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ORIGINAL ARTICLE

The 312N variant of the luteinizing hormone/choriogonadotropin receptor gene (*LHCGR*) confers up to 2·7-fold increased risk of polycystic ovary syndrome in a Sardinian population

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Summary

Objective Polycystic ovary syndrome (PCOS) is a frequent condition, affecting about 15% of women of reproductive age. Because of its familial occurrence, a multifactorial model of susceptibility, including both genetic and environmental factors, has been proposed. However, the identification of genetic factors has been elusive.

Design Case–control study aimed at evaluating possible associations between functionally relevant variants of the luteinizing hormone/choriogonadotrophin receptor gene (*LHCGR*) and PCOS phenotype.

Patients A total of 198 PCOS and 187 non-PCOS women, aged 14–35 years, of Sardinian origin, were referred to the outpatient clinic of the Department of Obstetrics and Gynaecology of the University of Cagliari (Sardinia). PCOS diagnosis was based on the Rotterdam criteria.

Measurements We determined the genotype of ins18LQ, S291N and S312N variants at the *LHCGR* locus. Genotype was related to the presence or absence of PCOS and to several clinical and biochemical characteristics.

Results The presence of at least one 312N allele was strongly associated with PCOS risk (OR, 2·04; 95% CI, 1·32–3·14; χ^2, 10·47; $P = 0·001$). 312N homozygosity was associated with a further risk increase (OR, 2·73; 95% CI, 1·25–5·95; χ^2, 6·65; $P = 0·01$). The number of ins18LQ alleles was associated with LH serum levels in controls (χ^2, 8·04, $P = 0·017$).

Conclusions For the first time, we have identified a genetic variant that is strongly associated with PCOS in an isolated population. These results, if confirmed in other cohorts, may provide the opportunity to test the S312N genotype at the *LHCGR* locus in fertile women to assess the risk of PCOS. The avoidance of triggering factors like weight increase may improve the reproductive outcome of potentially at-risk subjects.

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Introduction

Polycystic ovary syndrome (PCOS) is a very common endocrine disorder, affecting about 15% of women of reproductive age. The clinical picture is highly variable, resulting in several revisions of the diagnostic criteria. The most recent consensus on clinical criteria for the diagnosis of PCOS was reached in 2003 in the context of the Rotterdam workshop, sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine.[1,2] Based on these criteria, the diagnosis of PCOS is made when two of the following signs are present: oligo-amenorrhoea/anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovary (PCO) at ultrasound examination. However, PCOS is a diagnosis of exclusion, because other conditions resulting in hyperandrogenism should be ruled out, including non-classical adrenogenital syndrome, Cushing's syndrome and disease, and ovarian and suprarenal neoplasms.

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The aetiology of the condition is largely unknown; the existence of genetic factors responsible for or predisposing to the syndrome has been postulated on the basis of familial aggregation of PCOS and/or related conditions, such as hyperandrogenism or hyperinsulinism.[3–5] Some authors have proposed an autosomal dominant pattern of inheritance with incomplete penetrance and variable expressivity.[6,7] At present, the most commonly accepted model is that of a multifactorial aetiology with a strong genetic component but also with a relevant role of environmental factors.[8,9] Among the latter, the roles of diet, physical exercise and lifestyle have been implicated. Obesity may induce the switch from a subclinical form to the full clinical picture of PCOS.[10]

The identification of genetic factors predisposing to PCOS has been frustrated so far by several complicating elements, including clinical variability, the absence of universally accepted diagnostic criteria and of a confirmatory laboratory test, and the reduced fertility of affected subjects. On the other hand, the recent identification of metabolic abnormalities, similar to those found in PCOS, in male first-degree relatives of affected women,[11,12] might improve the yield of genetic association studies in the next future. The high prevalence of the condition allows us to hypothesize a wide genetic heterogeneity with several loci involved, rather than a major locus with a prevalent role in the predisposition to PCOS. Several case–control and linkage studies have been performed so far, aimed at identifying genetic susceptibility factors.[13,14] The majority of genes investigated as potential candidates for PCOS susceptibility belong to specific pathways mediating sex hormone response, peripheral sensitivity to insulin, inflammatory response, cardiovascular risk and steroid biogenesis.[15–17] However, these studies have not led to the identification of gene variants determining high risk of developing PCOS, and/or most studies were not confirmed in replicative populations. Very recently, a genome-wide association study has been performed in a Han Chinese population:[18] the authors identified three different single-nucleotide polymorphisms associated with PCOS, two with protective effect (rs13405728 and rs13429458 in 2p16·3 and 2p21, respectively) and one with predisposing effect (rs2479106 in 9q33·3). The functional effect of these variants has not been defined.

In this study, we have evaluated the potential role of three functionally relevant variants of the luteinizing hormone/choriogonadotrophin receptor (*LHCGR*) gene, located in 2p21: rs4539842 (c.54insCTCCAG, ins18LQ) in exon 1, rs12470652 (c. A872G, N291S) and rs2293275 (c.G935A, S312N) in exon 10. The rationale behind the choice of *LHGCR* as a candidate gene is based on several factors: it is expressed in thecal cells of the ovary and in Leydig cells of the testicle (as well as in adipose tissue[19]), and it encodes the receptor mediating the action of both luteinizing hormone (LH) and choriogonadotrophin (hCG) on steroid biosynthesis.[20,21] Mitogen-activated protein kinases (MAPK), mediating the signal transduction of LH, are downregulated in thecal cells of PCOS individuals,[22,23] and mutations in *LHCGR* have been reported in different disorders of sexual differentiation and maturation, both in human and in mouse.[24–35] *LHCGR* encodes a transmembrane integral protein, belonging to the G-protein-coupled receptor superfamily (GPCR), and is composed of 11 exons spanning about 70 kbp. Over 300 SNPs have been identified in the *LHCGR* gene (http://SNPper.chip.org) with an average spacing of 306 bp, the majority being located in intronic sequences. We have selected, among the most frequent SNPs located in the coding region, those which may modulate the receptor activity of the *LHCGR* gene product. In particular, ins18LQ, caused by the insertion of an hexanucleotide in position 54 of the mRNA, has a minor allelic frequency (MAF) of about 30% in Caucasian population, is located in the signal peptide of the receptor and has been associated with an increase in both expression and bioactivity of the LH receptor;[36] N291S (MAF: 3%) and S312N (MAF: 42%), located in exon 10, are involved in the transduction of LH but not of hCG signal and are next to the glycosylation signals of the protein and may thus affect translocation and stability of the receptor.[37] However, to our knowledge, no data are available on the functional effect of these variants on receptor activity.

In this study, we have analysed a selected population of cases and controls of Sardinian origin. We have chosen this geographic area of Italy, not because of a specific ethnic origin, but rather for the relative isolation of the population.[38] However, if on the one hand the choice of genetic isolates may increase the potential yield of genetic association studies by reducing genetic background noise, on the other hand the results need to be confirmed in replicative populations.

Materials and methods

Subjects

For all subjects, written informed consent for the participation to the study was obtained. This study was approved by the ethics committees of the University of Cagliari, and the Università Cattolica del Sacro Cuore. Three hundred and eighty-five unrelated women of Sardinian origin were recruited in a prospective study between 1 May 2006 and 30 October 2009 at the outpatient clinic of the Department of Obstetrics and Gynaecology of the University of Cagliari. Subjects were divided into two groups: 198 patients with PCOS (age range, 14–35 years; mean, 18·4) and 187 non-PCOS, included as controls (age range, 15–36; mean, 19·1). Twenty-one of 198 patients were $\leq$16 years of age. In this group, because menstrual disorders and/or multicystic ovaries are frequent findings during the first years of fertile life, subjects without clinical or biochemical signs of hyperandrogenism were not included. Among patients, the diagnosis of PCOS was made according to Rotterdam criteria.[1,2]

Biochemical hyperandrogenism was established by total testosterone (totT) levels $\geq$1·74 nM. In the absence of hirsutism, isolated acne was not considered a clinical sign of hyperandrogenism, because of the high frequency of this condition. The presence of signs of PCO was evaluated in all subjects by ultrasound examination (transabdominal or, when possible, transvaginal).[39] Control subjects were women with regular menstrual cycles, absence of clinical and biochemical signs of hyperandrogenism and no PCO signs at ultrasound examination, enrolled on a voluntary

basis during regular gynaecological check-up visits, covered by the regional health system. Exclusion criteria from the study were as follows: use of hormone preparations, including oral contraceptives, for at least 3 months preceding recruitment, hyperprolactinaemia, Cushing's disease, androgen-secreting neoplasms and thyroid disorders.

For healthy controls, blood sampling was performed morning of the third day of the menstrual cycle, while for patients with PCOS, blood was taken on the 5–7th day after progesterone-induced menstrual flow. DNA extraction and the following biochemical analyses were performed in all subjects: FSH, LH, LH/FSH ratio, estradiol (E2), totT, androstenedione (A), progesterone (P), dehydroepiandrosterone sulphate (DHEAS), 17-hydroxyprogesterone (17-OHP) and prolactin. Hormonal levels were evaluated by commercially available radioimmunoassays (RIA; Radim, Pomezia, and Ares Serono, Milan, Italy). Serum samples were immediately processed in a refrigerated centrifuge and stored at −20 °C until assay. The intra-assay and inter-assay coefficients of variation obtained were $\leq 9\%$ for all variables.

Genotype analysis

For genomic DNA (gDNA) extraction, 5–10 ml of whole blood was collected in EDTA tubes. Red blood cells were lysed twice in hypotonic solution (NH_4Cl 150 mM, $KHCO_3$ 10 mM, EDTA 0·1 mM). The white blood cell pellet was digested overnight in hypotonic solution (NaCl 75 mM, EDTA 25 mM) containing 200 mg/l of proteinase K and 1% SDS. Subsequently, DNA was extracted with one volume of chloroform, precipitated with isopropanol/NaCl 1·5 M, washed in ethanol 70% and resuspended in TE pH8 (Tris–HCl 10 mM, EDTA 1 mM) at a concentration of 100 mg/l. The concentration and purity of DNA samples were assessed by spectrophotometer absorbance at 260 and 280 nm (GeneQuant II Pro; Pharmacia, Uppsala, Sweden).

Genotype analysis at the three SNPs was performed by restriction fragment length polymorphisms–polymerase chain reaction analysis (RFLP–PCR). PCRs were performed in a final volume of 25 μl containing 100–200 ng of gDNA, 200 μM of each dNTP, 1 μM of each primer, 1 × reaction buffer, 1 mM $MgCl_2$ and 1 U of Taq polymerase (Fisher scientific, Illkirch, France).

For ins18LQ, a specific fragment of exon 1 (215 bp) of *LHCGR* gene was amplified by using the following oligonucleotides: *LHCGR-ex1 F:* 5′– GACACTGGCAAGCCGCAGAAGCCC – 3′; *LHCGR-ex1 R:* 5′ – GCTGTGTACTCACAGTCGAGTGAG – 3′. PCR products were digested with 0·2 U of *Pvu*II in a final volume of 20 μl. The presence of ins18LQ variant created an additional *Pvu*II restriction site, resulting in three fragments of 27, 65 and 128 bp. The digestion of wild-type alleles resulted in two fragments, of 65 and 149 bp.

For N291S and S312N SNPs, a fragment of 111 bp of exon 10 of *LHCGR* gene was amplified with the following primers: *LHR ex10-F:* 5′ – CCTCTTCTCTTTCAGACAGA – 3′; *LHR-ex10 R:* 5′ – CATGCAAATACTTACAGTGTTTTGGTA – 3′. When digested with *Apo*I restriction endonuclease, 291N allele remained uncut while 291S allele generated two fragments of 19 and 92 bp. For S312N variant analysis, PCR products were digested with 0·2 U of *Rsa*I, which generated, in the presence of the 312N allele, two fragments of 26 and 85 bp. In all cases, fragment size analysis was performed by 3·5% agarose gel/TAE 1 × electrophoresis.

Statistical analysis

The primary end-point was the comparison of allelic frequencies of S312N variant between PCOS and non-PCOS subjects. The statistical power of the study was established by Sample power version 2·0 (SPSS, Inc., Chicago, IL, USA). Based on the expected frequencies of two of the variants analysed (ins18LQ and S312N, about 30%, http://www.ncbi.nlm.nih.gov/snp), a χ^2-test of association at an alpha level = 0·05 would guarantee a power of at least 80%, with a sample size of about 180 patients, if assuming a delta of 15% in the frequency of allelic variants in PCOS *vs* control subjects.

We also analysed the distribution of allelic frequencies of N291S, Ins18LQ and haplotype for S312N and Ins18LQ; genotype was related to phenotypic characteristics.

χ^2 Pearson's tests were used to establish whether the allelic and genotypic frequencies of each SNP respected Hardy–Weinberg equilibrium. The odds ratio and relative risk associated with the genotype were calculated by contingency tables (confidence interval, CI, 95%). Continuous outcomes like hormonal levels were compared by means of Student's *t*-test in case of two groups and one-way ANOVA in case of more than two groups, upon verification of normality by Kolmogorov–Smirnov test. In case of non-normality, medians were compared by Mann–Whitney or Kruskal–Wallis tests. For allelic and genotypic frequencies, the cut-off of significance was fixed at $P < 0{\cdot}017$, after Bonferroni correction, while in all other cases, a *P*-value smaller than 0·05 was deemed as statistically significant. All statistical analyses were performed using SPSS v18·0 software (SPSS Inc.).

Results

Hormonal and clinical characteristics of controls and patients

Characteristics of the two populations are summarized in Table 1. As expected, we did not observe any difference between patients and controls for BMI, age, E2, prolactin and *P* levels ($P \geq 0{\cdot}11$). LH, totT, A, DHEAS and 17-OHP levels were significantly higher in PCOS ($P = 0{\cdot}001$), indicating the correct sampling of the two populations. Among patients, 92/198 (46·5%) fulfilled the three Rotterdam diagnostic criteria; 71/198 (35·8%) had amenorrhoea and PCO, while 30 (15·1%) had hyperandrogenism and PCO. The remaining five patients (2·5%) had hyperandrogenism and amenorrhoea, in the absence of ultrasound signs of PCO. Biochemical hyperandrogenism was found in 64% of cases. For all biochemical characteristics, except those related to the condition, the PCOS and non-PCOS groups were homogenously distributed.

Table 1. Clinical and hormonal characteristics of control and polycystic ovary syndrome (PCOS) women

	Controls (n = 187)			PCOS (n = 198)		
	Mean	SD	95%CI	Mean	SD	95%CI
Age (years)	19·10	3·99	18·3–19·8	18·40	3·62	17·8 – 19·0
BMI (kg/m^2)	23·35	5·02	22·37–24·32	23·11	4·93	22·18–24·03
FSH (IU/l)	5·63	1·42	5·33–5·92	5·53	1·82	5·20–5·85
LH (IU/l)*	4·13	2·41	3·63–4·63	5·76	2·99	5·18–6·33
LH/FSH*	0·73	0·39	0·65–0·81	1·07	0·60	0·95–1·18
Estradiol (pM)	100·26	63·77	87·22–113·21	103·96	62·59	92·73–115·16
Prolactin (pM)	923·04	386·08	845·65–999·99	1027·39	532·61	930·86–1123·91
Androstenedione (nM)*	6·67	2·69	6·11–7·19	9·56	3·56	8·90–10·19
Total testosterone (nM)*	1·56	1·04	1·32–1·84	3·16	1·53	2·88–3·43
DHEAS (nM)*	5·79	3·57	5·07–6·52	7·95	4·82	7·01–8·92
17-OH-Progesterone (nM)*	2·89	0·89	2·70–3·08	4·26	2·26	3·82–4·67
Progesterone (nM)	2·61	0·99	2·39–2·80	2·35	1·21	2·07–2·61

AD, androstenedione; DHEAS, dehydroepiandrosterone sulphate; P, progesterone; Prl, prolactin; Ttot, total testosterone.
*Statistically significant difference between PCOS and controls ($P \leq 0{\cdot}01$).

Ins18LQ variant is not associated with PCOS but is related to LH levels in controls

The ins18LQ genotype was assessed in 156 patients and 174 controls. No differences were observed in allelic and genotypic frequencies in the two cohorts (Table 2; OR, 1·16; CI, 0·83–1·64; $P = 0{\cdot}38$). Subsequently, we evaluated whether the ins18LQ genotype may be involved in the modulation of serum levels of hormones analysed. While in PCOS individuals no correlations were found, in controls we observed that median LH levels were 4·39, 3·36 and 1·89 IU/l in non-ins18LQ homozygous, ins18LQ heterozygous and ins18LQ homozygous individuals, respectively (Table 3, Kruskal–Wallis test, $P = 0{\cdot}017$).

N291S variant is not associated with PCOS phenotype

This SNP was analysed in 153 patients and 180 controls. As in the case of ins18LQ, both populations were in Hardy–Weinberg equilibrium. No differences were observed in the distribution of genotypic and allelic frequencies in the two cohorts (Table 2; OR, 1·16; CI, 0·83–1·64; $P = 0{\cdot}27$). Because of the rarity of this SNP, it was not possible to analyse possible correlation between genotype and hormonal levels.

312N allele is associated with higher PCOS risk

The S312N variant was analysed in 159 patients and 180 controls. Both populations were in Hardy–Weinberg equilibrium. Genotypic and allelic frequencies were significantly different in the two groups (Table 2). In particular, the 312N allele frequency was 0·46 and 0·35 in PCOS and controls, respectively. In the PCOS group, 60·4% were carriers of at least one 312N allele, compared to 42·8% of controls, conferring an increase in PCOS risk of about 2-fold (OR, 2·04; CI, 1·32–3·14; $P = 0{\cdot}001$). The frequency of individuals homozygous for 312N allele was significantly higher in the group of cases compared to controls

Table 2. Frequency of alleles and genotypes in polycystic ovary syndrome (PCOS) and controls

		PCOS		Controls			
Variant	Genotype	n	%	n	%	OR (95%CI)	P
ins18LQ	ins/ins	9	5·8	8	4·6		
	non-ins/ins	74	47·4	76	43·7		
	non-ins/non-ins	73	46·8	90	51·7		
	MAF (ins)	0·29		0·26		1·16 (0·83–1·64)	0·38
N291S	N/N	174	98·3	144	94·1		
	N/S	6	1·7	9	6·9		
	S/S	0	0	0	0		
	MAF (S)	0·03		0·02		1·78 (0·63–5·08)	0·27
S312N	S/S	63	39·6	103	57·2		
	S/N	76	47·8	65	36·1		
	N/N	20	12·6	12	6·7		
	MAF (N)	0·46		0·35		1·75 (1·26–2·43)	0·0009
		N/N and S/N *vs* S/S				2·04 (1·32–3·14)	0·001
		N/N *vs* S/S				2·73 (1·25–5·95)	0·01

Table 3. Serum LH levels (IU/l) in controls relative to ins18LQ genotype

	non-ins/non-ins	ins/non-ins	ins/ins	P*
n	71	72	8	
Median	4·39	3·36	1·89	0·005
Interquartile range	3·5–5·31	2·32–3·94	1·2–3·85	

*Kruskal–Wallis test.

(Table 2, 62·5% *vs* 37·5%; $P \leq 0{\cdot}01$). 312N homozygous individuals had a 2·73-fold increased risk of PCOS compared to controls (CI, 1·25–5·95; $P = 0{\cdot}01$.). To rule out the possible confounding effect of younger age in the diagnosis of PCOS, we have analysed data excluding patients and controls of age $\leq$ 16 years (21/198 and 6/187, respectively). The correlation with the 312N variant remains unaltered (OR, 2·01; CI, 1·29–3·15; $P = 0{\cdot}002$). Also, when evaluating hyperandrogenic ($n = 103$) and non-hyperandrogenic ($n = 56$) women separately, the percentage of subjects bearing at least one 312N allele remained substantially unchanged (63·1% and 55·4%, respectively), as well as the risk of PCOS in hyperandrogenic women (OR, 2·29; CI, 1·39–3·76; $P = 0{\cdot}001$).

No correlations were found between the genotype at S312N variant and hormonal or clinical variables, either in patients or in controls.

Haplotype analysis of polymorphic variants

To evaluate a possible combinatorial effect of the variants analysed, we determined the haplotypic frequency of the ins18LQ and S312N variants in cases and controls. N291S SNP was not included in the analysis because of the low frequency of the 291N allele. Data are summarized in Table 4. The distribution of haplotypes was very similar in the two groups. However, the statistical power of this analysis is low because of the small number of samples analysed.

Discussion

In the present study, we have evaluated the role of three polymorphic variants of the *LHCGR* gene as risk factors for PCOS, in a case–control study of Sardinian women. In our cohort of PCOS subjects, the prevalence of hyperandrogenism was lower than that observed in other series of patients evaluated;[40,41] this finding might be explained by the younger mean age of our patients, compared to the previously published cohorts (19·1 *vs* 25·5 years, respectively). Of the three polymorphisms investigated, ins18LQ did not associate with the phenotype, because the two cohorts displayed a similar genotypic distribution. Interestingly, the ins18LQ variant was correlated with serum LH levels in controls but not in patients. This finding may be related to the functional effect of the variant, which is located in the signal peptide of the LH receptor and is associated with higher receptor activity.[35] It can be speculated that the increase in receptor activity, linearly and inversely related to the number of ins18LQ alleles, determines the up-regulation of the hypophyseal negative feedback system, controlling LH secretion. On the other hand, because in PCOS individuals this feedback is altered, the control of LH receptor on hormonal levels is lost, and consequently also the correlation between genotype and LH levels. The lack of correlation between genotype of single variants at the *LHCGR* locus and different hormonal variables is not unexpected. Indeed, if on one side it is conceivable that functionally relevant variants at the *LHGCR* locus might play a pivotal role in androgen synthesis, on the other hand the regulation of hormonal production is likely to be modulated at different levels and thus should be evaluated in a multigenic/multifactorial context.

N291S appeared not to be related to the PCOS phenotype, because its genotypic distribution was similar in the two cohorts. However, this variant is rather rare, and thus, the number of patients analysed is not sufficient to generate meaningful results.

The most relevant finding of our study is the strong correlation between PCOS phenotype and the S312N variant. Differently from previous studies of genetic susceptibility factors, the presence of the 312N allele correlates with an increase in relative risk of PCOS in carriers of at least one copy of the variant. The significance of these data is strengthened by the finding that in homozygous individuals, the risk of PCOS increases further, up to 2·7-fold compared to homozygous wild-type individuals. The observed correlation is not influenced by the presence or absence of hyperandrogenism, because no differences were observed when analysing the two endophenotypes separately.

Table 4. Contingency table of haplotypes at S312N and ins18LQ variants

	Haplotype									
	homo non-ins/ homo 312S	het non-ins/ homo 312S	homo non-ins/ het 312S	het ins/ het 312S	homo non-ins/ homo 312N	homo ins/ homo 312S	het non-ins/ homo 312N	homo ins/ het 312S	homo ins /homo312N	Total
Controls										
n	27	33	22	29	6	4	4	2	0	127
%	21·3	26·0	17·3	22·8	4·7	3·1	3·1	1·6	0	100
PCOS										
n	27	32	30	40	6	6	10	2	2	155
%	17·4	20·6	19·4	25·8	3·9	3·9	6·5	1·3	1·3	100
Total										
n	54	65	52	69	12	10	14	4	2	282
%	19·1	23·0	18·4	24·5	4·3	3·5	5·0	1·4	0·7	100

Pearson's $\chi^2 = 5{\cdot}24$, $P = 0{\cdot}73$. Table legend: homo or het non–ins, homozygous or heterozygous wild type; homo or het 312S, homozygous or heterozygous wild type; homo ins, ins18LQ homozygous; homo 312N, 312N homozygous.

The S312N variant has already been investigated in a previous study by Valkenburg *et al.*[42] These authors did not find an increase in PCOS risk related to S312N genotype. The discrepancy between the results of the two studies is partially unexplained, because the same diagnostic criteria for PCOS were used. One hypothesis could be related to the different geographic origin of patients included or, more likely, to the fact that the control cohort in the study of Valkenburg was a sample from the general population, selected independently from the PCOS phenotype. Based on the prevalence of the condition in the general population, it can be expected that about 15% of women included as controls were affected by the condition, and this may have masked the association between the 312N variant and PCOS phenotype. This hypothesis may be supported by the finding that in our control cohort, the frequency of 312N allele is slightly lower than that reported by Valkenburg *et al.* (35% *vs* 42%, respectively).

Chen *et al.*[18] have very recently reported a genome-wide association study performed by SNP array. These authors found 21 SNPs in the 2p21 region strongly associated with PCOS phenotype. The strongest association was found with 3 SNPs in the thyroid adenoma–associated (*THADA*) gene. The putative functional relevance of these variants (two of which are located in intronic regions) remains to be investigated. Intriguingly, *THADA* is located at a distance of only 5 Mb from *LHCGR* gene, and it can be hypothesized either that S312N variant in *LHCGR* is in linkage disequilibrium with variants in other gene/genes in the same locus not yet identified, or that the prevalent PCOS-predisposing effect is played by *LHCGR* variants. The latter hypothesis is, in our opinion, more likely due to the fact that *LHCGR* is a strong functional candidate for PCOS susceptibility and that 312N allele confers the highest relative risk defined so far.

Our results need to be confirmed in an independent study, performed in a replicative population. In the context of translational medicine, if our data are confirmed, the increased relative risk of PCOS, associated with the presence of 312N allele, suggests the opportunity of testing the genotype at *LHCGR* locus in patients affected by PCOS and their relatives. This information may be useful for PCOS families carrying the 312N variant because, in case of homozygosity for this allele, the probability of being affected by a condition in the PCOS clinical spectrum rises to about 40%. Subsequently, in at-risk subjects, it may even more advisable than in the general population to avoid some detrimental behaviours, like excessive weight gain, to reduce the risk of worsening the clinical and reproductive outcome associated with the PCOS phenotype.

Competing interests/financial disclosure

Nothing to declare.

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