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ORIGINAL ARTICLE

The peroxisome proliferator activated receptor gamma Pro12Ala polymorphism is associated with a lower hirsutism score and increased insulin sensitivity in women with polycystic ovary syndrome

Susanne Hahn*, Anja Fingerhut†, Ulyana Khomtsiv*, Liliya Khomtsiv*, Susanne Tan*, Beate Quadbeck*, Burkhard L. Herrmann*, Birgit Knebel‡, Dirk Müller-Wieland‡, Klaus Mann* and Onno E. Janssen*

*Division of Endocrinology, Department of Internal Medicine, University Hospital of Essen, †Department of Pharmacology, University of Essen, Essen and ‡Department of Clinical Biochemistry, German Diabetes Research-Institute, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

Summary

Background Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism and chronic anovulation. The genetic background of the insulin resistance frequently associated with PCOS is unclear.

Objectives To examine the influence of the Pro12Ala polymorphism of the peroxisome proliferator activated receptor gamma (PPARγ), which is thought to play a role in the regulation of insulin sensitivity, on endocrine and metabolic parameters in PCOS patients.

Methods PPARγ alleles were analysed in 102 PCOS patients (age 27 ± 5·3 years) and 104 age matched control women. PCOS was defined by the NIH-criteria as the presence of chronic oligo- or anovulation and hyperandrogenism. Family history and clinical parameters were evaluated by personal interview and physical examination, parameters of insulin resistance [homeostasis model assessment (HOMA) and Matsuda-index] were evaluated with a glucose tolerance test.

Results Seventy-nine (77·5%) PCOS patients were carriers of the wild-type PPARγ allele (Pro/Pro), while 23 (22·5%) had at least one Ala allele (X/Ala), with an equal distribution in controls. X/Ala PCOS women were more insulin-sensitive, evidenced by lower fasting insulin, HOMA index and insulin secretion. Differences in insulin resistance did not depend on body mass index. The genotype had no influence on lipid status, leptin, adiponectin, ghrelin, or family history of type 2 diabetes. A significantly lower proportion of Pro/Ala patients had hirsutism and they had on average lower hirsutism scores than Pro/Pro patients. No relationship was found between the Pro/Ala polymorphism and other signs of hyperandrogenism.

Conclusion The Pro12Ala polymorphism of the PPARγ gene is associated with increased insulin sensitivity and lower hirsutism scores in PCOS women.

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Introduction

Polycystic ovary syndome (PCOS) is the most common endocrine disorder in young women, with a prevalence of 5–10% depending on their ethnic background.[1,2] Recently, controversy over the diagnostic criteria of PCOS has arisen. While both National Institutes of Health (NIH)[3] and Rotterdam 2003[4] definitions exclude other pituitary, adrenal and ovarian pathologies, the 1990 NIH criteria include chronic anovulation and hyperandrogenism, but the 2003 Rotterdam consensus meeting introduced polycystic ovaries (PCO) as an additional third criterion, requiring two out of three to diagnose PCOS. The latter definition, which is not without criticism,[5] expands the number of affected women by more than 30%. The difficulties in finding a common definition of PCOS reflect the heterogeneity of this syndrome. This also holds true for its association with insulin resistance and with the metabolic syndrome. The majority of PCOS patients[6,7] show an increased insulin resistance and compensatory hyperinsulinaemia, which in turn aggravates hyperandrogenism.[3,8] The impairment in insulin secretion and insulin action in PCOS is of great concern because this condition is associated with the metabolic syndrome and thought to be a risk factor for the development of type 2 diabetes mellitus in later years. The pathogenesis of insulin resistance in PCOS women is likely to be multifactorial. While a family history of obesity and insulin resistance seems to be more prevalent in affected patients, it is unclear whether this association is due to nutrition as an environmental factor or inherited, thus implicating a genetic background. Associations of PCOS with mutations in a variety of candidate genes involved in obesity,[9] insulin resistance,[10]

Correspondence: Onno E. Janssen, Division of Endocrinology, Department of Medicine, University of Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany. Tel: +49 201723 2854; Fax: +49 201723 5976; E-mail: onno.janssen@uni-essen.de

glucose metabolism,[11] reproduction[10] and hormone secretion[12] have been analysed, without evidence of a single common defect, again reflecting the heterogeneity of the PCOS.

The discovery that glitazones improve insulin resistance via activation of the peroxisome proliferator activated receptor gamma (PPARγ) has spurred analysis of PPARγ gene mutations as the cause of metabolic disorders, including insulin resistance, obesity and type 2 diabetes mellitus.[13] As PPARγ is a nuclear receptor regulating lipid and energy metabolism,[14–16] PPARγ mutations are likely candidates to affect insulin action. Indeed, the PPARγ Pro12Ala polymorphism has been shown to be associated with a lower degree of insulin resistance,[17] an increased insulin clearance[18] and a reduced risk of type 2 diabetes.[19,20] In mice heterozygous for PPARγ deficiency, a higher insulin sensitivity than in wild-type mice was found.[21] Eriksson and co-workers discovered that the Ala allele might protect against the insulin resistance that is associated with small body size at birth.[22] Thus, while insulin sensitivity is apparently determined by the Pro12Ala polymorphism, its effect on β-cell function and insulin secretion is unknown. In a Spanish study population the Pro12Ala polymorphism was also associated with lower total triglyceride levels.[23] In a Finnish cohort of patients with familial combined hyperlipidaemia the Ala12 allele was associated with a lower body mass index (BMI), lower triglycerides and an increased high-dsnsity lipoprotein (HDL)-cholesterol.[24] Simon *et al.*[25] demonstrated higher leptin levels in female carriers of the Pro12Ala mutation compared to noncarriers. In PCOS patients, Korhonen and co-workers[26] investigated the possible association between the PPARγ polymorphism and the occurrence of PCOS. They reported a significantly lower prevalence of the Ala isoform in PCOS women, hypothesizing that this genotype might protect against the development of PCOS. Another study[27] showed that Caucasian PCOS patients with the Ala allele were less insulin-resistant than those women with two Pro alleles.

The present study was designed to address the questions whether carriers of the Pro12Ala polymorphism have differences in family history of diabetes, insulin resistance and insulin secretion and biochemical or clinical parameters of hyperandrogenism compared to PCOS women with the wild-type PPARγ.

Materials and methods

Study population

PCOS-patients ($n = 102$, age 27 ± 5·3 years) seeking medical advice for cycle abnormalities, hirsutism, obesity or infertility were recruited from the outpatient clinics of the Division of Endocrinology, Department of Medicine and the Department of Gynecology at the University of Essen. Based on the criteria derived from the 1990 NIH conference, diagnosis of PCOS was established when either oligomenorrhoea (cycles lasting longer than 35 days) or amenorrhoea (absence of menstrual cycles in the past 6 months) and either clinical signs of hyperandrogenism [hirsutism with a Ferriman/Gallwey (FG) score of more than 5[28] or obvious acne or alopecia and/or an elevated total testosterone (normal range < 2·0 nmol/l)] were found, and other pituitary, adrenal or ovarian diseases could be excluded. Hirsutism was routinely graded by two physicians independently. FG scores never differed by more than 2 and when not identical were re-evaluated by a third physician and the median value used. According to the FG scores, hirsutism was classified as 0–5, none; 6–9, mild; 10–14, moderate; and 15+, severe. Age-matched healthy controls ($n = 104$) were recruited from a screening programme for employees at the University of Essen. All patients and controls were of Caucasian origin. The NIH-PCOS criteria were excluded in controls before entering the study. PCOS as well as control subjects were not taking any medication known to affect carbohydrate metabolism or endocrine parameters for at least 3 months before entering the study. The study protocol was approved by the Ethics Committee of the University of Essen. Written informed consent was obtained from all participants.

Clinical and biochemical data

In PCOS subjects, clinical parameters, including the degree of hirsutism, acne and alopecia, were verified by physical examination. Sitting blood pressure was measured after a 15-min rest. Birth weight, the age at menarche, menstrual cyclicity, familiy history of PCOS and family history of type 2 diabetes were evaluated in each patient by personal interview. Body fat was measured using the Body FAT Watcher (NAIS Wellnesslife GmbH, Düsseldorf, Germany) and BMI was calculated as weight/(height)^2 (kg/m^2). Parameters of insulin resistance and β-cell function were evaluated using a 3-h oral glucose tolerance test (OGTT). After an overnight fast of 12 h, patients ingested 75 g glucose and had their glucose and insulin levels determined at baseline and after 30, 60, 90, 120 and 180 min. Insulin resistance and β-cell function were defined by the HOMA-model,[29] hyperinsulinaemia by calculating the area under the insulin response curve (AUC-I) and insulin secretion by the 30-min increment in glucose concentration over the 30-min increment in glucose concentration (dI/dG). In addition the whole-body insulin sensitivity (ISI Matsuda)[30] was measured by the formula: 10·000/square root of [fasting glucose × fasting insulin] × [mean glucose × mean insulin during OGTT (times 0, 30, 60, 90, 120 minutes)]. Impaired glucose tolerance (IGT) and diabetes mellitus type 2 (T2DM) were defined by the classification from the German Diabetes Association.

For biochemical analysis, automated chemiluminescence immunoassay systems were used for the determination of LH, FSH, testosterone (T), oestradiol (E2), cholesterol (CHOL), LDL-cholesterol (LDL), HDL-cholesterol (HDL) and triglycerides (TGL; ADVIA Centaur, Bayer Vital, Fernwald, Germany), dehydroepiandrosteronesulphate (DHEAS), insulin and sex hormone binding globuline (SHBG; IMMULITE 2000, DPC Biermann, Bad Nauheim, Germany) and IGF-1 (Nichols Advantage, Nichols Institute Diagnostics, Bad Vilbel, Germany). Leptin, adiponectin and ghrelin were measured using specific RIA Kits (Linco Research Inc., St Louis, MO, USA). Blood glucose was measured by an automated hexokinase method on a Dimension RXL (Dade Behring Marburg GmbH, Marburg, Germany). The glycosylated fraction of haemoglobin-A1c (HbA1c) was determined by an automated high-performance liquid chromatography (HPLC) method on an A1C 2·2 glycohemoglobin analyser (TOSOH-Eurogenetics, Cologne, Germany). Intra-assay variation was < 5% and interassay variation was < 8% for all parameters.

Genotyping

For the molecular analysis of the PPARγ alleles genomic DNA was isolated from whole blood with the QIAmp DNA Blood Midi Kit (QIAGEN GmbH Germany, Düsseldorf, Germany). The respective part of the PPARγ gene was amplified by PCR using the upstream primer 5′GCCAATTCAAGCCCAGTC3′ and the mutagenic downstream primer 5′GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG3′ which introduces a *Bst*U-I restriction site only when the C to G substitution at nucleotide 34 is present as previously described.[13] PCR products were digested with *Bst*U-I and a 2·5% agarose gel electrophoresis was performed. For homozygote wild-type subjects a 270-bp product, for Pro12Ala a 227-bp and a 43-bp product and for heterozygote women 270-, 227- and 43-bp products were detected. All genotypes were confirmed by automated sequencing on an ABI Prism 377 DNA sequencer (Applied Biosystems, Darmstadt, Germany).

Statistical analysis

Differences of findings between PCOS subgroups were evaluated with the unpaired *t*-test for normally distributed parameters and by Wilcoxon rank sum test for non-normally distributed parameters using the Statistical Package for the Social Sciences (SPSS Inc. Chicago, IL, USA, version 11·0). Prevalence data, hirsutism score categories and genotype distribution were compared by χ^2 using SPSS. Data are presented as mean ± SD, median or number of patients. *P*-values < 0·05 were considered significant.

Results

A total of 102 women with PCOS (age 27 ± 5·3 years) and 104 aged-matched healthy women (age 28·0 ± 5·4 years) were recruited. The majority of individuals in both groups were nonsmokers, drank alcohol infrequently and rated their general health as being 'good' or 'very good'. In our study cohort the frequency of the Pro12Ala mutation of the PPARγ gene did not differ significantly between PCOS women and healthy controls (Table 1). Only one homozygous carrier of the Ala substitution was identified in the PCOS-group. Due to the low number of Ala/Ala patients only Pro/Pro patients were compared with Pro/Ala patients in all subsequent analyses.

The metabolic parameters of the PCOS study cohort are shown in Table 2. PCOS women with the Pro/Ala genotype were less insulin-resistant than Pro/Pro patients, evidenced by a lower HOMA-IR (2·9 ± 1·9 *vs.* 4·5 ± 4·1) and reduced fasting insulin levels (70·0 ± 52·6 *vs.* 110·0 ± 100·6 pmol/l). Both AUC-insulin (221·0 ± 99·5 *vs.* 372·0 ± 393·3) and AUC-glucose (309·0 ± 49·6 *vs.* 342·0 ± 83·5) were also lower in Pro/Ala than in Pro/Pro-subjects (Table 2). No significant differences were noted in BMI, body fat, parameters of insulin secretion (HOMA-β, dI/dG), 2-h glucose during OGTT, HbA1c and lipid status. Furthermore, serum adiponectin, leptin and ghrelin levels were similar within the two groups, even when comparing data from obese or lean Pro/Pro and Pro/Ala women. Leptin levels were positively correlated to BMI ($r = 0·74$) and body fat ($r = 0·72$) in all patients. In a subgroup analysis, comparing either lean PCOS women with and without the Ala allele or overweight/obese women with and without the Ala allele, differences in insulin resistance were significant only in the overweight/obese subgroup. In Pro/Ala patients with a BMI ≥ 25, HOMA-IR (3·8 ± 1·9 *vs.* 5·9 ± 4·4), fasting insulin (88·0 ± 51·2 *vs.* 146·0 ± 109·9 pmol/l) and AUC-I (252·0 ± 82·5 *vs.* 490·0 ± 442·9) were significantly lower than in wild-type women. ISI Matsuda as an index for hepatic and peripheral insulin sensitivity was significantly higher in Pro/Ala than in Pro/Pro genotypes (3·85 ± 1·69 *vs.* 2·43 ± 2·3; Table 3). No differences were found in total cholesterol or triglycerides. In lean women (Pro/Ala *vs.* Pro/Pro) no significant differences were found in the following parameters: HOMA-IR (1·6 ± 0·7 *vs.* 2·0 ± 0·9), ISI Matsuda (8·29 ± 5·02 *vs.* 6·72 ± 3·56), fasting insulin (39·0 ± 22·8 *vs.* 51·0 ± 29·9 pmol/l) and AUC-I (170·0 ± 102·4 *vs.* 176·0 ± 121·4). Parameters of hyperandrogenism and other endocrine biochemical values also did not differ between Pro/Pro and Pro/Ala women (Table 4). Data on clinical findings and family history of all genotypes are presented in Table 5. Features concerning PCOS and metabolic syndrome like birth weight, age at menarche, menstrual cyclicity, acne, alopecia and blood pressure were similar in both genotypes. The prevalence of impaired glucose tolerance and diabetes mellitus was also comparable in Pro/Pro and Pro/Ala subjects. Thirty-one out of 62 Pro/Pro PCOS women had a family history of T2DM and 25 out of the

Table 1. PPAR-γ genotype distribution in PCOS patients and controls

Genotype	Pro/Pro	X/Ala
PCOS ($n = 102$)	79 (77·5)	23 (22·5)
Controls ($n = 104$)	80 (76·9)	24 (23·1)

Values are number (% of total).

Table 2. Metabolic parameters in Pro/Pro and Pro/Ala-PCOS women

Variable	Pro/Pro	Pro/Ala	*P*-value
n	79	22	
Age (years)	27·0 ± 5·3	25·0 ± 5·8	NS
BMI (kg/m^2)	30·0 ± 9·4	30·0 ± 7·6	NS
Body fat (%)	37·1 ± 10·2	37·9 ± 8·1	NS
Fasting insulin (pmol/l)	110·0 ± 100·6	70·0 ± 52·6	0·0145*
HOMA-IR (mUmM/l)	4·5 ± 4·1	2·9 ± 1·9	0·0110*
HOMA-β (%)	273·0 ± 204·8	210·0 ± 126·1	NS
dI/dG	268·0 ± 203·3	260·1 ± 191·5	NS
AUC-insulin	372·0 ± 393·3	221·0 ± 99·5	0·0027*
AUC-glucose	342·0 ± 83·5	309·0 ± 49·6	0·0232*
2-h glucose (mmol/l)	5·79 ± 1·74	5·33 ± 1·21	NS
HbA1c (%)	5·0 ± 0·5	5·2 ± 0·7	NS
CHOL (mmol/l)	5·1 ± 1·0	4·91 ± 0·87	NS
LDL (mmol/l)	3·12 ± 1·09	3·27 ± 0·84	NS
HDL (mmol/l)	1·45 ± 0·47	1·32 ± 0·33	NS
TGL (mg/dl)	109·0 ± 75·1	89·0 ± 28·9	NS
Adiponectin (μg/ml)	28·6 ± 20·6	32·4 ± 21·9	NS
Leptin (ng/ml)	27·8 ± 19·6	30·8 ± 18·7	NS
Ghrelin (pg/ml)	32·6 ± 14·9	35·9 ± 15·9	NS

Values are mean ± SD (range). NS, not significant. *By Wilcoxon rank sum test. BMI, body mass index; HOMA, homeostatis model assessment; AUC, area under curve.

Table 3. Metabolic parameters in overweight and obese Pro/Pro and Pro/Ala PCOS subjects

Variable	Pro/Pro (BMI ≥ 25)	Pro/Ala (BMI ≥ 25)	*P*-value
n	50	12	
% of total	63	55	NS
BMI (kg/m^2)	35·0 ± 7·9	34·0 ± 6·5	NS
HOMA-IR (mUmM/l)	5·9 ± 4·4	3·8 ± 1·9	0·0153*
ISI Matsuda	2·43 ± 2·30	3·85 ± 1·69	0·0246*
Fasting insulin (pmol/l)	146·0 ± 109·9	88·0 ± 51·2	0·0100*
AUC-insulin	490·0 ± 442·9	252·0 ± 82·5	0·0008*
AUC-glucose	362·0 ± 91·7	318·0 ± 37·4	0·0124*

Values are mean ± SD (range). NS, not significant. *By Wilcoxon rank sum test. ISI, insulin sensitivity index; HOMA, homeostatis model assessment; AUC, area under curve; BMI, body mass index.

Table 4. Endocrine parameters in Pro/Pro and Pro/Ala-PCOS women

Variable	Pro/Pro	Pro/Ala	*P*-value
n	79	22	
LH : FSH ratio	2·4 ± 1·3	2·2 ± 1·2	NS
Testosterone (nmol/l)	2·8 ± 1·1	2·7 ± 1·1	NS
FAI	8·8 ± 6·4	7·4 ± 4·5	NS
E2 (pmol/l)	222·1 ± 160·2	224·2 ± 206·9	NS
DHEAS (µmol/l)	6·0 ± 3·2	5·8 ± 2·8	NS
Cortisol (nmol/l)	394·6 ± 181·8	386·4 ± 183·9	NS
IGF-1 (µg/l)	183·3 ± 56·7	169·9 ± 57·8	NS

Values are mean ± SD (range). NS, not significant; FAI, free androgen index.

79 PCOS patients also had a family history of PCOS, including premature balding as the male PCOS phenotype. Compared to the Ala allele carriers no significant differences in family history were found (Table 5).

No general differences in the distribution pattern of hirsutism were found in Pro/Pro and Pro/Ala patients. However, Pro/Pro PCOS women presented significantly more often with hirsutism (Table 5). Hirsutism scores of Pro/Ala PCOS women were significantly lower compared with Pro/Pro PCOS women (median 7 *vs.* 11; $P < 0{\cdot}05$ by Wilcoxon rank sum test). The differences in hirsutism scores remained significant even after performing subgroup analysis of lean and overweight/obese women (data not shown). Differences were also found comparing lean and obese Pro/Ala patients, implicating an additional effect of BMI on the degree of hirsutism only in Ala allele carriers (median 10 *vs.* 12). Analysis of FG scores by category revealed a highly significant difference in the proportion of mild, moderate and severe hirsutism in Pro/Pro and Pro/Ala PCOS patients (Table 6, $P < 0{\cdot}01$ by χ^2 test).

Discussion

In the present study the PPARγ Pro/Ala genotype frequency in German PCOS patients was similar to a Finnish PCOS cohort,[26] with 21·6% and 20·7% heterozygous women, respectively. While

Table 5. Clinical characteristics and family history of diabetes and PCOS

Variable	Pro/Pro	Pro/Ala	*P*-value
n	79	22	
Amenorrhoea	21 (26·6)	6 (27·3)	NS
Oligomenorrhoea	58 (73·4)	16 (72·7)	NS
Age at menarche (years)	12·8 ± 1·7	12·8 ± 1·3	NS
Acne	32 (40·5)	8 (36·4)	NS
Alopecia	23 (29·1)	9 (40·9)	NS
Hirsutism	65 (82·3)	13 (59·0)	0·025†
Birth weight (g)	3341·8 ± 591·0	3525·0 ± 318·2	NS
Family history of T2DM	31 of 62 (50)	5 of 14 (35·7)	NS
Family history of PCOS	25 (31·6)	3 (13·6)	NS
IGT	8 (10·1)	0 (0)	NS
T2DM	1 (1·3)	0 (0)	NS
DM1	1 (1·3)	1 (4·5)	NS
Systolic blood pressure (mmHg)	121·9 ± 12·1	120·0 ± 15·0	NS

Values are mean ± SD (range) or number (% of total). NS, not significant. †By Chi-square test. T2DM, type II diabetes mellitus.

Table 6. Evaluation of hirsutism by category in Pro/Pro and Pro/Ala PCOS patients

FG-score		Pro/Pro ($n = 79$)	Pro/Ala ($n = 22$)
FG 0–5	No hirsutism	14	9
FG 6–9	Mild hirsutism	18	9
FG 10–14	Moderate hirsutism	26	3
FG 15–36	Severe hirsutism	21	1

Values are number of total. FG, Ferriman/Gallwey.

homozygosity for the Ala allele is rare in the overall population, other ethnic groups have been found to vary from 1 to 25% in the frequency of Pro/Ala heterozygosity.[27] In our study cohort, the genotype distribution in PCOS patients and healthy controls did not differ significantly and, in addition, was comparable to that found in other healthy control populations.[20,22,31] In three large community-based populations including healthy relatives of patients with T2DM,[18] cardiovascular disease[17] and obesity,[23] and in a recent PCOS cohort,[27] the PPARγ Pro/Ala polymorphism was found to be associated with lower insulin resistance and lower hyperinsulinaemia. Concordant results were obtained from animal studies, showing greater insulin sensitivity in heterozygous PPARγ knock-out mice.[21] Insulin resistance is a predictor of diabetes development even when the individual has a normal glucose tolerance, suggesting that carriers of the Ala allele may be at reduced risk to develop T2DM. In our study, all parameters of IR and hyperinsulinaemia were lower in obese Pro/Ala compared to Pro/Pro PCOS subjects. However, in lean PCOS women, no differences were found. This held true for both the HOMA-IR, reflecting hepatic insulin sensitivity and hepatic glucose production, and the Matsuda index. The Matsuda index combines hepatic and peripheral insulin sensitivity and is thought to be a better parameter to determine insulin sensitivity and resistance in subjects with normal glucose tolerance.[32] IGT was found in eight out of 79 Pro/Pro patients but in none of 22 Pro/Ala PCOS women, and obese

Pro/Ala patients had a lower AUC-glucose, further indicating a possible protective effect of the PPARγ Ala allele against the manifestation of T2DM. As no differences in lean PCOS subjects were detected, the benefit of lower IR associated with the Pro12Ala polymorphism may either be revealed only in obesity or depends on a certain environmental background, such as nutritional status, or even on gene–gene interactions.[33]

The family history for T2DM or PCOS did not depend on the PPAR genotype. Furthermore, in our German PCOS cohort, no differences in total cholesterol, HDL-cholesterol, LDL-cholesterol or triglycerides were found, in agreement with another German[34] and a Spanish[25] cohort of T2DM patients but in contrast to a study in elderly Finnish nondiabetic subjects, who were reported to have differences in their lipid and lipoprotein profiles depending on their genotypes.[24] In keeping with a recent study in a healthy German cohort,[31] adiponectin levels of our PCOS women did not differ in Pro/Pro and Pro/Ala carriers, even after subgroup analysis of lean and obese patients, compatible with the findings of Orio *et al.*[35] Again, ethnic differences are obvious, as Yamamoto reported lower adiponectin levels in Ala carriers in a healthy Japanese population.[36] This result was unexpected, as the lower IR associated with the Ala allele, if at all, would predict higher adiponectin levels. In contrast to data recently published by Simon *et al.*,[25] we did not find an association of leptin serum levels with the PPARγ polymorphism, in accordance with similar recent data from Orio *et al.*,[37] while we did find a correlation of leptin with BMI and body fat. As ghrelin has been shown to stimulate the differentiation of preadipocytes *in vitro* and to increases the expression of the PPARγ2 gene,[38] an effect of PPARγ mutations on the ghrelin expression might be expected. However, in our PCOS cohort, ghrelin levels were not influenced by the Pro12Ala genotype.

Glitazones have turned out to be an option to improve insulin resistance in patients with T2DM[39] or PCOS.[28] Troglitazone has also been shown to improve hyperandrogenaemia[40] and hirsutism.[28] *In vitro*, glitazones inhibit 17-hydroxylase (P450c17) and 3β-hydroxysteroiddehydrogenase type II (3βHSDII), key enzymes in androgen biosynthesis and bind with different affinity to the nuclear receptor PPARγ.[41] Furthermore, activation of PPARγ inhibits insulin-stimulated theca cell androgen production *in vitro*.[42] In our PCOS patients, neither androgen and gonadotropin levels nor the prevalence of acne or alopecia were affected by the Pro12Ala genotype. However, hisutism scores were lower in PCOS women carrying the Ala allele, even if subgroup analysis of lean and obese as well as of hirsute (FG > 5) or nonhirsute women were performed. In Pro/Ala, but not in Pro/Pro, subjects a higher BMI was associated with higher hirsutism scores. Thus, while the common Pro12 Ala PPARγ polymorphism does not affect ovarian and adrenal androgen levels, it appears to increase the prevalence and modulate the severity of hirsutism in PCOS-affected women.

Both in men[43] and mice,[44] expression of all three PPAR isotypes has been found in the pilosebaceous unit (PSU), which comprises a single morphological entity of the sebaceous gland and the hair follicle.[45,46] PPAR ligands have been found specifically to affect sebaceous cell growth, differentiation and lipogenesis,[47] and PPARs appear to be involved in the mechanisms associated with chronic inflammation present in acne,[46] psoriasis[48] and wound healing.[49]

The majority of steroid hormones and other local or systemic factors can directly and indirectly regulate hair growth. As androgens are responsible both for the vellous-terminal transition of pubic follicles and the terminal-vellous transition and apoptosis in balding follicles, and androgen levels correlate poorly with the prevalence and degree of hirsutism, local factors appear differentially to regulate PSU response, such as variations in 5-α-reductase.[50–52] Our study is the first to implicate PPARs as a possible new local factor in hair growth, as the prevalence and degree of hirsutism were different in PCOS patients depending on their PPARγ genotype. While the molecular biology of hair morphogenesis has been studied extensively, and at least three signalling pathways are involved in hair follicle induction and differentiation,[45] the role of PPARs for differentiation of the PSU or growth of terminal hair is unclear.[44,46]

In summary, our data confirm a beneficial effect of the PPARγ 12Ala allele on insulin resistance and glucose metabolism in PCOS women. Furthermore, the Ala allele appears to be associated with less severe hirsutism, independent of hyperandrogenaemia. Further studies are required to evaluate these findings in other ethnic cohorts and to elucidate the underlying molecular mechanisms.

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