Common variants in the sex hormone-binding globulin gene (SHBG) and polycystic ovary syndrome (PCOS) in Mediterranean women

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STUDY QUESTION: Is there an association between polycystic ovary syndrome (PCOS) and the sex hormone-binding globulin (SHBG) rs1799941, rs6257, rs6259 and rs727428 variants in a large series of Mediterranean women?

SUMMARY ANSWER: The rs727428 and rs6259 variants are associated with PCOS in Mediterranean women.

WHAT IS KNOWN ALREADY: The level of SHBG, the primary plasma transport protein for sex steroids, which regulates the bioavailability of these hormones to target tissues, is reduced in patients with PCOS. Single-nucleotide polymorphisms in the SHBG gene influence circulating SHBG levels in American patients with PCOS and may predict the development of type 2 diabetes.

STUDY DESIGN, SIZE AND DURATION: This was a genetic case–control association study including 1004 premenopausal Mediterranean women.

PARTICIPANTS/MATERIALS, SETTING AND METHODS: In an Academic setting, we genotyped a clinical cohort consisting of 281 patients with PCOS and 142 women without any evidence of androgen excess, and a population-based cohort comprised of 581 unselected female blood donors from Spain and Italy. The latter included 31 patients with PCOS and 550 controls, of whom 298 had no evidence of any androgen excess disorder and were considered hyper-normal controls.

MAIN RESULTS AND THE ROLE OF CHANCE: Mutant alleles of the rs727428 variant were more frequent in patients with PCOS compared with controls and with hyper-normal controls. This association was independent of obesity. Carrying mutant alleles of rs727428 was found to be associated with a 1.29 odds ratio (OR) for PCOS, whereas carrying mutant alleles of rs6259 associated with a 0.68 OR for PCOS. The rs1799941 and rs6257 variants were not associated with PCOS. None of the SHBG variants influenced serum SHBG concentrations.

LIMITATIONS AND REASONS FOR CAUTION: The associations found here were relatively weak and, arising from a case–control study, do not necessarily indicate a causative role of the SHBG variants in the development of PCOS. Also, we studied different patients and controls from different sources, making some of the interpretations difficult. Finally, the rs1799941 variant was not in Hardy–Weinberg equilibrium in the small group of patients with PCOS recruited from the general population, yet this variant was not associated with PCOS.

WIDER IMPLICATIONS OF THE FINDINGS: SHBG variants that influenced circulating SHBG levels in American patients with PCOS are also associated with this syndrome in Mediterranean women, pointing to SHBG as a candidate gene for PCOS.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by grants PI080944 and PI110357 from Instituto de Investigación Carlos III, Spanish Ministry of Economy and Competitiveness. CIBERDEM is also an initiative of Instituto de Investigación Carlos III. The Authors have no competing interests to declare.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among premenopausal Mediterranean women (Diamanti-Kandarakis et al., 1999; Asuncion et al., 2000; Sanchon et al., 2012). Considered nowadays a predominantly androgen excess disorder, PCOS is characterized by clinical or biochemical hyperandrogenism in association with ovulatory dysfunction and/or polycystic ovarian morphology (Azziz et al., 2006, 2009).

Sex hormone-binding globulin (SHBG) plays a central role in the pathophysiology of PCOS (Botwood et al., 1995). SHBG binds testosterone with high affinity, thus regulating free testosterone levels (Rosner et al., 1991). The free fraction of testosterone enters target cells and, after being transformed into dihydrotestosterone, binds the nuclear receptor exerting androgenic effects.

Circulating SHBG concentrations are characteristically low in patients with PCOS because these women have increased androgen levels and often present with insulin resistance and compensatory hyperinsulinemia and both androgens and insulin inhibit the hepatic synthesis and secretion of SHBG (Pugeat et al., 1996). In conceptual agreement with the importance of SHBG for the development of PCOS, a single non-fasting measurement of SHBG shows an excellent accuracy for the diagnosis of PCOS: a SHBG level below 36 nmol/l identifies patients with PCOS with a 0.88 sensitivity and a 0.87 specificity in epidemiological studies, even superior to those of total or free testosterone in such settings (Escobar-Morreale et al., 2001).

Aside from the inhibitory influences of androgens and insulin and the stimulatory effects of estrogens and thyroid hormones (Pugeat et al., 1996), circulating SHBG levels are also influenced by genetic variation in the SHBG gene (Ring et al., 2005; Xita and Tsatsoulis, 2010).

Recently, four single-nucleotide polymorphisms (SNPs; rs1799941, rs6257, rs6259 and rs727428) have been identified as modifiers of serum SHBG concentrations and as predictors of the development of type 2 diabetes in men and women (Ding et al., 2009; Perry et al., 2010). Furthermore, a family-based study conducted in the USA showed that, although these SHBG polymorphisms were not directly associated with PCOS, SNPs rs1799941 and rs727428 influenced serum SHBG concentrations after controlling for BMI and indexes of androgen excess and insulin resistance (Wickham et al., 2011).

Considering the emerging scenario of PCOS as a complex endocrine and metabolic disorder resulting from the interaction of multiple protective and predisposing variants with strong environmental influences that are heavily dependent on diet and life-style (Escobar-Morreale et al., 2005), we decided to explore the association between these SHBG variants and PCOS and their possible influence on circulating SHBG levels in a large sample of premenopausal women from the Mediterranean area.

Materials and Methods

Subjects

The study included two different cohorts of premenopausal women. The first cohort consisted of unselected premenopausal women from the general population who were recruited when reporting for blood donation to the facilities of the participating Hospitals in Spain and Italy. These women participated in a study addressing the prevalence of functional disorders of androgen excess, including PCOS (Sanchon et al., 2012). The present study describes the SHBG variants of the 581 women from whom we were able to extract genomic DNA.

The second cohort consisted of 281 Spanish patients with PCOS attending the clinical practice of one of the authors (H.F.E.-M.) and 142 premenopausal women selected from healthy lean volunteers and overweight and obese patients referred for dietary advice, who presented with regular menstrual ovulatory cycles and no evidence—clinical or biochemical—of androgen excess. The controls were selected as to match patients with PCOS for BMI.

All women gave written informed consent and we obtained approval from the Ethics Committees of Hospital Universitario Ramón y Cajal and Policlinico S. Orsola-Malpighi.

Phenotyping and criteria for health and disease

Because ultrasound examination of ovarian morphology was not possible in all the women, the diagnosis of the women relied on the criteria derived from the 1990 National Institute of Child Health and Human Development conference (Zawadzki and Dunaf, 1992).

For the population and clinical cohorts, PCOS was defined by the presence of menstrual dysfunction, clinical hyperandrogenism and/or hyperandrogenemia and exclusion of specific etiologies (Zawadzki and Dunaf, 1992). Menstrual dysfunction was considered when the women had oligomenorrhea, defined by more than six cycles with a length of >35 days per year, and/or when the patient had no menstrual bleeding for 3 consecutive months (Goodman, 2007). Clinical hyperandrogenism was defined by the presence of hirsutism, represented by a modified Ferriman–Gallwey score of 8 or more, persistence of acne during the third decade of life or later or the presence of androgenic alopecia (Zawadzki and Dunaf, 1992). Specific etiologies were excluded in all the women who presented with both criteria for the diagnosis of PCOS. Hyperprolactinemia and thyroid dysfunction were excluded by the finding of serum prolactin and thyroid-stimulating hormone levels within the normal range. Basal or cosyntrophin-stimulated 17-hydroxyprogesterone levels served to rule out non-classic 21-hydroxylase deficiency (Azziz et al., 1999). Clinical assessment served to rule out androgen secreting tumors, Cushing’s syndrome and anabolic drug use or abuse. We considered women presenting without menstrual and ovulatory dysfunction and who had no evidence of androgen excess, either clinical or biochemical, as hyper-normal controls.

Within the population cohort, there were women who did not fulfill all the criteria for the diagnosis of PCOS, but could not be considered as hyper-normal controls either: women presenting with idiopathic hyperandrogenism (increased androgen levels and signs of androgen excess in women presenting with regular menstrual cycles of normal length), idiopathic hirsutism (normal androgen levels and regular menstrual cycles of normal length), isolated acne or oligomenorrhea, and women under hormonal contraception. These women were considered together with hyper-normal controls in a population-based group of non-PCOS controls for certain sub-analyses (Hattersley and McCarthy, 2005).

The precise methods used to evaluate clinical and biochemical hyperandrogenism and menstrual and ovulatory dysfunction and to exclude secondary etiologies have been described in detail elsewhere (Escobar-Morreale et al., 2008; Sanchon et al., 2012). Serum total testosterone...
concentrations were measured by direct radioimmunoassay (Spectria Testosterone RIA, Orion Diagnostica Oy, Espoo, Finland) in the clinical cohort and by automated immunochemiluminescence (Immulite 2000, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) in the general population cohort, whereas SHBG concentrations were analyzed by automated immunochemiluminescence (Immulite 2000) in both cohorts. The lower limits of detection and intra- and inter-assay coefficients of variation were 0.1 nmol/l, 5.3 and 5.4% for the Spectria testosterone radioimmunoassay, 0.5 nmol/l, 9.8 and 12.0% for the Immulite 2000 testosterone assay and 0.02 nmol/l, 3.0 and 5.0% for the Immulite 2000 SHBG assay, respectively. Serum-free testosterone concentrations were calculated from total testosterone and SHBG concentrations (Vermeulen et al., 1999).

Although ovarian morphology was not analyzed, by having hyperandrogenism and oligo-ovulation, all the patients fulfilled all the current definitions of PCOS (Zawadzki and Dunaif, 1992; The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004; Aziz et al., 2006), and PCOS was ruled out reliably in the hyper-normal controls because all these women did not have clinical or biochemical hyperandrogenism and showed regular menstrual cycles.

Genotyping
Genomic DNA was obtained from whole blood samples using QIAamp DNA Blood Kit silica-membrane-based spin columns (Qiagen Iberia, S.L., Las Matas, Madrid, Spain) or an automated DNA isolation process based on magnetic beads with a Chemagic DNA Blood Kit Special in conjunction with a Magnetic Separation Module I (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany).

Blood donors were genotyped for four polymorphisms in the SHBG gene as previously described (Wickham et al., 2011). SNPs rs1799941, rs6257, rs6259 and rs727428 were determined using a Real-Time PCR system with pre-developed TaqMan SNP Genotyping Assays (C_8727483_10, C_11955742_10, C_11955739_10 and C_3290005_10, respectively) following a standard protocol of TaqMan allelic discrimination (Applied Biosystems, Foster City, CA, USA). Each allele-specific probe is labeled with VIC or FAM fluorescent dyes. PCR was carried out in a total reaction volume of 10 μl with the following amplification protocol: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. After PCR, the genotype of each sample was attributed automatically by measuring the allele-specific fluorescence in a StepOne Plus Real-Time PCR instrument using the StepOne Software v2.1 (Applied Biosystems).

Statistical analysis
Power analysis used the online facilities provided by R.V. Lenth, Department of Statistics, University of Iowa (http://www.stat.uiowa.edu/~rlenth/Power, last accessed 27 January 2012). Considering the minor allele frequencies already published (Wickham et al., 2011), our sample size would permit the detection of 7–10% differences in the allele frequencies of the SHBG SNPs studied here, with a power of 0.05 and ß = 0.20.

Statistically significant deviations of observed allele frequencies from those expected by Hardy–Weinberg equilibrium were evaluated using the Pearson χ² test separately in the clinical and general population cohorts. Pairwise linkage disequilibrium (D’ and r² statistics) was estimated using Haploview v4.2. The association between PCOS and SHBG genotypes was analyzed using Pearson’s χ² tests.

Continuous variables are reported as means ± SDs unless otherwise stated. The Kolmogorov–Smirnov statistic was applied to continuous variables. Logarithmic or square-root transformations were applied as needed to ensure normal distribution of the variables. One-way analysis of variance, followed by the Games–Howell post hoc test when three or more groups were being compared, served to analyze differences among groups of women.

A multivariate logistic regression analysis was used to determine the effects of SHBG genotypes on PCOS or control status. P < 0.05 was considered statistically significant. Statistical analyses were computed using SPSS Statistics 17.0 for Windows (SPSS, Chicago, IL, USA).

Results
Phenotypic characteristics of patients and controls in the clinical and population series
As expected from design, there were no differences in BMI among patients with PCOS and controls in the clinical series (Table I). In contrast, patients with PCOS were heavier compared with controls in the unselected population series and the mean BMI of the patients was in the overweight range (Table I). Considering the clinical and population series as a whole, and compared with the controls, PCOS patients had an increased BMI (PCOS 28.9 ± 7.2 versus controls 25.4 ± 5.6 kg/m², P < 0.001) and were younger (PCOS 25.2 ± 6.5 versus controls 31.5 ± 7.6 year, P < 0.001) (Table I).

Because in both series patients were younger than controls, age was introduced as a covariate in the comparison of other phenotypic variables, whereas BMI was included as a covariate only in the population series. Waist circumference was similar in patients and controls from the clinical and population series, whereas the waist–hip ratio was increased in patients only in the clinical series (Table I). The hirsutism score, total and free testosterone levels and androstenedione concentrations were higher in patients compared with controls in both series (Table I). Serum dehydroepiandrosterone-sulfate (DHEAS) levels were increased and serum SHBG levels decreased in patients compared with controls but only in the clinical series (Table I).

Association of PCOS with SHBG variants
All SHBG variants were in Hardy–Weinberg equilibrium in PCOS patients and in controls from the clinical and general population cohorts with the exception of the rs1799941 variant that was in disequilibrium only in the small group of patients with PCOS in the general population cohort.

Considering the clinical and population series as a whole, we found an association between PCOS and T alleles of the rs727428 variant (Table II), whereas genotypes of the rs1799941, rs6257 and rs6259 were similarly distributed among the patients and the controls (Table II). The association existed when comparing PCOS with non-PCOS controls or with hyper-normal controls (Table II).

We confirmed this association using a binary logistic regression model that included PCOS or control status as an independent variable (coded as 1 for PCOS and 0 for non-PCOS controls) and the SHBG rs1799941, rs6257, rs6259 and rs727428 genotypes as independent variables (coded as 0 for homozygosity for wild-type alleles, 1 for heterozygosity and 2 for homozygosity for mutant alleles). A backward stepwise likelihood ratio method was used to introduce independent variables (probability of entering ≤ 0.05; probability of removing ≥ 0.10). The model (Nagelkerke’s R² = 0.011;
Martínez-García et al.

Accordingly, the SHBG haplotype composed of GTGT alleles of rs1799941, rs6257, rs6259 and rs727428 variants was associated with PCOS (χ² = 6.185; P = 0.013).

Since the patients with PCOS were heavier than controls, the association between PCOS and the SHBG rs727428 variant could be spurious, being actually derived from the association between PCOS and obesity. The fact that the association between PCOS and the SHBG rs727428 variant persisted after restricting the analysis to the clinical series, in which patients with PCOS and controls were matched for BMI, suggests that weight excess did not influence this association (Table III).

### Table I Phenotypic characteristics of patients with PCOS and controls in the clinical and population series.

<table>
<thead>
<tr>
<th>SHBG SNP</th>
<th>Genotype</th>
<th>Patients with PCOS (n = 312)</th>
<th>Non-PCOS controls (n = 692)</th>
<th>P-value</th>
<th>Hyper-normal controls (n = 440)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799941</td>
<td>GG</td>
<td>184 (59.0)</td>
<td>383 (55.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>116 (37.2)</td>
<td>271 (39.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>12 (3.8)</td>
<td>38 (5.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs6257</td>
<td>TT</td>
<td>246 (78.8)</td>
<td>567 (81.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>65 (20.8)</td>
<td>121 (17.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1 (0.3)</td>
<td>4 (0.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs6259</td>
<td>GG</td>
<td>258 (82.7)</td>
<td>551 (79.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>52 (16.7)</td>
<td>129 (18.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>2 (0.6)</td>
<td>12 (1.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs727428</td>
<td>CC</td>
<td>93 (29.8)</td>
<td>260 (37.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>171 (54.8)</td>
<td>323 (46.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>48 (15.4)</td>
<td>109 (15.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are counts (percentage). PCOS, polycystic ovary syndrome; SNP, single-nucleotide polymorphism.
Influence of SHBG variants on phenotypic variables

For these analyses, PCOS patients and controls were considered as a whole yet the influence of the SHBG rs727428 variant on phenotypic variables was analyzed separately in patients with PCOS and non-PCOS controls to avoid a spurious effects derived from the association between this SNP and PCOS. We did not find any influence of SHBG variants on BMI, waist circumference, waist-hip ratio or hirsutism score, either considering all genotypes or considering dominant or recessive models of inheritance (data not shown).

The possible influence on hormonal variables was analyzed similarly, but excluding the women who were taking hormonal contraception at the time of sampling. None of the SHBG variants influenced serum SHBG concentrations (Table IV). However, the rs6259 variant was associated with decreased serum androstenedione concentrations in a recessive model of inheritance (GG + GA subjects: 8.8 ± 4.8 versus AA subjects: 7.8 ± 5.5 nmol/l, \( P = 0.040 \)), although a similar decrease in serum SHBG levels was far from reaching statistical significance (Table IV). Finally, none of the variants studied here influenced serum-free testosterone concentrations (data not shown).

Influence of SHBG variants on metabolic variables

We analyzed the influence of SHBG variants on serum lipids and indexes of glucose metabolism only in the clinical series, because...
the women in the population series were not in the fasting state at the time of sampling.

The rs6259 variant decreased serum low-density lipoprotein (LDL) cholesterol levels (GG subjects: 2.7 ± 0.7 versus GA + AA subjects: 3.0 ± 0.8 mmol/l, \( P = 0.019 \)) and increased serum triglycerides (GG subjects: 1.1 ± 0.7 versus GA + AA subjects: 0.9 ± 0.4 mmol/l, \( P = 0.040 \)) in a dominant model of inheritance. The rs1799941 variant increased serum triglycerides (GG subjects: 1.0 ± 0.7 versus GA + AA subjects: 1.3 ± 0.7 mmol/l, \( P = 0.008 \)) in a dominant model of inheritance. The rs727428 variant increased serum concentrations of total cholesterol (CC subjects: 4.5 ± 0.9 versus CT + TT subjects: 4.7 ± 0.7 mmol/l, \( P = 0.010 \)) and LDL cholesterol (CC subjects: 2.7 ± 0.8 versus CT + TT subjects: 3.0 ± 0.7 mmol/l, \( P = 0.015 \)) in a dominant model of inheritance.

None of the SHBG variants influenced fasting glucose and insulin concentrations or homeostasis model assessment of insulin resistance (data not shown).

**Discussion**

Our present results confirm, in women from the Mediterranean area, the association between common SHBG SNPs and PCOS described earlier in women from the USA (Wickham et al., 2011). Mutant alleles of the rs727428 variant that have been associated with reduced serum SHBG concentrations in women with PCOS (Wickham et al., 2011) were more frequent in Mediterranean patients with PCOS, and this association was independent of obesity. According to the logistic regression model, carrying rs727428 mutant alleles associated a 1.29 OR of having PCOS. Controls carrying rs727428 mutant alleles tended toward showing reduced SHBG concentrations in our series, and their total testosterone levels were reduced in conceptual agreement with the effects described in American women (Wickham et al., 2011).

The rs6259 variant also influenced the risk of PCOS in Mediterranean women, but in this case the influence of the rs6259 variant was protective, with a 0.68 OR for having PCOS in carriers of mutant alleles. SHBG rs6259 mutant alleles associate higher serum SHBG concentrations (Dunning et al., 2004; Berndt et al., 2007; Ding et al., 2009). An increase in SHBG levels would be protective against PCOS by decreasing free testosterone concentrations and the amount of circulating testosterone reaching target tissues.

In any case, according to the parameters of the logistic regression model, the SHBG rs727428 and rs6259 variants are not strongly associated with PCOS. Moreover, in case we had applied a correction to the level of statistical significance to compensate for having analyzed four SNPs (i.e. setting statistical significance at the \( P < 0.0125 \) level), the association between rs727428 and PCOS would have retained statistical significance only as a dominant model in the BMI-matched clinical cohort \( (P = 0.009, \text{Table III}) \), but not when considering all genotypes within the clinical cohort \( (P = 0.032, \text{Table III}) \) or when analyzing the clinical and populations cohorts as a whole \( (P = 0.037 \) for all genotypes, \( P = 0.017 \) for a dominant model, \( \text{Table II} \)).

Furthermore, these associations do not necessarily indicate a causative role of the SHBG variants in the development of PCOS. PCOS is characterized by reduced circulating SHBG concentrations (Escobar-Morreale et al., 2001) and patients with PCOS frequently present with increased androgen levels and hyperinsulinemia, which are negative regulators of hepatic SHBG synthesis and secretion (Pugeat et al., 1996). Therefore, the association between PCOS and SHBG variants that influence circulating SHBG may also be spurious, resulting from the individual associations of both PCOS and SHBG variants with reduced serum SHBG concentrations.

In contrast to other genetic studies (Dunning et al., 2004; Berndt et al., 2007; Ding et al., 2009; Perry et al., 2010; Wickham et al., 2011), we have not found an influence of any of the four SHBG SNPs studied here on serum SHBG concentrations. The lack of replication of such findings may be related to differences in the population under study (i.e. sex, age and menopausal status), the disease under study (i.e. type 2 diabetes, hormone-dependent cancers), differences in the experimental design particularly with regard to sample size and differences in the assays used to measure SHBG concentrations. Furthermore, this lack of replication is not surprising when considering that not all studies have found the same influences and magnitude of effects (Dunning et al., 2004; Berndt et al., 2007; Ding et al., 2009; Perry et al., 2010; Wickham et al., 2011).

The sample sizes in our present study and in a previous family-based PCOS study conducted in American women (Wickham et al., 2011) were much smaller compared with previous reports (Dunning et al., 2004; Perry et al., 2010) and possibly lacked the power needed to detect small effects of SHBG variants on circulating SHBG concentrations. But also, androgen excess and PCOS-associated metabolic derangements (Escobar-Morreale and San Millan, 2007) might confound the influences of SHBG variants on circulating SHBG levels observed in other populations explaining, at least partly, the lack of replication in our PCOS case–control study. In conceptual agreement, only the influences of the rs727428 and rs1799941 variants on SHBG levels were found in a family-based PCOS study in American women, in whom the SHBG raising effects of the rs1799941 variant were much weaker (Wickham et al., 2011) compared with those reported earlier (Perry et al., 2010). Moreover, the strong effects of the rs6259 and rs6257 variants on circulating SHBG concentrations described in American men and women (Ding et al., 2009) were not replicated in the US family-based PCOS study (Wickham et al., 2011) as occurred in our present study.

Although we did not find any influence of the SHBG variants on SHBG levels, we found statistically significant effects on serum total androgen concentrations and on plasma lipid levels. The decrease in total testosterone concentration found in the controls carrying T alleles of the rs727428 variant would be the expected result of a putative SHBG lowering effect (Wickham et al., 2011). However, it is difficult to explain the lower androstenedione and DHEAS levels found in carriers of the rs1799941 and rs6259 variant alleles, because circulating androstenedione and DHEAS bind mainly to albumin and not to SHBG (Sidropoulou et al., 2012). This finding could result from a spurious association because androgen levels and SHBG concentrations are inversely related in women and SHBG concentrations may increase in carriers of SHBG rs1799941 and rs6259 variants (Ding et al., 2009; Perry et al., 2010). Similar considerations apply to the influence of SHBG variants on plasma lipid levels.

Finally, our study was not free of limitations. First, our study included different patients and controls from different sources, making some of the interpretations difficult. Second, the rs1799941 variant was not in Hardy–Weinberg equilibrium in the small group of patients with PCOS recruited from the general population.
Although SNP rs1799941 was not associated with PCOS, this disequilibrium may relate to population sampling issues or to genotyping issues with this particular polymorphism.

In summary, SHBG variants that influenced circulating SHBG levels in American patients with PCOS are also associated with this syndrome in Mediterranean women. The involvement of these SHBG variants in PCOS and PCOS-associated traits in different ethnic and geographic populations points to SHBG as a candidate gene for PCOS. However, that the associations are relatively weak suggests a minor role of SHBG variants in the pathogenesis of PCOS, in conceptual agreement with the current perception of this syndrome as a complex multigenic metabolic and reproductive disorder in which multiple predisposing and protective variants interact with very important environmental influences leading to the PCOS phenotype (Escobar-Morreale et al., 2005).

Authors’ roles

M.A.M.-G. conducted genotyping and genetic association studies. A.G., M.A., R.S. and H.F.E.-M. recruited and phenotyped subjects. M.A.M.-G., M.A., R.S. A.G. and H.F.E.-M. contributed to data-mining, statistical analysis and drafting of the manuscript. R.P. and H.F.E.-M. designed the study and provided administrative support and funding. H.F.E.-M. wrote the final version of the manuscript. All the authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest

None declared.

References


