

# Tryptophan 64 → arginine polymorphism of $\beta$ -3-adrenergic receptor in Chilean women with polycystic ovary syndrome

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## Summary

**Objective** To establish the frequency of the Trp64Arg polymorphism of the  $\beta$ 3 adrenergic receptor (ADRB3) in women with polycystic ovary syndrome (PCOS) from a Chilean population, focusing particularly on the interaction with body weight. In addition, we evaluated the relationship of the Trp64Arg variant with other metabolic components of this syndrome.

**Patients and Design** In a case–control design study, a total of 106 women with clinical and hormonal evidence of PCOS and 82 healthy women (HW) were evaluated.

**Measurements** An oral glucose tolerance test (OGTT) was performed and serum glucose and insulin were measured before the glucose load and 30, 60, 90 and 120 min after. Lipid profile was determined in the basal sample. Insulin resistance was assessed by the homeostatic model assessment ( $\text{HOMA}_{\text{IR}}$ ) and insulin sensitivity index (ISI) composite. A polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis was performed to determine the Trp64Arg polymorphism of ADRB3.

**Results** The frequency of the heterozygous condition was similar between PCOS and HW (39% vs. 35%). Only two subjects were homozygous for arginine, both belonging to the PCOS group and having a body mass index (BMI)  $> 30 \text{ kg/m}^2$ . In the crude analysis, hypothesis tests and odds ratios show that there is no evidence of association between the ADRB3 Trp64Arg variant and PCOS ( $P = 0.47$ ). Moreover, when data were stratified by BMI categories, the statistical test for interaction between Trp64 carrier status and obesity was not significant ( $P = 0.29$ ). This variant was present in 52% of the obese PCOS patients and 40% of the obese HW. In normal weight and obese PCOS carriers, the presence of the Trp64Arg variant was associated with high triglyceride (TG) levels. A major effect of the Trp64Arg variant on insulin resistance parameters could not be demonstrated.

**Conclusions** The frequency of the Trp64Arg polymorphism was similar in healthy women and PCOS women, and a possible interaction between the effect of this variant and obesity in PCOS could not be demonstrated. However, our results showed an association between triglyceride levels and the presence of this genetic variant in PCOS women.

## Introduction

In recent years, several studies have reported links between insulin resistance and polycystic ovary syndrome (PCOS), one of the most common endocrine disorders affecting 5–10% of premenopausal women.<sup>1–3</sup> The most widely accepted definition of PCOS is the association of chronic anovulation and hyperandrogenism in the absence of specific diseases of the ovaries, adrenals and pituitary.<sup>4</sup> In addition, most women with PCOS also exhibit peripheral insulin resistance, affecting predominantly muscle and adipose tissue, and a compensatory hyperinsulinaemia independent of obesity.<sup>5–7</sup> It is currently accepted that insulin resistance and pancreatic  $\beta$ -cell dysfunction, with increased risk of type 2 diabetes, are usual comorbidities in PCOS patients.<sup>8–10</sup> Approximately 50% of PCOS women are overweight or obese and most of them exhibit abdominal fat distribution.<sup>10</sup> Studies have shown that 25–35% of obese women with PCOS will have either impaired glucose tolerance or type 2 diabetes by 30 years of age, and that the history of diabetes in a first-degree relative appears to define a subset of PCOS subjects with a greater prevalence of insulin secretory defects.<sup>11,12</sup> Therefore, PCOS is a major health issue for women with implications well beyond the reproductive endocrine abnormalities that usually bring women with PCOS to clinical attention at an early age. This offers the opportunity to detect metabolic abnormalities earlier in these women.<sup>8,13</sup>

Obesity has both environmental and genetic determinants, and there is some evidence that molecular defects in the  $\beta$ 3 adrenergic receptor (ADRB3) may predispose subjects to obesity and insulin resistance by decreasing energy expenditure.<sup>14,15</sup> A missense mutation

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in codon 64 of the ADRB3 gene tryptophan → arginine at base amino acid (Trp64Arg) has been reported in several ethnic groups, associated with an early onset of type 2 diabetes and increased weight gain.<sup>16–24</sup> The clinical importance of this defect could lead to alterations in signal transduction resulting in decreased adipose tissue lipolysis and resting metabolic rates.<sup>25,26</sup>

Because of the high prevalence of obesity in PCOS women and the potential role of the Trp64Arg polymorphism in predisposition to obesity, we decided to evaluate as a first step the relationship between this variant and obesity in PCOS women. The aim of this study was to establish the frequency of the Trp64Arg polymorphism of ADRB3 in women with PCOS in a Chilean population, focusing particularly on the interaction with body weight. In addition, we evaluated the relationship of the Trp64Arg variant with other metabolic components of this syndrome.

## Subjects and methods

### Subjects

One hundred and six unrelated women with PCOS, with an age range of 15–35 years, were consecutively recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile, between 2000 and 2002.

Diagnosis of PCOS was made if subjects had chronic anovulation, hyperandrogenism without any other specific causes of adrenal or pituitary disease, and met the diagnostic criteria for PCOS of the NIH consensus.<sup>4</sup>

Inclusion criteria for cases were: chronic oligo- or amenorrhoea, hirsutism, serum androstenedione concentration > 10.5 nmol/l, total testosterone concentration > 2.08 nmol/l and/or free androgen index (FAI) > 5.0. All women were amenorrhoeic and anovulatory according to progesterone measurements and ultrasound examination. The presence of characteristic ovarian morphology on ultrasound was not considered an inclusion criterion. Hyperprolactinaemia, androgen-secreting neoplasm, Cushing's syndrome and attenuated 21-hydroxylase deficiency, as well as thyroid disease, were excluded by appropriate tests.

Eighty-two healthy women (HW), with normal cycles and between 15 and 35 years of age (Table 1), acted as a control group. All HW had a history of regular 28- to 32-day menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism, and absence of galactorrhoea and/or thyroid dysfunction. The women of the control group were recruited from the same city area as the patients and had the same socio-economic status. The Chilean population contains three sociogenetic strata: Strata I (high income, pure Caucasian, representing 7% of the population); Strata II (medium income, mixed population, representing 65% of the population); and Strata III (low income, high percentage of aboriginal ancestry, 18% of the population). The groups of patients and controls represent Strata II, which is considered the most representative 'Chilean Admixture Population'.<sup>27</sup>

All women had given their written consent to participation in the study, which was approved by the local ethics committee.

After a 3-day 300-g carbohydrate diet and an overnight fast of 10 h, all women were admitted to the Clinical Research Centre in

**Table 1.** Clinical and endocrine–metabolic parameters in healthy women (HW) and PCOS women (PCOS)

	HW (n = 82)	PCOS (n = 106)
Age (years)	25.10 ± 5.64	23.58 ± 5.19
BMI (kg/m <sup>2</sup> )	26.65 ± 5.34	29.11 ± 6.08*
WC (cm)	83.63 ± 12.96	89.24 ± 14.15*
Fasting glucose (mmol/l)	4.45 ± 0.68	4.85 ± 0.77*
Insulin (pmol/l)	84.80 ± 51.30	151.32 ± 110.0*
Androstenedione (nmol/l)	5.71 ± 3.16	13.15 ± 5.40*
Testosterone (nmol/l)	1.25 ± 0.42	2.84 ± 1.53*
SHBG (nmol/l)	60.4 ± 31.44	29.27 ± 18.98*
FAI	2.98 ± 1.89	13.69 ± 10.82*

Data are mean ± SD.

\*P < 0.05, HW vs. PCOS.

FAI, free androgen index.

the morning (08:30–09:00 h). A clinical history was obtained and a physical examination was conducted. A 75-g oral glucose tolerance test (OGTT) was performed and subjects were classified according to the World Health Organization (WHO) criteria. Serum glucose and insulin were measured before the glucose load and 30, 60, 90 and 120 min after. SHBG, testosterone, androstenedione and lipid concentrations were also measured before the glucose load. The FAI was calculated as the quotient of the molar concentrations of testosterone (nmol/l) and SHBG (nmol/l).

### Data analysis

The measurements derived from the OGTT included:

1. Serum fasting glucose, serum fasting insulin, homeostatic model assessment (HOMA<sub>IR</sub>) and ISI composite.<sup>28,29</sup>
2. Serum lipid profile, total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDLc).

### Assays

Serum glucose was determined by the glucose oxidase method (Photometric Instrument 4010; Roche, Basel, Switzerland). The coefficient of variation (CV) of this method was less than 2.0%. Serum insulin and testosterone were assayed by radioimmunoassay (RIA) (Diagnostic System Laboratories, TX, USA), and androstenedione was also assayed by RIA (Diagnostic Products Corp., LA, USA). The intra- and interassay CVs were 5 and 8% for insulin, 7.0 and 11.0% for testosterone, 3.7 and 4.9% for androstenedione, respectively. The lipid profile was determined by standard colorimetric assays (Photometric Instrument 4010). The CV of this method was less than 3.0%.

### Anthropometric measurements

Anthropometric measurements were performed in all subjects; height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and weight was measured to the nearest 0.1 kg using a hospital balance beam scale. Body mass index (BMI) was used as a

measure of overall adiposity and was defined as weight (kg)/height<sup>2</sup> (m<sup>2</sup>). The obesity status (normal, overweight or obese) was defined by the WHO criterion.<sup>30</sup> Waist circumference (WC) was measured to the nearest 0.5 cm at the point of narrowing (as viewed from behind) between the umbilicus and xiphoid process.

### Molecular analysis

Genomic DNA was extracted from peripheral blood leucocytes. The Trp64Arg polymorphism of ADRB3 was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using Bst NI endonuclease digestion, according to the method described in the literature.<sup>17,31</sup>

### Statistical evaluation

Continuous variables are expressed as mean  $\pm$  standard deviation (SD). Differences among study groups were assessed through the Student *t*-test. Fisher's exact test and odds ratios were calculated to compare allele and genotype frequencies between PCOS and HW. Hardy-Weinberg equilibrium was evaluated through an exact method. The Breslow-Day test for interaction was performed to assess homogeneity of odds ratios across levels of obesity status (normal, overweight or obese). All statistical analyses were performed with STATA 7.0 package. The significance level was set at 5%.

### Results

Table 1 shows the clinical and hormonal characteristics of healthy and PCOS women. Mean age was not different between PCOS women and the HW group. BMI was significantly higher in PCOS women compared to HW ( $P < 0.05$ ). As expected, WC, serum androstenedione concentrations, total serum testosterone concentrations and FAI were significantly higher and SHBG was significantly lower in PCOS women compared to HW ( $P < 0.01$ ). Moreover, PCOS women showed significantly higher fasting glucose and insulin concentrations than control women.

The frequency of the Trp64Arg variant was not different between HW and PCOS women. Both groups were in Hardy-Weinberg

**Table 2.** Genotypic frequency of the Trp64Arg polymorphism in Chilean healthy (HW) and PCOS women (PCOS)

ADRB3 genotype	HW (n = 82)		PCOS (n = 106)	
	n	(%)	n	(%)
Trp64Trp	53	65.0	63	59.0
Trp64Arg	29	35.0	41	39.0
Arg64Arg	0	0.0	2	2.0

Fisher's exact test ( $P$ -value = 0.52).

Hardy-Weinberg equilibrium: PCOS = 0.15 and HW = 0.06.

equilibrium, as shown in Table 2. The allelic frequency for the Arg64 carriers was 0.21 in PCOS and 0.18 in HW.

In the HW group, no differences were observed in BMI between Trp64Arg carriers and noncarriers (Table 3). The same tendency was observed in the PCOS group. We found only two subjects homozygous for the Arg64Arg variant, both from the PCOS group. Both homozygous carriers showed a BMI of over 30 kg/m<sup>2</sup>.

With regard to WC in the HW and the PCOS groups, carriers and noncarriers of the Trp64Arg mutation displayed similar WC (HW: 85.6  $\pm$  14.1 cm vs. 82.6  $\pm$  12.4 cm; PCOS: 90.2  $\pm$  15.2 cm vs. 88.5  $\pm$  13.8 cm). However, among the PCOS patients, mean values of WC tended to increase with the Arg dosage compared to noncarriers: Trp64Trp (88.5  $\pm$  13.8 cm), Trp64Arg (90.2  $\pm$  15.2 cm) and Arg/Arg (95.0  $\pm$  7.1 cm) ( $P = 0.12$ ).

Regarding insulin resistance parameters (fasting insulin, HOMA<sub>IR</sub> and ISI composite), no differences were established between carriers and noncarriers in each group. With regard to the lipid profile, TG concentration was higher in PCOS carriers than in noncarriers ( $P = 0.01$ ) (Table 3).

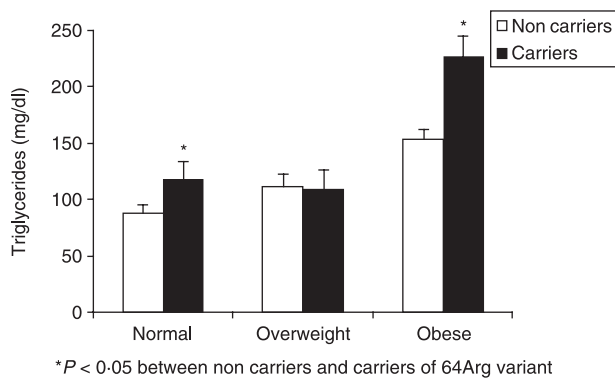
Figure 1 shows the TG concentrations in carriers and noncarriers of the Trp64Arg polymorphism in PCOS women according to BMI categories. TG levels were higher in normal weight carriers vs. noncarriers of the codon 64 variant (1.33  $\pm$  0.39 mmol/l vs. 0.97  $\pm$  0.32 mmol/l,  $P = 0.008$ ). Moreover, elevated TG levels were also observed in obese PCOS carriers vs. obese PCOS noncarriers (2.56  $\pm$  1.00 mmol/l

**Table 3.** Clinical and endocrine-metabolic parameters of healthy (HW) and PCOS women (PCOS) according to the Trp64Arg variant

	HW carriers (n = 29)	HW noncarriers (n = 53)	PCOS carriers (n = 43)	PCOS noncarriers (n = 63)
BMI (kg/m <sup>2</sup> )	27.7 $\pm$ 6.3	26.1 $\pm$ 4.7	29.9 $\pm$ 6.3	28.5 $\pm$ 5.9
WC (cm)	85.6 $\pm$ 14.1	82.6 $\pm$ 12.4	90.2 $\pm$ 15.2	88.5 $\pm$ 13.8
Fasting glucose (mmol/l)	4.55 $\pm$ 0.58	4.40 $\pm$ 0.73	4.81 $\pm$ 0.78	4.88 $\pm$ 0.77
Fasting insulin (pmol/l)	78.20 $\pm$ 35.8	88.25 $\pm$ 58.8	158.5 $\pm$ 105.5	138.5 $\pm$ 92.6
HOMA <sub>IR</sub>	2.3 $\pm$ 1.2	2.5 $\pm$ 1.9	5.0 $\pm$ 3.8	4.4 $\pm$ 3.6
ISI composite	8.5 $\pm$ 5.6	7.3 $\pm$ 5.6	4.0 $\pm$ 2.7	3.9 $\pm$ 3.3
Triglycerides (mmol/l)	1.27 $\pm$ 0.70	1.41 $\pm$ 0.55	1.72 $\pm$ 0.97	1.32 $\pm$ 0.67*
Total cholesterol (mmol/l)	4.50 $\pm$ 1.04	4.57 $\pm$ 0.91	4.79 $\pm$ 1.33	4.65 $\pm$ 1.15
HDL cholesterol (mmol/l)	1.09 $\pm$ 0.36	1.07 $\pm$ 0.28	0.86 $\pm$ 0.30	0.86 $\pm$ 0.40

Data are mean  $\pm$  SD.

\* $P < 0.05$ , PCOS carriers vs. noncarriers.



**Fig. 1** Triglyceride concentrations in carriers and noncarriers of the Trp64Arg polymorphism in PCOS women according to BMI categories (normal weight: BMI < 24.9 kg/m<sup>2</sup>, overweight: BMI 25–29.9 kg/m<sup>2</sup>, and obese: BMI > 30 kg/m<sup>2</sup>).

l vs. 1.73 ± 0.41 mmol/l,  $P = 0.03$ ). However, in overweight patients, TG concentrations were similar in carriers and noncarriers of the Trp64Arg variant.

On the other hand, in the HW group, we did not observe differences between carriers and noncarriers according to BMI categories of the Trp64Arg variant regarding lipid profile and insulin resistance parameters.

Finally, Table 4 shows the odds ratios for the association with their 95% confidence intervals across groups defined by BMI categories. In the crude analysis, hypothesis tests and odds ratios show that there is no evidence of association between the ADRB3 Trp64Arg variant and PCOS ( $P = 0.47$ ). The same was observed when the data were stratified by BMI categories. As shown in the table, this variant was present in 52% of the obese PCOS patients and 40% of the obese HW.

## Discussion

In the present study, we examined the prevalence of the Trp64Arg polymorphism in a Chilean population of PCOS women and the relationship of the Trp64Arg variant with metabolic features of this syndrome. We found a similar frequency of this polymorphism in healthy and PCOS women, and a possible interaction between the effect of this variant and obesity in PCOS could not be demonstrated.

However, in the PCOS group, the presence of the Trp64Arg variant was associated with high TG levels in normal weight and obese women.

The genetic Trp64Arg polymorphism of ADRB3 has been reported in several populations and ethnic groups.<sup>19</sup> In general, the prevalence of Arg64 carriers in our population (41% in PCOS and 35% in HW) is higher than that described in other studies, in which it has been demonstrated that the prevalence of Arg64 carriers is not different between normal weight, overweight and obese subjects (12, 13 and 10%, respectively).<sup>24</sup>

A previous study of our group in an Aymara population (one of the three ethnic groups that currently live in Chile) showed a low prevalence of this polymorphism with an estimated allele frequency of the Arg64 variant of 0.13.<sup>32</sup> However, it is interesting to note that this ethnic group represents only 2% of the population in Chile.

In the present study, a similar genotypic frequency of this polymorphism in PCOS (41%) and HW (35%) was observed. Moreover, the polymorphism was present in 52% of the obese PCOS women and in 40% of the obese HW, suggesting that there is no interaction between the effect of this variant and obesity in PCOS women.

The data obtained in this study present insufficient evidence to support the association of this polymorphism with BMI, similar to what was observed in our Aymara population.<sup>32</sup> Nevertheless, some populations, such as the Japanese, show a significant association between homozygous carriers of the Arg64Arg variant and BMI compared with heterozygous and wild-type carriers, suggesting that this mutation contributes directly to weight gain and susceptibility to type 2 diabetes.<sup>33</sup> Other reports demonstrate a strong association of this variant with BMI and gender.<sup>16–18,31,33–35</sup> However, another study did not show differences between carriers and noncarriers with regard to BMI, insulin levels or TG concentrations.<sup>36</sup>

Our results show an association between TG levels and the presence of this genetic variant in PCOS women. Conflicting results have been published relating to the association between this genetic variant and plasmatic lipids. A Danish study showed that Arg64 carriers had elevated TG and low density lipoprotein (LDL) levels, while in China an association of this polymorphism with low HDL cholesterol was observed.<sup>23,37</sup> However, in other studies, this association has not been reported.<sup>17,38–40</sup>

In the present study, PCOS women exhibited central obesity reflected by a high WC. This latter parameter has been proposed as

**Table 4.** Odds ratios (95% confidence intervals) for the association between the Trp64Arg variant and PCOS across BMI categories

	Stratification by BMI cut-off points							
	Crude analysis		BMI < 25 kg/m <sup>2</sup>		BMI 25–29.9 kg/m <sup>2</sup>		BMI ≥ 30 kg/m <sup>2</sup>	
	PCOS	HW	PCOS	HW	PCOS	HW	PCOS	HW
Arg64 carrier	43	29	11	9	8	14	24	6
Noncarrier	63	53	20	26	21	18	22	9
	OR = 1.40 (1.3–1.5)		OR = 1.60 (0.5–4.6)		OR = 0.50 (0.2–1.4)		OR = 1.64 (0.50–5.35)	

$P$ -value for association in crude analysis = 0.47 by the Breslow–Day test.

$P$ -value for BMI × Arg64 interaction = 0.29 by the Breslow–Day test.

an indicator of abdominal adiposity and has been associated with several metabolic complications, such as insulin resistance, hyperinsulinaemia and alterations in the plasma lipoprotein/lipid profile including increased plasma triglycerides.<sup>41,42</sup>

A previous study reported sexual differences in visceral fat lipolysis, which was 12 times higher in men than in women.<sup>14</sup> A particular distribution of ADRB3 has been proposed in men according to the higher intra-abdominal fat mass.<sup>15</sup> ADRB3 is expressed in visceral fat and several studies with selective  $\beta_3$  agonist have shown a decreased lipolytic rate in human omental fat derived from samples homozygous for the Arg64 mutation.<sup>26,43</sup>

In the present study, a tendency for association between Arg dosage and waist circumference was observed. Even though the number of homozygous patients was low, from a clinical point of view it is interesting to note that, in PCOS patients, WC increases with the Arg dosage. Therefore, PCOS women with abdominal obesity might express ADRB3 in visceral fat similarly to men, showing high levels of TG and probably a decreased lipolytic rate.

Another study on the relation of this polymorphism to a hyperandrogenic condition was carried out in children with premature pubarche. In the study, a major effect of this polymorphism on BMI, using Trp64Arg as a candidate gene, was not demonstrated.<sup>44</sup> In this context, in our adult population, a possible lipolysis imbalance could explain, in part, our results regarding the high TG levels observed in the PCOS group, in accordance with the android obesity and hyperandrogenism characteristic of adult PCOS.

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