Int J Clin Exp Pathol 2019;12(9):3514-3520
www.ijcep.com /ISSN:1936-2625/IJCEP0099467

Original Article

Association between sex hormone binding globulin gene polymorphism and type 2 diabetes mellitus

Li Quan[1], Liang Wang[2], Jing Wang[1], Binya Yuwen[1], Jun Zhu[1]

Departments of [1]Endocrinology, [2]Clinical Laboratory, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

Received July 10, 2019; Accepted August 26, 2019; Epub September 1, 2019; Published September 15, 2019

Abstract: This study aims to investigate the relationship between single nucleotide polymorphisms (SNPs) of sex hormone binding globulin (SHBG) and type 2 diabetes mellitus (T2DM) in an Uighur population. One hundred and fourteen T2DM male patients (with a history of diabetes or diagnosed as diabetic by the oral glucose tolerance test) and 173 healthy males from the Uighur ethnic group were included in the study to test the following SNPs of SHBG: rs727428, rs1799941, rs6259, rs6257, rs858521, rs858518, rs3760213, and rs11078701. The body mass index (BMI), blood pressure, and waist circumference, and lipid, glucose, HbA1c, insulin, HOMA-IR, testosterone, and SHBG levels of enrolled individuals were measured. We used the t-test or rank sum test and Chi-square test to analyze the difference and compare numeration data, respectively, between the case and control groups. Comparisons among multiple groups were carried out using analysis of variance, and the correlation between variables was determined by nonparametric Spearman rank correlation analysis; multivariate logistic regression analysis was used to assess the risk of abnormal glucose in the two groups. There was a significant difference ($P < 0.05$) in BMI, blood pressure, and waist circumference, and lipid, glucose, HbA1c, insulin, and HOMA-IR levels between the case and control groups. The risk factors for diabetes included testosterone ($P = 0.042$) and SHBG ($P = 0.001$). The distribution of rs858521 ($P = 0.001$), rs1799941 (2.3%, $P = 0.032$), rs6259 (2.5%, $P = 0.040$), and rs727428 (3.4%, $P = 0.016$) was significantly different between the case and control groups ($P < 0.05$). In the control group, there was linkage disequilibrium (LD) between rs727428 and rs6259, while in the case group LD was found among rs858518, rs3760213, rs1799941, and rs6257. The frequency of rs858518-rs3760213-rs1799941-rs6257 haplotype TCGC was significantly different between the two groups ($P = 0.033$). Both testosterone and SHBGwere found to be risk factors of diabetes in the Uighur population, and SNPs of SHBG may contribute to T2DM.

Keywords: Diabetes, single Nucleotide Polymorphism (SNPs), sex hormone binding globulin (SHBG), Uighur ethnic group

Introduction

The increasing incidence of type 2 diabetes (T2DM) is a major problem [1, 2]. T2DM is a metabolic syndrome characterized by hyperglycemia. In T2DM, insufficient insulin secretion and/or insulin resistance (IR) affect the sugar, fat, protein, water, and electrolyte balance in patients [3]. T2DM is not caused by a single pathophysiologic mechanism but is a result of multiple factors. Although genetic variations and environmental factors are vital for the development and progression of T2DM [4], the mechanism of their involvement in T2DM has not yet been fully elucidated due to the complexity of T2DM etiology and pathogenesis.

Sex hormone binding globulin (SHBG) is an important protein involved in cellular signaling pathways [5] and is mainly produced by the liver. Certain metabolic diseases affect serum SHBG levels [6]. A role of SHBG as a risk factor for T2DM has been observed in many clinical trials and large prospective cohorts of men and women across ethnic populations [7]. It has been demonstrated that circulating SHBG is strongly associated with systemic metabolism and is predictive of insulin resistance and diabetes [8]. A meta-analysis reported that SHBG and estradiol are independent risk factors for the development of T2DM in women [9]. Some studies report that diabetes risk is potently determined by obesity and glycemia rath-

er than the sex hormones and that SHBG and its polymorphisms (rs6259, rs6257 and rs179-9941) do not predict any risks in men or women [10].

In this study, we comparatively studied single nucleotide polymorphisms (SNPs) of the SHBG gene between subjects with T2DM and those without T2DM, to explore the correlation between the polymorphisms of the SHBG gene and the risk of T2DM in the Uygur population of Xinjiang, China.

Materials and methods

Subjects

Our methodology was designed as a case-control study on the outpatients visiting the Department of Endocrinology at the First Affiliated Hospital of Xinjiang Medical University. The individuals receiving a physical examination at the same department between Sep 2016 and Dec 2016 were also included in the study to record the general data of the patients and the results of their physical and laboratory examination. Informed consent was obtained from all the subjects, and the research plan was approved by the ethics committee of the First Affiliated Hospital of Xinjiang Medical University.

Inclusion criteria and exclusion criteria

The inclusion criteria were as follows: 1) male patients over 40 years old; 2) patients of pure ethnic Uygur origin; 3) resident of Xinjiang for more than 5 years; 4) meeting the diagnostic criteria for T2DM. The healthy controls had similar criteri but had no history of diabetes and did not show an abnormal result on the oral glucose tolerance test (OGTT).

The exclusion criteria included: 1) patients with serious complications of the heart, lung, kidney, liver, brain or any other organ; 2) patients with thyroid disorders; 3) patients with psychological and mental diseases; 4) patients with a history of tumors related to the gonads or receiving exogenous hormone (including glucocorticoid, sex hormone, thyroid hormone, growth hormone) treatment at the time of screening; 5) patients with a history of pituitary disease (including pituitary tumor, increased pituitary hormone secretion or hypopituitarism); 6) patients with type 1 diabetes and other special types of diabetes; 7) those who did not provide their consent to draw blood.

Questionnaire survey

All subjects received a standardized face to face questionnaire survey, which was carried out in the native language of the subjects by the trained staff of our group. The survey included general information, such as age, address, education, marital status, job nature, and family income; lifestyle such as smoking history and drinking history; childbearing history; personal and family history of diabetes; history of diabetic complications or hypertension; history of metabolic syndrome such as dyslipidemia, hyperuricemia, and the medication they are currently using.

Anthroposomatological information

The anthroposomatological information including height, weight, blood pressure and waist circumference of all the subjects was collected by the trained staff of our group.

Sample collection and determination of the T2DM-related biochemical index

Fasting blood was collected from the anterior elbow vein of each subject after 8-10 h of fasting for determining the biochemical index: serum lipid levels, testosterone, SHBG, insulin levels, glycosylated hemoglobin, and plasma glucose levels. Fasting blood glucose (FBG) was evaluated by OGTT for all subjects except those who had already been diagnosed with diabetes. In OGTT, the blood glucose was tested at 2 h after consumption of glucose water (75 g anhydrous glucose powder or 82.5 g glucose monohydrate dissolved in 200-300 mL warm water). The post-prandial glucose (PBG) was tested in patients 2 h after consumption of food.

T2DM diagnostic criteria

The diagnoses of T2DM and abnormal blood sugar were based on the diagnostic criteria fordiabetes recommended by WHO in 1999.

SNP selection

SNPs in the SHBG gene were selected from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and the International HapMap database (http://hapmap.ncbi.nlm.nih.gov/) according to the following criteria: 1) at least 5% minor allele frequency (plus) in the Chinese population, and 2) an r^2 threshold of less than 0.8 as the lower limit for linkage disequilibrium (LD). The tag SNPs were chosen

Table 1. Values of metabolic measures (BMI, blood pressure, total cholesterol and waist circumference) in the groups

	BMI	blood pressure (mmHg) (high pressure/low pressure)	Total cholesterol (mmol/L)	waist circumference (cm)	*P* value
Healthy group	24.77±9.5	130.0±42/76.9±25.0	4.85±2.2	90.8±10	0.034
T2DM group	27.58±9.9	142.5±47.1/82.8±36	5.65±2.5	98.88±30	

to constitute a set of minimal height information markers to minimize redundant data.

Finally, we searched the published literature for SNPs that were already reported to affect the levels of circulating SHBG. Eight SNPs, including rs727428, rs1799941, rs6259, rs6257, rs858521, rs858518, rs3760213, and rs11078701 were selected for further analysis.

Statistical analysis

SPSS 21.0 statistical software was used for data processing. The data were represented as mean ± standard deviation (SD) if the data were normally distributed, and the data that were not normally distributed were expressed as median and interquantile range. The difference between the two groups was analyzed using *t*-test or rank sum test. The enumeration data were compared using Chi-square test. Comparisons among multiple groups were carried out using analysis of variance. The correlation between the variables was analyzed by non-parametric Spearman's rank correlation analysis, and multivariate logistic regression analysis was used to analyze the risk of T2DM. The significance level was set as $\alpha = 0.05$, and the difference was considered significant when $P < 0.05$.

The original data collected on the ABI3730XL sequencer were analyzed with GeneMapper 4.1 software (Applied Biosystems Co., Ltd., USA). The genotype data of SHBG gene were obtained from the HapMap database, and the r^2 values were compared using post hoc multiple comparisons. Haploview 4.2 software was used for LD and haplotype analysis. LD Block was estimated using the solid spine of the LD algorithm. Thus, the LD map and haplotype data were obtained.

Results

Comparison of clinical data

A total of 287 subjects were enrolled in the study and were divided into case group (type 2 diabetes group) and control group according to the diagnostic criteria for diabetes. BMI, blood pressure, serum lipid levels and plasma glucose levels in the case group were all significantly ($P < 0.05$) higher than those determined in the control group (**Table 1**). The case group had significantly ($P < 0.05$) higher levels of SHBG and testosterone as compared to their levels in the control group. Fasting insulin levels were significantly ($P < 0.05$) lower in the case group compared to their levels in the control group.

Multivariate non-conditional logistic regression analysis of T2DM indicators

Logistic regression analysis showed that BMI, blood pressure, serum lipid levels and waist circumference were risk factors for T2DM. Additionally, SHBG (P = 0.001, 95% CI = 0.863-0.965) and testosterone (*P* = 0.042, 95% CI = 0.977-1.058) were also risk factors for T2DM.

Hardy-Weinberg equilibrium test of genotype distribution

The allele distribution of the SNP loci (rs11078701, rs858521, rs858518, rs3760213, rs1799941, rs6257, rs6259, and rs727428) of the *SHBG* gene was tested by the goodness of fit Chi-square test for Hardy-Weinberg (H-W) genetic equilibrium. The results showed that all SNP loci rs11078701 (HWP = 0.068/0.160), rs858521 (HWP = 0.000/0.167), rs858518 (HWP = 0.143/0.197), rs3760213 (HWP = 0.062/0.110), and rs1799941 (HWP = 0.887/1.000) were in compliance with the Hardy-Weinberg equilibrium. Thus, the samples were a good representative of the population.

Comparisons of the distribution of SNP loci

The distributions of alleles among the case and control groups were compared. The results showed that there was a significant difference in the allele distribution of rs858521 (P = 0.003), rs1799941 (P = 0.032), rs6259 (P = 0.040), and rs727428 (P = 0.016) between the two groups. The distribution of rs1799941 A/A

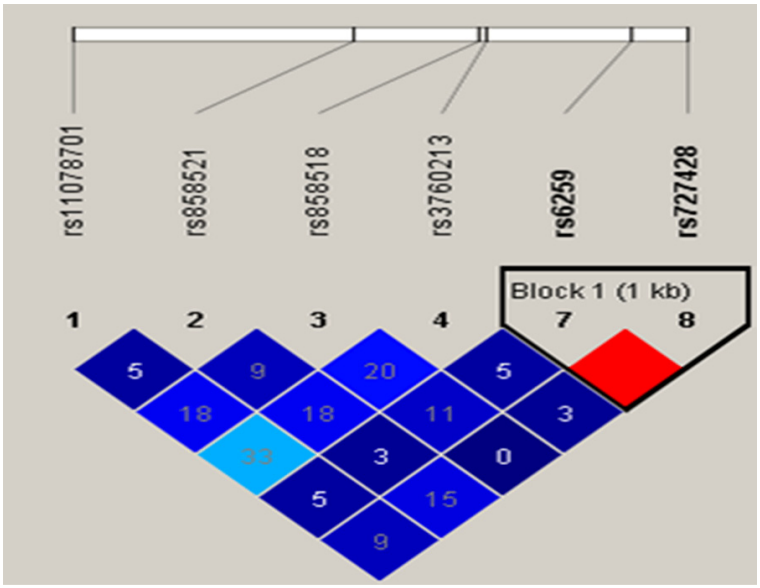

Figure 1. LD map of SNPs in SHBG in the control group.

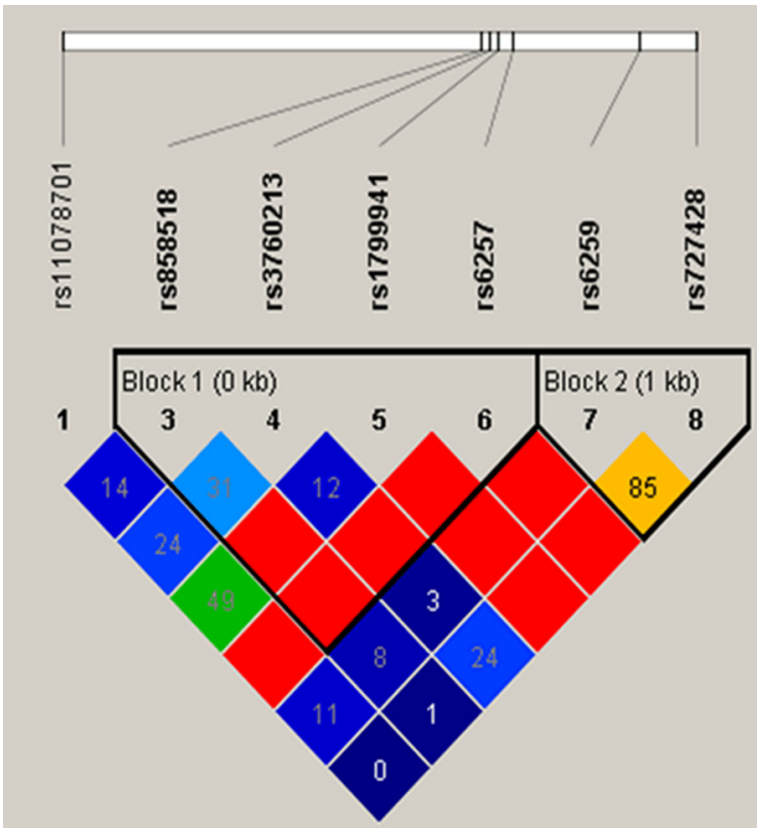

Figure 2. LD map of SNPs in SHBG in the case group.

and rs6257 T/T was not found in the case group, while rs1799941 G/A and A/A and rs6257C/T and T/T were not found in the control group.

Distribution and odds ratio of alleles of SNP loci in case and control groups

Analysis of the odds ratio of the distribution of different alleles of 8 SNP loci showed that the differences in distribution of different alleles of rs8585-21 (χ^2 = 10.131, *P* = 0.001), rs6259 (χ^2 = 4.851, *P* = 0.028) and rs727428 (χ^2 = 6.378, P = 0.012) were significant between the case and control groups, while the allele distribution of the remaining 5 SNP loci was not observed in the case group or control group. There was no significant difference in the allele distribution of rs-11078701, rs858518 and rs 3760213 between the case and control groups.

Analysis of linkage disequilibrium of 8 SNPs in SHBG gene

Linkage disequilibrium (LD) analysis was performed on the SNP loci in case and control groups that matched with the Hardy-Weinberg equilibrium. The results showed that there was a LD among the SNP loci rs727428 and rs6259 in the control group (D' = 1). In the case group, there was a LD among the loci rs8585-18-rs3760213-rs1799941-rs-6257 (D' = 1) (**Figures 1** and **2**; **Tables 2** and **3**).

Construction of haplotype of SHBG gene and its distribution frequency analysis

Haplotypes were constructed for the SNP loci obtained from the LD analysis, and their distribution frequencies were analyzed. The results showed that there was a significant difference in the distribution frequency of haplotype TCGC (rs858518-rs3760213-rs1799941-rs6257) in the *SHBG* gene between the case and control groups in the Uygur population (OR

Table 2. SHBG gene SNP site chain relationship |D'| value in control group

Loci	rs858521	rs858518	rs3760213	rs6259	rs727428
rs11078701	0.050	0.183	0.337	0.052	0.098
rs858521	-	0.093	0.182	0.037	0.155
rs858518	-	-	0.209	0.117	0.004
rs3760213	-	-	-	0.057	0.030
rs6259	-	-	-	-	1.000

Table 3. SHBG gene SNP site chain relationship |D'| value in case group

Loci	rs858518	rs3760213	rs1799941	rs6257	rs6259	rs727428
rs11078701	0.141	0.249	0.490	1.000	0.117	0.009
rs858518	-	0.313	1.000	1.000	0.081	0.017
rs3760213	-	-	0.126	1.000	0.030	0.244
rs1799941	-	-	-	1.000	1.000	1.000
rs6257	-	-	-	-	1.000	1.000
rs6259	-	-	-	-	-	0.853

= 0.697, 95% CI = 0.867-1.743, *P* = 0.033). The differences in the distribution frequencies of rs6259-rs727428 haplotype GA (OR = 0.468, 95% CI = 0.320-0.683, P < 0.001) and GG (OR = 1.478, 95% CI = 1.053-2.074, P = 0.037) were also statistically significant between the two groups.

Discussion

The specific nucleotide polymorphism loci in the SHBG gene associated with the pathogenesis of T2DM may act through a mechanism that affects the synthesis and functions of these proteins. Our results showed that the difference in the distribution of different genotypes of rs858521 loci in the Uygur middle-aged and elderly men was significant between the case and control groups. Allele C was associated with a higher risk for T2DM compared to the risk associated with allele G. The difference in the distribution of rs1799941 and rs6259 genotypes was also significant (P < 0.05) between the case and control groups. However, allele G was associated with a higher risk for T2DM compared to the risk associated with allele A. The difference in the distribution frequency of s858518-rs3760213-rs1799941-rs6257 haplotype TCGC was significant (P < 0.05) between the case and control groups. The difference in the frequency of rs6259-rs727428 haplotype GA and GG was statistically significant between the case and control groups. Our results provide important clues to explore the relationship between SNPs of SHBG and glucose metabolism at a genetic level.

SHBG and liver enzyme levels are both risk factors of T2DM. SHBG affects the glucose and lipid levels and this association depends on the liver fat content, liver enzymes or sex hormone concentrations [11]. By analysis of the data from the women's health study and doctors' health study II, SHBG was confirmed to play an important role in the pathogenesis of T2DM at both genetic and phenotypic levels [12]. Studies have shown that rs1799941 SNP is located at the 5' terminal of the non-translated region (G > A) 8 nucleotides upstream of the translation initiation site in the promoter sequence [13]. Investigation on the relationship between the rs179994 genotype and the serum SHBG level suggested this position plays a crucial role in determining the SHBG splicing variants in circulating SHBG levels. However, the association between rs1799941 SNP and diabetes is unclear. A meta-analysis suggested that there is a differential effect of the rs1799941 polymorphism-related risk of T2DM among the genders [14]. Studies on Caucasian women and Middle Eastern women suggested that gene polymorphisms of rs6257, rs6259 (D356N), rs727428 and rs1799941 loci are associated with reduced SHBG levels and the occurrence of PCOS [15-19]. rs13894, rs858521 and rs9898876 are also considered to be associated with achange of SHBG level in the pathogenesis of T2DM and insulin resistance [20-22]. These specific SHBG polymorphisms are not associated with BMI, suggesting that SNPs affect the risk of T2DM independently of lipid factors.

In summary, SHBG was previously believed to be associated with the transportation of sex steroids. The elucidation of the internal structure of SHBG and the discovery of secondary messenger systems activated by unrelated SHBG indicate that SHBG is involved in additional

functions [23-25]. Epidemiological studies have revealed an association between low SHBG levels and the increased risk of T2DM. Recent genetic research suggests that this association may be causal.

Acknowledgements

This work was supported by the State Key Laboratory of Pathogenesis, Prevention, Treatment of High Incidence Diseases in Central Asia Fund, China (SKL-HIDCA-2017-Y10).

Disclosure of conflict of interest

None.

Address correspondence to: Jun Zhu, Department of Endocrinology, The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830000, China. Tel: +86 991 4362607; Fax: +86 991 4361060; E-mail: yunzhudoc@163.com

References

[1] Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U and Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 2014; 103: 137-149.

[2] Zimmet P, Alberti KG and Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001; 414: 782-787.

[3] Chiba Y, Yamakawa T, Tsuchiya H, Oba M, Suzuki D, Danno H, Takatsuka Y, Shigematsu H, Kaneshiro M and Terauchi Y. Effect of anagliptin on glycemic and lipid profile in patients with type 2 diabetes mellitus. J Clin Med Res 2018; 10: 648-656.

[4] Zhou Z, Sun B, Li X and Zhu C. DNA methylation landscapes in the pathogenesis of type 2 diabetes mellitus. Nutr Metab (Lond) 2018; 15: 47.

[5] Weil PA. The Diversity of the Endocrine System. In: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA, editors. Harper's Illustrated Biochemistry. 28th edition. New York: McGrawHill; 2009.

[6] Goldštajn MŠ, Toljan K, Grgić F, Jurković I and Baldani DP. Sex hormone binding globulin (SHBG) as a marker of clinical disorders. Coll Antropol 2016; 40: 211-218.

[7] Liu S and Sun Q. Sex differences, endogenous sex-hormone hormones, sex-hormone binding globulin, and exogenous disruptors in diabetes and related metabolic outcomes. J Diabetes 2018; 10: 428-441.

[8] Wang Q, Kangas AJ, Soininen P, Tiainen M, Tynkkynen T, Puukka K, Ruokonen A, Viikari J, Kähönen M, Lehtimäki T, Salomaa V, Perola M, Davey Smith G, Raitakari OT, Järvelin MR, Würtz P, Kettunen J and Ala-Korpela M. Sex hormone-binding globulin associations with circulating lipids and metabolites and the risk for type 2 diabetes: observational and causal effect estimates. Int J Epidemiol 2015; 44: 623-637.

[9] Muka T, Nano J, Jaspers L, Meun C, Bramer WM, Hofman A, Dehghan A, Kavousi M, Laven JS and Franco OH. Associations of steroid sex hormones and sex hormone-binding globulin with the risk of type 2 diabetes in women: a population-based cohort study and meta-analysis. Diabetes 2017; 66: 577-586.

[10] Mather KJ, Kim C, Christophi CA, Aroda VR, Knowler WC, Edelstein SE, Florez JC, Labrie F, Kahn SE, Goldberg RB and Barrett-Connor E; Diabetes Prevention Program. Steroid sex hormones, sex hormone-binding globulin, and diabetes incidence in the diabetes prevention program. J Clin Endocrinol Metab 2015; 100: 3778-3786.

[11] Bonnet F, Velayoudom Cephise FL, Gautier A, Dubois S, Massart C, Camara A, Larifla L, Balkau B and Ducluzeau PH. Role of sex steroids, intrahepatic fat and liver enzymes in the association between shbg and metabolic features. Clin Endocrinol (Oxf) 2013; 79: 517-22.

[12] Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM and Liu S. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med 2009; 361: 1152-1163.

[13] Xita N and Tsatsoulis A. Genetic variants of sex hormone-binding globulin and their biological consequences. Mol Cell Endocrinol 2010; 316: 60-65.

[14] White MJ, Eren F, Agirbasli D, Williams SM and Agirbasli M. Shbg gene polymorphism (rs1799941) associates with metabolic syndromein children and adolescents. PLoS One 2015; 10: e0116915.

[15] Martínez-García MÁ, Gambineri A, Alpañés M, Sanchón R, Pasquali R and Escobar-Morreale HF. Common variants in the sex hormone-binding globulin gene (SHBG) and polycystic ovary syndrome (PCOS) in Mediterranean women. Hum Reprod 2012; 27: 3569-3576.

[16] Pau C, Saxena R and Welt CK. Evaluating reported candidate gene associations with polycystic ovary syndrome. Fertil Steril 2013; 99: 1774-1778.

[17] Wickham EP 3rd, Ewens KG, Legro RS, Dunaif A, Nestler JE and Strauss JF 3rd. Polymorphisms in the SHBG gene influence serum SHBG levels in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2011; 96: E719-727.

[18] Ferk P, Teran N and Gersak K. The (TAAAA) n microsatellite polymorphism in the SHBG gene influences serum SHBG levels in women with polycystic ovary syndrome. Hum Reprod 2007; 22: 1031-1036.

[19] Abu-Hijleh TM, Gammoh E, Al-Busaidi AS, Malalla ZH, Madan S, Mahmood N and Almawi WY. Common variants in the sex hormone-binding globulin (SHBG) gene influence SHBG levels in women with polycystic ovary syndrome. Ann Nutr Metab 2016; 68: 66-74.

[20] Riancho JA, Valero C, Zarrabeitia MT, García-Unzueta MT, Amado JA and González-Macías J. Genetic polymorphisms are associated with serum levels of sex hormone binding globulin in postmenopausal women. BMC Med Genet 2008; 9: 112.

[21] Sunbul M, Eren F, Nacar C and Agirbasli M. Sex hormone binding globulin gene polymorphisms and metabolic syndrome in postmenopausal Turkish women. Cardiol J 2013; 20: 287-293.

[22] Thompson DJ, Healey CS, Baynes C, Kalmyrzaev B, Ahmed S, Dowsett M, Folkerd E, Luben RN, Cox D, Ballinger D, Pharoah PD, Ponder BA, Dunning AM and Easton DF; Studies in Epidemiology and Risks of Cancer Heredity Team. Identification of common variants in the SHBG gene affecting sex hormone binding globulin levels and breast cancer risk in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2008; 17: 3490-3498.

[23] Chen Z, Tao S, Gao Y, Zhang J, Hu Y, Mo L, Kim ST, Yang X, Tan A, Zhang H, Qin X, Li L, Wu Y, Zhang S, Zheng SL, Xu J, Mo Z and Sun J. Genome-wide association study of sex hormones, gonadotropins and sex hormone-binding protein in Chinese men. J Med Genet 2013; 50: 794-801.

[24] Zhou JY, Shi R, Yu HL, Zheng WL and Ma WL. Association between SHBG Asp327Asn (rs6259) polymorphism and breast cancer risk: a meta-analysis of 10, 454 cases and 13,111 controls. Mol Biol Rep 2012; 39: 8307-8314.

[25] Fang X, Wang L, Wu C, Shi H, Zhou Z, Montgomery S and Cao Y. Sex hormones, gonadotropins, and sex hormone-binding globulin in infants fed breast milk, cow milk formula, or soy formula. Sci Rep 2017; 7: 4332.