the *PPARG* gene did not differ significantly between PCOS women and healthy controls. Thirty-four control women (20.2%) were carriers of the Pro12Ala G allele versus 54 (20.2%) PCOS women (P=0.99). Thirty-eight (22.1%) control women were carriers of the His447His T allele versus 55 (19.9%) PCOS women (P=0.57). Similarly, covariate-adjusted analyses showed no significant association of either variant with PCOS diagnosis. Carriers of the Pro12Ala G allele had an odds ratio of 1.28 for PCOS (95% confidence interval 0.68-2.43, age- and BMI-adjusted P value = 0.45); carriers of the His447His T allele had an odds ratio for PCOS of 0.71 (95% confidence interval 0.39-1.31, age- and BMI-adjusted P value=0.28).

Analysis of the Pro12Ala polymorphism in relation to quantitative traits showed no significant associations within PCOS and controls separately. Carriers of the G allele did not differ from non-carriers in terms of total or free testosterone, DHEAS, SHBG, mFG score, hirsutism, or any of the adiposity or insulin-related traits (fasting insulin, fasting glucose, HOMA-IR, HOMA-%B). Analyzing only the 228 cases with hyperandrogenemia plus ovulatory dysfunction, the G allele similarly showed no association with PCOS status or quantitative traits, such as androgen levels (data not shown).

The silent exon 6 (His447His) T allele was significantly associated with lower total testosterone levels within the control group (C/C: 48.4 ± 21.6; T carriers: 34.7 ± 14.9 ng/dL, age-adjusted P value<0.0001) (Table 2). Furthermore, in the control group a significant decrease in mean free testosterone levels was also present in His447His T allele carriers (C/C: 0.42 ± 0.20; T carriers: 0.31 ± 0.16 pg/mL, age-adjusted P value=0.0004). Control women with the C/T or T/T genotype also had significantly decreased HOMA-IR (C/C: 1.3 ± 0.8; T-carriers: 1.0 ± 1.1, BMI-adjusted P value=0.0056) and a trend towards lower mean fasting insulin levels (C/C: 9.4 ± 6.2; T-carriers: 7.8 ± 8.0 μ?U/mL, BMI-adjusted P value=0.014), when compared to those with a C/C genotype. Although carriers of the T allele within the PCOS group also had modestly lower mean total testosterone levels (C/C: 86.2 ± 30.0; T carriers: 78.6 ± 29.0 ng/dL, P=0.023), following adjustment for multiple comparisons, that difference no longer remained

significant. A similar trend was seen in the 228 cases with hyperandrogenemia and ovulatory dysfunction (C/C: 92.3 ± 29.7; T carriers: 84.7 ± 25.3 ng/dL, P=0.062).

A small number (n=19) of genotyped postmenopausal women were present in the control group. Compared to the premenopausal controls, the postmenopausal women had lower total testosterone (postmenopausal: 27.1 ± 12.3; premenopausal: 47.3 ± 20.5 ng/dL, P<0.0001) and free testosterone (postmenopausal: 0.25 ± 0.15; premenopausal: 0.40 ± 0.19 pg/mL, P<0.0001) levels but similar HOMA-IR (postmenopausal: 1.14 ±0.87; premenopausal: 1.18 ± 0.86, P=0.75).

Therefore, we excluded the postmenopausal controls and repeated the His447His association analyses with total and free testosterone. This had minimal effect on the association of the T allele with total (C/C: 51.4 ± 21.5; T carriers: 36.2 ± 14.6 ng/dL, age-adjusted P value=0.0002) and free (C/C: 0.43 ± 0.20; T carriers: 0.32 ± 0.16 pg/mL, age-adjusted P value=0.0008) testosterone.

Given that significant genotype-phenotype associations were observed with the silent variant, we conducted haplotype-based analyses to better understand the nature of these associations. All four possible haplotypes based on the two variants (Pro12Ala(C/G)-His447His(C/T)) were observed, with the following frequencies: C-C 85.6%, G-T 7.5%, C-T 3.6%, G-C 3.4%. These frequencies were not different between women with and without PCOS. In control subjects, two haplotypes, both carrying the His447His T allele, demonstrated association with total and free testosterone: The G-T haplotype was associated with decreased total testosterone (G-T carriers: 35.7 ± 15.9; non-carriers: 46.4 ± 21.3 ng/dL, age-adjusted P value=0.012) and decreased free testosterone (G-T carriers: 0.30 ± 0.14; non-carriers: 0.40 ± 0.20 pg/mL, age-adjusted P value=0.015). The C-T haplotype was also associated with decreased total testosterone (C-T carriers: 30.4 ± 12.3; non-carriers: 46.0 ± 21.0 ng/dL, age-adjusted P value=0.0027) and decreased free testosterone (C-T carriers: 0.27 ± 0.14; non-carriers: 0.39 ± 0.19 pg/mL, age-adjusted P value=0.010).

In subjects with PCOS, no haplotype demonstrated statistically significant phenotypic associations. However, we observed a trend for the same two haplotypes as associated with decreased total testosterone in PCOS (G-T carriers: 77.2 ± 24.3; non-carriers: 86.0 ± 30.7 ng/dL, P value=0.082; C-T carriers: 80.8 ± 40.4; non-carriers: 85.0 ± 24.3 ng/dL, P value=0.12).

DISCUSSION

In the present study, we investigated the possible association between *PPARG* variants and various hormonal and metabolic variables known to be altered in PCOS. In size, our cohort of PCOS women is the largest examined for either the Pro12Ala or His447His polymorphisms. Neither variant was associated with risk of developing PCOS or with insulin-related traits or androgen levels in women with PCOS. Specifically, we did not confirm a role of Pro12Ala in the insulin resistance of PCOS. On the other hand, this study is the first to demonstrate that control women carrying the His447His T allele had improved insulin sensitivity and decreased mean levels of free and total testosterone

Though several previous studies have examined the effects of the Pro12Ala and His447His polymorphisms in PCOS cohorts, most produced differing and sometimes completely contrasting results. Problematic to genetic association studies are differences in ethnic background and small sample sizes, both of which can lead to spurious results. Whereas most studies (9-12,14,15), present study included, reported no association between Pro12Ala genotype and PCOS diagnosis, a few reported that the Pro12Ala G allele was significantly less frequent in PCOS versus control women (13,16). Additionally, some studies reported significant increases in insulin sensitivity (decreased HOMA-IR) and decreases in fasting

insulin and glucose levels (8-10) and lower hirsutism score (9) in PCOS women with the Pro12Ala G allele; whereas others (7,11,14), including this study, reported no association with fasting glucose and insulin or changes in HOMA-IR in those with PCOS. Only a subset of the subjects in our study was phenotyped for insulin-related traits, which may explain why we did not observe association with these traits in subjects with PCOS; however, this subset was still greater in number than each of the reports that did find an effect of Pro12Ala on insulin-related traits. Thus, an important result herein is the non-replication, in a larger cohort, of previously reported association results for Pro12Ala within PCOS.

Here we report that variation in *PPARG* influenced testosterone levels in control women, but only weakly, if at all, in PCOS. *PPARG* is primarily known as a type 2 diabetes mellitus gene both because of the decreased risk conferred by the Pro12Ala G allele (6) and because it is the target of the thiazolidinedione (TZD) class of pharmaceuticals that reverse insulin resistance in target tissues. Clinical trials of TZDs in PCOS demonstrated improvement in ovulatory dysfunction, hirsutism, and hyperandrogenemia (27). Acting as *PPARG* agonists, they have been shown to decrease mean serum testosterone levels when administered to both obese and non-obese PCOS women, and even men (28-32). Therefore, it is plausible that a genetic polymorphism affecting PPARγ activity can influence testosterone production in our control women. *PPARG* mRNA has been shown to be expressed in mouse, rat, porcine, bovine, and human ovarian tissues, where they can influence androgen biosynthesis (33). *In vitro*, troglitazone administration to both porcine and rat theca cell cultures produced significant decreases in basal and hormone-stimulated (LH and/or insulin) androgen production (34,35). In human ovarian cell cultures, pioglitazone and rosiglitazone also significantly reduced testosterone levels and abolished the insulin-induced stimulation of testosterone production (36).

The TZD-induced decrease in testosterone production has been attributed to inhibition of 3β-hydroxysteroid dehydrogenase (3βHSD) and cytochrome P450 17α-hydroxylase/17,20-lyase (P450c17) activity in the ovaries. In porcine granulosa cell cultures, administration of troglitazone inhibited progesterone production in a dose and time dependent manner and was accompanied by an elevation of pregnenolone, suggesting an inhibition of the 3βHSD enzyme (37). Furthermore, 3βHSD activity was significantly reduced in human ovarian homogenates incubated with troglitazone (38). Lastly, Arlt, et al. studied “humanized yeast” that express steroidogenic enzymes in microsomal environments and noted that three TZDs (troglitazone, pioglitazone, and rosiglitazone) inhibited 3βHSD and both activities of P450c17, independent of their effects in lowering insulin levels (39).

We observed an association between the His447His silent exon 6 T allele and reduced androgen levels and lower insulin resistance in our control women. However, because this variant does not change the amino acid sequence, we hypothesize that this SNP must be in linkage disequilibrium with another functional variant. In other words, individuals who inherit the T allele may also inherit a functional allele at a location elsewhere in the gene because these two alleles are present on the same chromosome. The haplotype analysis suggests that the T allele effect on testosterone is not due to linkage with the Pro12Ala G allele, because both haplotypes (C-T and G-T) carrying the T allele were associated with decreased testosterone level, regardless of what allele was present at the Pro12Ala locus. We hypothesize that both haplotypes carrying the T allele also carry an as-yet unknown functional allele that alters *PPARG* expression and/or function such that it decreases testosterone level.

Alternatively, the silent His447His variant itself may have functional consequences if it lies in a regulatory site within the gene, such that transcription of the gene is altered or if splicing of the mRNA is altered. Given that the *PPARG* gene has multiple transcript variants via the use of alternate promoters and mRNA splicing, it is conceivable that the His447His lies in a

regulatory region affecting these processes. With the current data, we cannot determine which mechanism (linkage disequilibrium versus altered regulatory site) explains the His447His effect on testosterone levels.

The only other study to examine His447His in PCOS did not report association with circulating androgens (11). The His447His T allele has been associated with BMI and leptin levels (11, 40); however, no associations with BMI were found in the present study. We found no association of His447His genotype with PCOS; however, Orio, et al. reported a significant increase in the T allele among Italian PCOS patients (11). The differences between studies may reflect different patterns of linkage disequilibrium among different ethnic groups, such that the T allele is linked to a variant that influences testosterone level in some populations but not others. That the His447His genotype also appeared to influence carotid artery and coronary artery atherosclerosis (41,42) supports our hypothesis that it lies in linkage disequilibrium with another functional variant. Further studies are required to clarify the role this polymorphism may be playing in women with and without PCOS. Sequencing of the *PPARG* promoter and exons should be performed in subjects with and without the His447His T allele to attempt to identify the putative unknown functional variant elsewhere in the gene that is being inherited with the T allele.

Whereas the effect of the His447His T allele to reduce testosterone levels and insulin resistance in controls was highly significant, the effect of the T allele on testosterone level in PCOS was moderate and when adjusted for multiple comparisons, did not attain statistical significance. This more modest effect suggests other genes are more important for insulin resistance and testosterone production in PCOS. Any effect *PPARG* variation may be having on insulin resistance or testosterone production in PCOS may be masked by the effects of such genes. Abnormalities in these as yet unknown genes may uniquely predispose women to develop PCOS. That such genes exist is supported by the evidence from familial aggregation studies demonstrating that testosterone levels and insulin sensitivity are inherited traits in PCOS families (2,43,44).

This is the largest published study to date to examine the roles of these two polymorphisms in PCOS and controls. We did not replicate any of the published results that suggested a role of the Pro12Ala variant in PCOS. While the His447His T allele did not appear to affect risk of PCOS or component phenotypes in women with PCOS, it significantly reduced mean free and total testosterone levels and insulin resistance in controls. Of note, numerous clinical and *in vitro* studies have illustrated the effects of *PPARG* agonists on circulating testosterone and ovarian androgen biosynthesis, supporting the notion that genetic variants in *PPARG* may influence testosterone production. Therefore, *PPARG* emerges as an important modifier gene in the general population, but not in PCOS, wherein His447His or a functional variant in linkage disequilibrium with His447His, but not Pro12Ala, influences phenotypes of insulin resistance and testosterone production.

Acknowledgements

This study was supported in part by the Helping Hand of Los Angeles, and by grants R01-HD29364 and K24-HD01346 from the National Institutes of Health (to RA).

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Table 1

Hormonal characteristics by diagnosis and Pro12Ala genotype.

	Controls			PCOS		
	C/C n=134	C/G or G/G n=34	P-Value	C/C n=213	C/G or G/G n=54	P-Value
Age (yr)	33.6 ± 11.5	37.7 ± 11.7	0.076	27.6 ± 7.4	27.1 ± 7.3	0.72
Body mass index (kg/m2)	25.6 ± 5.8	25.8 ± 6.3	0.73	34.9 ± 9.1	34.5 ± 9.9	0.88
Waist/Hip ratio	0.79 ± 0.06	0.79 ± 0.07	0.37	0.84 ± 0.08	0.85 ± 0.13	0.30
Hirsutism (%)	0	0	n/a	75	69	0.32
Ferriman-Gallwey Score	0.16 ± 0.46	0.09 ± 0.29	0.54	8.34 ± 4.83	7.80 ± 5.05	0.39
Total Testosterone [ng/dL (nmol/L)]	46.4 ± 21.1 (1.61 ± 0.73)	37.7 ± 18.3 (1.31 ± 0.63)	0.058	86.1 ± 31.1 (2.98 ± 1.08)	78.1 ± 25.5 (2.71 ± 0.88)	0.11
Free Testosterone [pg/mL (pmol/L)]	0.40 ± 0.19 (13.87 ± 6.59)	0.34 ± 0.19 (11.79 ± 6.59)	0.12	0.92 ± 0.41 (31.90 ± 14.21)	0.87 ±0.33 (30.16 ± 11.44)	0.51
DHEAS (ng/mL)	1137.4 ± 778.4	992.7 ± 726.9	0.84	2241.9 ± 1270.5	2383.1 ± 1261.9	0.41
SHBG (nmol/L)	224.9 ± 81.5	227.1 ± 78.7	0.66	171.5 ± 61.9	173.5 ± 82.7	0.90

Note: Comparisons are made between the 2 genotypes within each group, using logistic regression (hirsutism) or analysis of covariance (other traits). Multiple testing-corrected P value<0.01 is significant. Values expressed with a plus/minus sign are mean ± SD.

Table 2

Metabolic characteristics by diagnosis and Pro12Ala genotype.

	Controls			PCOS		
	C/C n=72	C/G or G/G n=22	P-Values	C/C N=148	C/G or G/G n=40	P-Values
Age (yr)	34.8 ± 13.1	40.6 ± 12.4	0.032	27.1 ± 7.2	27.0 ± 7.7	0.84
Body mass index (kg/m2)	26.9 ± 6.5	25.4 ± 5.7	0.13	35.9 ± 8.8	36.6 ± 10.2	0.64
Fasting Insulin [μIU/mL (pmol/L)]	9.7 ± 7.3 (69.7 ± 52.7)	6.8 ± 3.7 (48.9 ± 26.4)	0.094	20.5 ± 15.4 (147.2 ± 110.6)	24.3 ± 17.8 (174.5 ± 127.9)	0.54
Fasting Glucose [mg/dL (mmol/L)]	87.0 ± 11.3 (4.83 ± 0.63)	87.4 ± 9.7 (4.85 ± 0.54)	0.67	88.0 ± 10.6 (4.88 ± 0.59)	87.8 ± 11.4 (4.87 ± 0.63)	0.93
HOMA-IR	1.30 ± 0.97	0.94 ± 0.49	0.10	2.31 ± 1.36	2.74 ± 1.68	0.43
HOMA-%B	118.9 ± 51.4	93.4 ± 30.3	0.13	177.5 ± 84.2	200.8 ± 101.8	0.39

Note: Comparisons are made between the 2 genotypes within each group, using logistic regression (hirsutism) or analysis of covariance (other traits). Multiple testing-corrected P value<0.01 is significant. Values are mean ± SD.

Table 3

Hormonal characteristics by diagnosis and His447His genotype.

	Controls			PCOS		
	C/C n=134	C/T or T/T n=38	P-Values	C/C n=222	C/T or T/T n=55	P-Values
Age (yr)	34.6 ± 11.9	33.4 ± 9.6	0.29	27.3 ± 7.4	28.2 ± 7.4	0.66
Body mass index (kg/m2)	25.3 ± 5.4	26.9 ± 7.1	0.080	34.4 ± 9.4	35.9 ± 9.2	0.38
Waist/Hip ratio	0.79 ± 0.06	0.79 ± 0.08	0.89	0.84 ± 0.08	0.85 ± 0.13	0.42
Hirsutism (%)	0	0	n/a	75	69	0.33
Ferriman-Gallwey Score	0.11 ± 0.39	0.30 ± 0.64	0.10	8.32 ± 4.68	8.02 ± 5.45	0.58
Total Testosterone [ng/dL (nmol/L)]	48.4 ± 21.6 (1.68 ± 0.75)	34.7 ± 14.9 (1.20 ± 0.52)	<0.0001	86.2 ± 30.0 (2.99 ± 1.04)	78.6 ± 29.0 (2.72 ±1.01)	0.023
Free Testosterone [pg/mL (pmol/L)]	0.42 ± 0.20 (14.56 ± 6.93)	0.31 ± 0.16 (10.75 ± 5.55)	0.0004	0.92 ± 0.40 (31.90 ± 13.87)	0.88 ± 0.34 (30.51 ± 11.79)	0.46
DHEAS (ng/mL)	1115.4 ± 766.9	1092.8 ± 753.7	0.59	2233.5 ± 1263.3	2295.4 ± 1269.1	0.36
SHBG (nmol/L)	225.6 ± 79.9	225.6 ± 84.0	0.93	174.9 ± 69.2	160.0 ± 46.0	0.15

Note: Comparisons are made between the 2 genotypes within each group, using logistic regression (hirsutism) or analysis of covariance (other traits). Multiple testing-corrected P value<0.01 is significant. Values expressed with a plus/minus sign are mean ± SD.

Table 4

Metabolic characteristics by diagnosis and His447His genotype.

	Controls			PCOS		
	C/C n= 73	C/T or T/T n= 23	P-Values	C/C n= 153	C/T or T/T n= 42	P-Values
Age (yr)	35.7 ± 13.9	36.0 ± 10.2	0.99	27.0 ± 7.1	28.0 ± 8.0	0.54
Body mass index (kg/m2)	26.4 ± 6.2	27.0 ± 6.4	0.72	35.5 ± 9.3	37.2 ± 9.1	0.36
Fasting Insulin [μIU/mL (pmol/L)]	9.4 ± 6.2 (67.4 ± 44.8)	7.0 ± 8.0 (56.0 ± 57.3)	0.014	20.4 ± 15.6 (144.5 ± 112.0)	24.6 ± 16.8 (176.6 ± 120.8)	0.16
Fasting Glucose [mg/dL (mmol/L)	87.4 ± 11.2 (4.85 ± 0.62)	85.3 ± 9.7 (4.74 ± 0.54)	0.43	87.7 ± 10.7 (4.87 ± 0.59)	89.2 ± 11.8 (4.95 ± 0.66)	0.68
HOMA-IR	1.27 ± 0.82	1.03 ± 1.06	0.0056	2.28 ± 1.41	2.82 ± 1.54	0.090
HOMA-%B	117.5 ± 50.7	100.8 ± 37.0	0.13	175.9 ± 85.8	201.2 ± 96.0	0.15

Note: Comparisons are made between the 2 genotypes within each group, using logistic regression (hirsutism) or analysis of covariance (other traits). Multiple testing-corrected P value<0.01 is significant. Values are mean ± SD.