Decreased Risk of Ovarian Cancer Associated With rs9898876 Sex Hormone-binding Globulin Gene Variant

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Abstract

Background. Ovarian cancer (OC) is one of the most common gynecologic cancers, with significant morbidity and mortality. The risk of OC is influenced by hormone status, of which sex hormone-binding globulin (SHBG), which influences the serum availability of steroid sex hormones, is implicated in the pathogenesis and evolution of OC. The aim of this study is to evaluate the involvement of common *SHBG* gene variants in OC susceptibility and evolution.

Materials. A case control study including 71 OC patients and 74 cancer-free controls, who were genotyped for rs9898876, rs13894, rs1799941 and rs6257 *SHBG* SNP. Genotyping was done by the allelic discrimination method, using VIC- and FAM-labeled primers.

Results. The minor allele frequencies of rs9898876, rs13894, rs1799941 and rs6257 *SHBG* SNP was comparable between OC cases and control women, implying no significant associations of the tested variants and overall OC risk. Taking homozygous wild-type genotype as reference (OR=1.00), heterozygous rs9898876 (G/T), and minor allele-carrying genotypes [G/T+T/T] were associated with reduced risk of OC. While rs9898876 heterozygosity (G/T) was predictive of OC occurrence, no significant association of the remaining three tested SNPs was noted with altered risk of OC. Irrespective of FIGO staging, the four tested *SHBG* SNPs were not associated with the clinical progression of OC.

Conclusion. In conclusion, SHBG rs9898876 is associated with a decreased risk of OC, and thus constitutes a potential diagnostic biomarker of OC.

1. Introduction

Ovarian cancer (OC) is a significant gynecological malignancy, and accounts about 2.5% of all malignancies among women, and 5% of deaths of female cancers [1]. It was reported that 184,799 deaths were attributed due to OC in 2018, which is predicted to rise significantly by 2040 [2]. While asymptomatic in its early stages, high mortality rates were reported for later stages of OC (Stage III, Stage IV), resulting in 46.2% 5-year survival of [3, 4]. OC is multifactorial and complex in nature, with a poorly defined etiology [3, 5], and several modifiable and non-modifiable factors linked with its development and progression were reported.

Despite considerable efforts to delineate its origin, the exact etiology of OC remains poorly understood [4, 5]. Two major theories were proposed for the pathogenesis of OC, namely the incessant ovulation, and the gonadotropin connection [5, 6]. Both epidemiologic and experimental evidence support the contribution of altered steroid sex hormone balance to the pathogenesis of OC, acting through stimulation of cell proliferation and telomerase expression. These in turn precipitate angiogenesis and greatly enhance carcinogenesis and metastasis, principally through the PI3K/AKT pathway [7, 8]. In support of the role of sex hormones in enhanced risk of OC was the findings that altered serum levels of circulating steroid sex hormones increase the overall incidence of OC [6, 7, 9], and that women on hormone replacement therapy are at increased risk for OC development [10].

The principal steroid sex hormones, testosterone and estrogen, are found in three pools: albumin-bound, free hormone, and a fraction tightly bound to sex hormone-binding globulin (SHBG), a 373-amino acid homodimeric glycoprotein with three carbohydrate side chains produced by the liver [11, 12]. SHBG is a key regulator of steroid-signaling system, and acts by binding circulating steroid sex hormones (testosterone and estradiol) [10, 11], thus limiting their tissue bioavailability [10, 12]. SHBG is encoded by *SHBG* gene which is organized into eight exons separated by seven introns, and spans 4-kb on the short arm of chromosome 17 (17p13-1p12) [13].

Insofar as the action of sex steroids depends on their bioavailability to target organs and tissues, including ovaries [12, 14], a role for SHBG was proposed for OC. Several *SHBG* gene polymorphisms that control SHBG production and protein stability, and steroid-binding activity were identified throughout human *SHBG* gene, especially in the promoter region [15–17]. Earlier gene association studies that investigated the association of specific *SHBG* SNP with altered risk of OC often yielded inconclusive results due, in part, to the relatively low number of study participants in most of these studies, and the failure to address the impact of key covariates on the association of *SHBG* variants with OC risk [11, 18–19]. In addition, few studies investigated the effect of different *SHBG* variants on circulating SHBG levels in both healthy control women and OC patients, but with inconclusive results [11, 15].

This study aims to examine the association of rs9898876, rs13894, rs1799941 and rs6257 common *SHBG* variants with the risk of OC and its evolution in 71 Tunisian women with OC and 74 cancer-free women who served as controls. This is the first study that addresses this association in a North African (Tunisian) population.

2. Subjects And Methods

2.1. Study Subjects. This retrospective case–control study was approved on 25 June 2017 by Research & Ethics Committee of Salah Azeiz Oncology Institute (IRB number: ISA/2018/19, granted). Study subjects comprised 71 women with histologically-confirmed OC, who were recruited from the outpatient oncology clinics of Salah Azeiz Oncology Institute (Tunis, Tunisia). Diagnosis and staging of OC were performed as per International Federation of Gynaecology and Obstetrics (FIGO) classification guidelines (www.figo.org), and with histological types (serous-papillary, endometrioid, mucinous, and mixed/others). The 74 age- and ethnically-matched control women were recruited from Dispenser of Ettadhamen City, and consisted of employees, or healthy women undergoing routine check-up. Control women were negative for allergies, diabetes, hypertension, or cardiovascular disease, with (self-reported) negative personal or family history of OC. OC cases and control women originated from the four major governorates of Tunisia, and were required to provide written consent for participating in the study, which was performed as per Helsinki II guidelines.

2.2. *SHBG* Genotyping. Genomic DNA was extracted from peripheral venous blood specimens were recovered from participants in EDTA-containing tube for genomic DNA extraction before start of radiation or chemotherapy. Genomic DNA was extracted using QIAamp DNA blood mini kit, according to

manufacturer's instruction (Qiagen GmbH, Hilden, Germany). Selection of *SHBG* polymorphisms (rs9898876, rs13894, rs1799941 and rs6257) was based on their clinical relevance (*NCBI Entrez Gene SNP Geneview*), and minor allele frequency (MAF) equal to or exceeding 5% in Caucasians (CEU-HapMap data), and were ordered as Taq-Man assay on demand VIC- and FAM-labelled primer pairs (ABI, ThermoFisher, Foster City, CA). PCR was performed in 6- μ l volume on StepOne Plus real-time PCR system (ThermoFisher). We included blinded replicate quality control samples for assessment of the genotyping reproducibility; concordance was 100%.

2.3. Statistical Analysis. Statistical analysis was performed on SPSS 21.0 (SPSS Inc., Chicago, IL), Epi info-7 (www.cdc.gov/epiinfo), SNPStats (www.bioinfo.iconcologia.net/snpstats) and Haploview 4.2 (www.broad.mit.edu/mpg/haploview). Categorical data were presented as percentages of total, while continuous variables were presented as mean ($\pm$ SD). Student's t-test analyzed differences in means, while Pearson χ^2 and Fisher's exact tests were used for assessment of inter-group significance. *SHBG* genotypes were analyzed for conformity with Hardy–Weinberg equilibrium (HWE) using Haploview 4.2. Analyses performed under the assumption of additive genetic effect was carried out using SNPStats. Linkage disequilibrium (LD) between all pairs of SNP was measured by Haploview 4.2, with 4-locus *SHBG* haplotype reconstruction done by the principle of expectation-maximization on Haploview 4.2. Taking the control women as reference, logistic regression analysis was used in odds ratios (OR) and 95% confidence intervals (CI) determination linked with the risk of OC; statistical significance set at $p < 0.05$.

3. Results

3.1. Study Subjects. The demographic and clinical characteristics of the 71 OC cases and 74 control women are shown in Table 1. Mean age was comparable between ovarian cancer cases and control women ($P = 0.58$). The frequency of smokers, oral contraceptive users, breastfeeding women, and post-menopausal women was comparable between ovarian cancer cases and cancer-free control women. The histological types identified among ovarian cancer cases were serious-papillary carcinoma (49; 69.0%), followed by endometrioid (14; 19.7%), mixed/others (7; 9.9%), and one single case of mucinous (1.4%). OC staging, as per FIGO staging, showed that 10 patients (14.1%) presented with stage I, 25 (35.2%) with stage II, and 30 (42.3%) with stage III, and the remaining 6 cases (8.5%) with stage IV.

3.2. Allelic and Genotypic Association Studies. Table 2 summarizes the association between rs1799941 and rs6257 *SHBG* variants and OC among case-control subjects. Apart from rs9898876 ($P = 0.04$), the genotype distribution of rs13894 ($P = 0.74$), rs1799941 ($P = 0.40$), and rs6257 ($P = 0.59$) were in HWE among study subjects. Of the tested *SHBG* SNPs, higher minor allele frequency (MAF) of rs9898876 ($P = 0.04$; $\chi^2 = 4013$) were seen in OC cases compared to control women; MAF of the remaining SNPs were comparable between OC cases and controls (Table 2).

Table 1
Demographic and clinical characteristics of the study subjects

		Study subjects (n=145)		<i>p</i> ^a
		Cases (n = 71)	Controls (n = 74)	
Demographic characteristics				
Age (mean±SD)		51.1 ±14.0	51.7±14.2	0.58
≥50 years		36 (50.7%)	39 (52.7%)	0.47
Menopausal status	Pre-menopausal	28 (39.4%)	27 (36.5%)	0.42
	Post-menopausal	43 (60.6%)	47 (63.5%)	
Oral contraception	Users	6 (8.5%)	6 (8.1%)	
	Non-users	65 (91.5%)	68 (91.9%)	
Breast feeding		20 (28.2%)	16 (21.6%)	
Pregnancy		32 (45.1%)	-	
Clinical characteristics				
Lymph nodes		60 (84.5%)	NA	
Chemotherapy		60 (84.5%)	NA	
FIGO staging of cancer				
	Stage I	10 (14.1%)	NA	—
	Stage II	25 (35.2%)	NA	—
	Stage III	30 (42.3%)	NA	—
	Stage IV	6 (08.5%)	NA	—
Histological type				
	Seriouspapillary	49 (69.0%)	NA	—
	Endometrioid	14 (19.7%)	NA	—
	Mixed/others	7 (9.9%)	NA	—
	Mucinous	1 (1.4%)	NA	—
FIGO International Federation of Gynecology and Obstetrics, NA not applicable				
a Student's t-test (continuous variables), Pearson χ2 test (categorical variables)				
b Number of subjects (percent total within group)				

Table 2
SHBG SNPs allelic distribution in patient and control groups

SNPs	Location ¹	Alleles	Cases ₂	Controls ₂	HWE ₃	χ^2	<i>p</i>	OR (95% CI)
rs9898876	17:7526962	G:T	0.37	0.26	0.05	4.13	0.04	1.68 (0.99-2.86)
rs13894	17:7529902	C:T	0.39	0.37	0.63	0.11	0.74	1.08 (0.66-1.79)
rs1799941	17:7533423	A:G	0.84	0.80	0.11	0.70	0.40	1.29 (0.68-2.46)
rs6257	17:7533717	C:T	0.15	0.13	0.21	0.29	0.59	1.20 (0.59-2.48)
1. Location on chromosome (GRCh37)								
2. Minor allele frequency (MAF) of OC cases (n = 71) and control women (n = 74).								
3. HWE, Hardy-Weinberg equilibrium <i>P</i> value.								

Significant difference in rs9898876 ($P < 0.001$) genotype distribution was seen between OC cases and control women (Table 3); the genotype distribution of the remaining *SHBG* SNPs was comparable between patients and controls. The negative association of rs9898876 with OC susceptibility persisted irrespective of the genetic models of analysis tested, namely codominant ($P < 0.001$), dominant ($P = 1.0 \times 10^{-4}$), recessive ($P = 8.8 \times 10^{-3}$) and overdominant ($P < 0.001$). No significant association with altered risk of OC were noted with the remaining three tested SNPs (Table 3).

Table 3
SHBG genotype distribution in cases and controls according 4 models of analysis

SNP		Study subjects			<i>p</i> _{value}	OR (95% CI)
Model	Genotypes	Cases ¹	Controls ¹			
rs9898876	Codominant	G/G	42 (59.10%)	20 (27.00%)	<0.001	1.00
		G/T	21 (29.60%)	53 (71.60%)		0.19 (0.09-0.39)
		T/T	8 (11.30%)	1 (1.40%)		3.81 (0.45-32.57)
	Dominant	G/G	42 (59.10%)	20 (27.00%)	1.0 × 10 ⁻⁴	1.00
		(G/T+T/T)	29 (40.90%)	54 (73.00%)		0.26 (0.13-0.51)
	Recessive	(G/G+G/T)	63 (88.70%)	73 (98.70%)	8.8 × 10 ⁻³	1.00
		T/T	8 (11.30%)	1 (1.40%)		9.27 (1.13-76.15)
	Overdominant	(G/G+T/T)	50 (70.40%)	21 (28.40%)	<0.001	1.00
		G/T	21 (29.60%)	53 (71.60%)		0.17 (0.08-0.34)
	rs13894	Codominant	C/C	30 (42.20%)	27 (36.50%)	0.64
C/T			29 (40.90%)	36 (48.60%)	0.73 (0.36-1.48)	
T/T			12 (16.90%)	11 (14.90%)	0.98 (0.37-2.59)	
Dominant		C/C	27 (36.50%)	30 (42.20%)	0.48	1.00
		(C/T+T/T)	47 (63.50%)	41 (57.80%)		0.79 (0.40-1.53)
Recessive		(C/C+C/T)	63 (85.10%)	59 (83.10%)	0.74	1.00
		T/T	11 (14.90%)	12 (16.90%)		1.16 (0.48-2.84)
Study subjects comprised 74 control women, and 71 OC patients						
1: Number of subjects (percent total within group)						

rs1799941	Overdominant	(C/C+T/T)	38 (51.40%)	42 (59.10%)	0.34	1.00
		C/T	36 (48.60%)	29 (40.90%)		0.73 (0.38-1.41)
	Codominant	G/G	49 (69.00%)	52 (70.30%)	0.28	1.00
		G/A	16 (22.50%)	20 (27.00%)		0.85 (0.40-1.82)
		A/A	6 (8.40%)	2 (2.70%)		3.18 (0.61-16.53)
	Dominant	G/G	49 (69.00%)	52 (70.30%)	0.87	1.00
		(G/A+A/A)	22 (31.00%)	22 (29.70%)		1.06 (0.52-2.15)
	Recessive	(G/G+G/A)	65 (91.50%)	72 (97.30%)	0.12	1.00
		A/A	6 (8.40%)	2 (2.70%)		3.32 (0.65-17.05)
	Overdominant	(G/G+A/A)	55 (77.50%)	54 (73.00%)	0.53	1.00
		G/A	16 (22.50%)	20 (27.00%)		0.79 (0.37-1.67)
rs6257	Codominant	T/T	57 (80.30%)	53 (71.60%)	0.07	1.00
		T/C	10 (14.10%)	20 (27.00%)		0.46 (0.20-1.08)
		C/C	4 (5.60%)	1 (1.14%)		3.72 (0.40-34.35)
	Dominant	T/T	57 (80.30%)	53 (71.60%)	0.22	1.00
		(T/C+C/C)	14 (19.70%)	21 (28.40%)		0.62 (0.29-1.34)
	Recessive	T/T+T/C	67 (94.40%)	73 (98.70%)	0.15	1.00
		C/C	4 (5.60%)	1 (1.40%)		4.36 (0.48-39.98)

Study subjects comprised 74 control women, and 71 OC patients

1: Number of subjects (percent total within group)

Overdominant	(T/T+C/C)	61 (85.90%)	54 (73.00%)	0.05	1.00
	T/C	10 (14.10%)	20 (27.00%)		0.44 (0.19- 1.03)
Study subjects comprised 74 control women, and 71 OC patients					
1: Number of subjects (percent total within group)					

3.3. FIGO stratification and OC Evolution. In order to evaluate the possible implication of *SHBG* gene polymorphisms on OC evolution, we performed analysis of genotype and allele frequencies of the tested *SHBG* polymorphisms between mild-moderate (stages I + II) versus severe stage (stages III + IV). Data from Table 4 demonstrated lack of association between *SHBG* SNPs and clinical progression of OC according FIGO classification.

3.4. Haploview Analysis. We assessed the interaction between tested gene variants by calculating LD, and analyzed 4-locus (rs9898876, rs13894, rs1799941 and rs6257) *SHBG* haplotype distribution in OC cases and control women using Haploview. As shown in Fig. 1, haploview analysis demonstrated strong linkage disequilibrium (LD) between rs9898876, rs13894, rs1799941 and rs6257. Of the 16 possible haplotypes, 11 were found to be common as their frequencies exceeded 1%. Data from Table 5 identified reduced prevalence of **GCGC** haplotype in OC cases than in control women ($P=0.047$), suggesting an OC-protective nature to this haplotype. The prevalence of the remaining haplotypes was not statistically different between OC cases and control women.

Table 4
Implication of SHGB polymorphisms in the evolution of ovarian cancer

SHGB SNPs	Early stage ¹	Advanced stage ¹	<i>p</i> -value	OR (CI 95%)
rs9898876				
G/G	19 (54.3%) ²	23 (63.9%)	0.67	1.00 (Reference)
G/T	12 (34.3%)	09 (25.0%)		0.62 (0.22-1.78)
T/T	04 (11.1%)	04 (11.1%)		0.83 (0.18-3.75)
T	20 (29.0%)	17 (24.0%)	0.50	0.77 (0.34-1.75)
rs13894				
C/C	17 (48.6%)	13 (36.1%)	0.50	1.00 (Reference)
C/T	12 (34.3%)	17 (47.2%)		1.85 (0.66-5.21)
T/T	06 (17.1%)	06 (16.7%)		1.31 (0.34-5.01)
T	24 (34.0%)	29 (40.0%)	0.46	1.29 (0.62-2.71)
rs1799941				
G/G	25 (71.4%)	23 (65.7%)	0.85	1.00 (Reference)
G/A	07 (20.0%)	09 (25.7%)		1.40 (0.45-4.36)
A/A	03 (08.6%)	03 (08.6%)		1.09 (0.20-5.94)
A	13 (19.0%)	15 (21.0%)	0.67	1.15 (0.59-2.24)
rs6257				
T/T	28 (80.0%)	29 (80.6%)	0.48	1.00 (Reference)
T/C	06 (17.1%)	04 (11.1%)		0.64 (0.16-2.53)
C/C	01 (02.9%)	0.3 (08.3%)		2.90 (0.28-29.53)
C	08 (11.0%)	10 (14.0%)	0.66	1.22 (0.51-2.90)
1. Study subjects comprised 35 early stage and 36 advanced stage OC patients				
2.Number of women (percent of total within group).				

Table 5
Distribution of 4-Locus *SHBG* haplotypes in OC cases and controls

Haplotype ¹	Cases	Controls	χ^2	<i>P</i>
GCAC	0.283	0.301	0.114	0.735
GTAC	0.163	0.178	0.111	0.739
TCAC	0.161	0.128	0.658	0.417
GCGC	0.078	0.152	3.933	0.047
TTAC	0.082	0.068	0.214	0.643
GTAT	0.053	0.053	0.000	0.982
TTAT	0.054	0.037	0.468	0.494
GCAT	0.031	0.032	0.000	0.988
TCGC	0.044	0.010	3.199	0.073
GTGC	0.020	0.024	0.037	0.847
TTGC	0.019	0.013	0.165	0.684
<p>Boldface indicates statistically significant differences</p> <p>Underlined indicates minor allele</p> <p>1. <i>SHBG</i> haplotype rs9898876, rs13894, rs1799941 and rs6257 frequency</p> <p>2. Pearson chi-square test</p>				

4. Discussion

Previous studies demonstrated a key, but complex role of SHBG in regulating androgen and estrogen availability [15, 19]. Since genetic factors control SHBG bioavailability, which can range from 12%-76% for individual gene variant [20, 21], an interest in the association of specific *SHBG* variants in regulating SHBG production or activity was reported for several pathobiologies [15, 22, 23], and role for these variants as diagnostic or prognostic markers was proposed. The present study examined the association of *SHBG* variants with the susceptibility to, and evolution of OC. While allelic distribution of rs13894, rs1799941 and rs6257 was similar between OC cases and control women, differential association of rs9898876 minor allele and genotypes was noted between cases and controls. Results obtained confirmed the negative association of this variants with OC presence, though not its evolution.

Same results were reported for rs1799941 and rs6257 variants among Polish women [11]. Although the rs1799941 and rs6257 *SHBG* variants were associated with elevation in circulating SHBG levels [23, 24] resulting in a reduced bioavailability of estrogen or other sex-steroids, and likely reduction in risk of hormonally-related cancers, the genotype frequencies of these variants was lower in cases than controls,

although these differences were not statistically significant [11]. A more recent study pointed to a possible link between polycystic ovary syndrome (PCOS) and OC risk. However, the genetic impact of SHBG variability was poorly investigated in OC contrary to PCOS [25]. The *SHBG* SNPs investigated in our study, were also studied in an earlier study on the contribution of *SHBG* genetic variability to altered SHBG levels and PCOS risk among Arab-speaking subjects [26], and negative conclusions were revealed. Furthermore, rs1799941 [27, 28] and rs6257 [27] were studied in Mediterranean [27] and USA (Virginia) [28] women with PCOS, and neither were shown to influence the physiopathology of PCOS or SHBG serum concentrations.

The study of Ruixia *et al.* involving 248 OC subjects demonstrated variable expression of SHBG in OC patients, with augmented production of SHBG expression in ovarian carcinoma associated with poor clinicopathological outcome [29]. Higher SHBG expression was significantly associated with aggressive histological subtypes ($P = 0.022$), higher FIGO staging ($P = 0.018$), and higher histological grade ($P = 0.020$). Future well controlled studies involving a larger sample size are needed for better evaluation of the implication of those variants on OC susceptibility.

We did not observe any notable difference in the distribution of *SHBG* variants between premenopausal and postmenopausal cases, suggesting lack of association. Given the relatively low number of cases (71) and controls (74), caution is warranted in adopting these findings, and future studies involving large cohort are needed to confirm, or alternatively rule out a role of specific *SHBG* variants in modulating the disease risk according to menopausal status. An apparent lack of association between the tested *SHBG* variants and the clinical progression of OC according to FIGO was also seen, which is the first study to report this. Furthermore, haploview analysis showed strong LD between rs9898876, rs13894, rs1799941 and rs6257, and identified GCGC as a disease-modifying haplotype risk.

Estrogens and androgens are produced in gonads, adrenal glands and placenta [30–31], and human SHBG plays an important role in transporting testosterone to target tissues and cells [16, 30–31]. Altered serum SHBG levels controls the extent of hyperandrogenemia, and the risk of associated endocrine pathologies [14, 15]. It was previously demonstrated that altered steroid and non-steroid sex hormone levels is key to the pathogenesis of OC [7, 31]. In this regard, it was shown that androgen levels correlate positively with heightened risk of OC development, with high androgen levels in pre- and post-menopausal stage correlating positively with OC initiation [32, 33]. This was in contrast to estradiol, high levels of which were related to endometrioid tumors [34, 35].

We recently reported the association of polymorphisms in several pro-inflammatory mediators with altered risk of OC [36]. Effective treatment of OC is often hampered by the advanced stage of OC at diagnosis, coupled with the establishment of drug resistance to chemotherapeutic therapy [37], and development of adverse effects [37, 38]. Antagonism of estrogen pathway was proposed as a treatment alternative, based on the relative low toxicity, and high compliance to and tolerability of anti-estrogen and aromatase inhibitor therapies, especially in post-menopausal breast cancer patients [39–41]. It is tempting to propose that changes in circulating SHBG levels, stemming from carriage of functional *SHBG*

genetic variants may serve diagnostic and prognostic role, thereby representing novel therapeutic strategies for OC management.

This study has some strengths. OC cases and control women had similar ethnic background, which reduced the chances of racial/ethnic admixture, and only new OC cases were recruited before the start of chemotherapy and/or radio-therapy. Histological typing, and OC staging of cases was based FIGO classification, which allowed comparison of our results with those that utilized similar staging. However, our study had alsosome limitations. This was mostly due to the relatively limited sample size, due to the fact that most referred patients already received treatment. Despite the incidence of OC in Tunisia, the uniqueness of this pathology lies in its quiet character responsible for a delay in diagnosis and therapeutic difficulty especially in its extended forms. Therefore, the low sample size cannot lowered the power of our study.

Conclusions

In conclusion, despite these shortcomings, our study is the first report that demonstrates an association of SHBG rs9898876 with a decreased risk of OC. This variant can be added to the panel of potential future diagnostic biomarker of OC.

Abbreviations

CI, confidence interval; **FIGO**, Federation of Gynaecology and Obstetrics; **LD**, linkage disequilibrium; **MAF**, minor allele frequency; **OC**, Ovarian cancer; **SHBG**, sex hormone-binding globulin

Declarations

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Compliance with Ethical Standards:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

Conflict of interest:

All authors declare that they have no conflict of interest.

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Figures

Figure 1

Haploview analysis of the typed SHBG variants. The nucleotide positions of the included SHBG variants are shown above Haploview output. The color scheme denotes LD between pairs of SHBG variants. LD calculation was based on D' values (D divided by the theoretical maximum for the observed allele frequencies) multiplied by 100. Absence of LD is expressed as values near zero, while values close to 100 suggest complete LD. The colored red square represents varying degrees of LD (< 1) and logarithm of odds (LOD; > 2) scores; darker shades presenting stronger LD.