Correlation between *SHBG* gene polymorphism and male infertility in Han population of Henan province of China
A STROBE-compliant article

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Abstract

Human sex hormone binding globulin (SHBG) level alteration and *SHBG* gene mutations, especially in rs6259 and rs727428 loci, are associated with male infertility. In this study, the rs6259 and rs727428 loci in *SHBG* gene were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to explore the direct relation between these 2 loci and male infertility in Han population of Henan province and to provide information for the pathogenesis, diagnosis, and treatment of male infertility.

A total of 366 male Han individuals in Henan province were enrolled in this study. Of the 366 male individuals, 183 infertility patients were served as infertility group and other 183 normal individuals as a control group. *SHBG* gene rs6259 and rs727428 locus polymorphisms were detected by PCR-RFLP in all patients. Also, genotype frequencies, allele frequency, and haplotype were all analyzed in both groups.

There were statistical differences in A allele frequency ($P = .017$) and GA genotype frequency ($P = .016$) of *SHBG* gene rs6259 locus and in CC genotype frequency of *SHBG* gene rs727428 locus ($P = .034$) between the 2 groups.

Male infertility is associated with GA genotype and A allele of rs6259 locus, as well as CC genotype of rs727428 locus in *SHBG* gene.

Abbreviations: CI = confidence interval, GWAS = genome-wide association study, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SHBG = sex hormone binding globulin, SNP = single nucleotide polymorphism.

Keywords: gene polymorphism, male infertility, sex hormone binding globulin

1. Introduction

According to the World Health Organization (WHO), male infertility refers to the infertility of couples caused by male factors when they have lived together for more than 1 year without any contraceptive methods. In recent years, large number of studies have shown that chromosomal abnormalities, Y chromosome microdeletion, and related gene mutation all are associated with spermatogenesis impairment.[1,2] Human sex hormone binding globulin (SHBG), a kind of glycoprotein, is produced and secreted by the liver, and can bind with circulating steroid hormones. It has been reported that SHBG could not only bind with steroid hormones but also mediate steroid hormone signaling, and SHBG level alteration and *SHBG* gene mutations, especially in rs6259 and rs727428 loci, are associated with male infertility.[3] In this study, the rs6259 and rs727428 loci in *SHBG* gene were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to explore the direct relation between these 2 loci and male infertility in Han population of Henan province and to provide information for the pathogenesis, diagnosis, and treatment of male infertility.

2. Subjects and methods

All study methods were approved by the Ethics Committee of the third Affiliated Hospital of Zhengzhou University. All subjects gave written informed consent to participate in this study.

2.1. Subjects

A total of 208 male patients aged 25 to 45 years, who visited the Reproductive Center or the Clinical Laboratory of the First Affiliated Hospital of Zhengzhou University due to infertility from March 2011 to August 2011, were observed in this study. All patients were subjected to routine semen analysis and were diagnosed with azoospermia or severe oligozoospermia after exclusion of orchitis, liver and kidney diseases, endocrine diseases, chromosome abnormalities and genital diseases, etc. The final diagnosis of azoospermia or oligozoospermia was based on the consistent results of 3 continuous routine semen analyses.

The inclusion criteria were the patients with azoospermia or oligozoospermia; the infertility did not caused by surgical and endocrine factors; and the patients without Y chromosome microdeletion. Of the 208 patients, 25 had Y chromosome microdeletion (see Results section), so a total of 183 male patients were enrolled in the infertility group. The age and BMI (body mass index) in the 2 groups are summarized in Table 1. Randomly selected 183 healthy men aged 25 to 45 years, who had had child, were served as control group. All subjects in this study were Han people in Henan province.

2.2. Y chromosome microdeletion analysis

DNA was extracted from 1 mL of semen samples in all subjects, and Y chromosome microdeletion was detected by multiple PCR.[4]

2.3. SHBG gene polymorphism detection

In this study, all subjects without Y chromosome microdeletion underwent *SHBG* gene polymorphism detection. Mixture of 500 μL semen and 500 μL normal saline was centrifuged at 3000g for 10 minutes. After removal of supernatant, 200 μL TNE (100 mmol/L Tris-Hcl(pH8.0), 10 mol/L EDTA, 100 mmol/L Nacl), 40 μL of 10% Sodium dodecyl sulfate, 10 μL of 1 mol/L DTT (DL-Dithiothreitol), and 10 μL of 10 mg/mL Proteinase K were added in the remaining precipitation,[5] and then digested at 37°C overnight (more than 4 hours). Genomic DNA was extracted by conventional phenol-chloroform method. The upstream primer sequence of rs6259 was 5′-AGCCACCT-TAATGCTCTAATGC-3′ and the downstream primer sequence was 5′-CAAGAGGGTAGGA ACCAGAGTG-3′. The upstream primer sequence of rs727428 was 5′-GCAAGTGGACCAA-GACTA GGAG-3′ and the downstream primer sequence was 5′-AACCCCGACAGACAGACATACT-3′. PCR was performed in volume of 20 μL, including 1 μL of DNA, 1 μL of upstream and downstream primers, respectively, 10 μL of 2 × Taq PCR Master, and the final volume was adjusted to 20 μL using deionized water. The amplification conditions were as follows: 94°C for 3 minutes, 94°C for 30 seconds, 53°C for 30 seconds, elongation at 72°C for 30 seconds, 35 cycles; elongation at 72°C for 7 minutes. Samples were stored at 4°C. Taking 10 μL of PCR products, and then 2 μL of 10 × buffer and 10 U of restriction enzyme were added and the final volume was adjusted to 20 μL using deionized water. The rs6259 and rs727428 loci were digested using HinfI enzyme at 37°C water for 3 or 4 hours. PCR products were electrophoresed on 2.5 g/L agarose gel and then analyzed using gel image analyzer.

2.4. Genotype interpretation

The amplified fragment of rs6259 locus was 339 bp, and then 3 genotypes [G/G (52, 92, 195 bp), G/A (52, 92, 144, 195 bp), A/A (144, 195 bp)] were obtained after it was digested using HinfI. The amplified fragment of rs727428 locus was 345 bp, and then 3 genotypes [C/C (148, 197 bp), C/T (148, 197, 345 bp), T/T (345 bp)] were obtained after it was digested using HinfI.

2.5. Statistical analysis

Statistical treatment was performed using SPSS17.0 software (Chicago, IL) and SHEsis on-line software (Chicago, IL). Gene frequencies were analyzed by mathematical counting method and the size of test was set at $\alpha = 0.05$. Chi-square test was used in the comparison of genotype frequency and allele frequency between the 2 groups. The reliability of data was evaluated using Hardy–Weinberg equilibrium law. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to evaluate the disease risk contributed by gene mutation.

3. Results

3.1. Y chromosome microdeletion

Among the 208 male infertility patients, 25 patients (12.02%) had Y chromosome microdeletion in AZF region. However, no Y chromosome microdeletion was detected in the control group, showing statistical difference in Y chromosome microdeletion between the 2 groups ($P < .05$) (Table 2).

3.2. Hardy–Weinberg equilibrium test

The genotype frequencies of control group rs6259 locus ($\chi^2 = 2.644$, $P = .104$) and rs727428 locus ($\chi^2 = 0.089$, $P = .766$), as well as patient group rs6259 locus ($\chi^2 = 0.001$, $P = .982$) and rs727428 locus ($\chi^2 = 2.029$, $P = .154$), were compatible with Hardy–Weinberg equilibrium, indicating that the samples had good representativeness of the population.

3.3. The genotypes in rs6259 and rs727428 loci

These results are shown in Figs. 1 and 2. In rs6259 locus, GG genotype was 52, 92, and 195 bp, GA genotype was 52, 92, 144, and 195 bp, AA genotype was 144 and 195 bp. In rs727428 locus, CC genotype was 148 and 197 bp, CT genotype was 148, 197, and 345 bp, TT genotype was a band of 345 bp.

3.4. The distributions of genotype frequency and allele frequency in rs6259 and rs727428 loci

These results are summarized in Table 3. The frequency of A allele in 6259 locus was significantly higher in infertility group (28.7%) than in control group (21.0%) ($P = .017$, OR = 1.510, 95% CI: 1.076–2.118). CC genotype frequency in rs727428 locus was significantly lower in infertility group (9.8%) than in control group (16.9%) ($P = .034$, OR = 0.485, 95% CI: 0.248–0.951).

Table 1

Age and body mass index in the 2 groups.

	Control group	Infertility group	P
Age	29.6 ± 4.8	28.5 ± 4.7	.295
Body mass index	23.71 ± 2.90	23.99 ± 3.43	.544

Table 2

Y chromosome microdeletion in AZF region in the 2 groups.

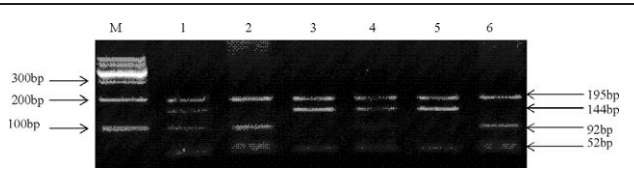| Grouping	Microdeletion	No microdeletion	Total	Microdeletion rate (%)	P
Infertility group	25	183	208	12.02	<.05
Control group	0	183	183	0	
Total	25	366	391		

Figure 1. Electrophoregram of rs6259 locus in *SHBG* gene in a patient from infertility group. Notes: Lanes 1 and 4: GA genotype; Lanes 2 and 6: GG genotype; Lanes 3 and 5: AA genotype; M: DNA Makerl.
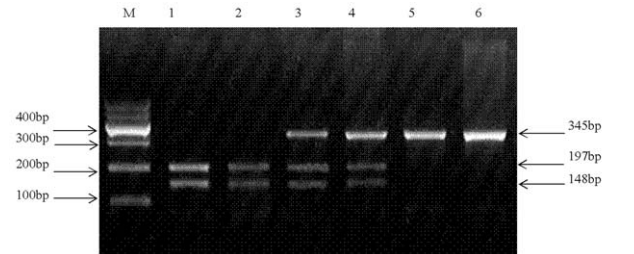

Figure 2. Electrophoregram of rs727428 locus in *SHBG* gene in a patient from infertility group. Notes: Lanes 1 and 2: CC genotype; Lanes 3 and 4: CT genotype; Lanes 5 and 6: TT genotype; M: DNA Makerl.

3.5. The genotype distributions of rs6259 and rs727428 loci under different genetic modes

These results are summarized in Tables 4 and 5. In 6259 locus, GA genotype was associated with male infertility under dominant mode and overlap mode, but it was not related to male infertility under recessive mode. In 727428 locus, GA genotype was associated with male infertility under recessive mode, but it was not related to male infertility under dominant mode and overlap mode.

3.6. Haplotype analysis of rs6259 and rs727428 loci

Pair-wise linkage disequilibrium test performed using SHEsis software showed no linkage disequilibrium between rs6259 and rs727428 loci (D'=1.00, r^2=0.211).

Haplotype analysis of *SHBG* rs6259A/G and rs727428C/T loci was further performed using SHEsis software and the haplotypes with a frequency <0.03 were ignored. The frequency of A-T haplotype was significantly higher in the infertility group than in the control group (P=.000). Risk analysis revealed that A-T was a risk factor (OR=2.024, 95% CI: 1.367–2.997) (Table 6).

4. Discussion

Approximately 15% couples at child-bearing age suffer from subfertility worldwide, and 50% of subfertility may be attributed to male factors.[6] The main cause of male infertility is spermatogenesis impairment. A large number of recent studies have shown that chromosome abnormality, Y chromosome deletion, and related gene mutation are associated with spermatogenesis impairment. By analyzing 250,000 single nucleotide polymorphism (SNP) sites using genome-wide association study (GWAS) method, Kosova et al[7] found 9 SNP sites that are closely associated with male infertility.

Human SHBG, a kind of glycoprotein, is produced and secreted by the liver, and can bind with circulating steroid hormones. *SHBG* gene is also expressed in other tissues, such as ovary, testis, endometrium, etc. It has been reported that SHBG could not only bind with steroid hormones but also mediate steroid hormone signaling, and SHBG level alteration and *SHBG* gene mutations are associated with male infertility.[3]

SHBG gene is located in 17p12–p13 region and encodes a polypeptide containing 402 amino acid residues. The serum SHBG concentration shows significant variation among individuals, thus influencing sex hormone behaviors. This gene harbors 11 SNP sites, including 7 SNPs and microsatellite sequences in genomic region. *SHBG* gene polymorphism could result in individual SHBG concentration alteration by changing protein products and metabolites. Safarinejad et al[3] found that in Iran population, the mutation in rs6259 locus resulted in the addition of an extra sugar chain, which then decreased the metabolic clearance rate of SHBG and prolonged SHBG half-life with the elevation of SHBG level, and which also changed the biological activities of androgen and estrogen by influencing their utilization and behaviors, further leading to male infertility. In a study on the

Table 3

Genotype frequencies and allele frequencies of rs6259 and rs727428 loci in *SHBG* gene in the 2 groups.

Items	Infertility group (n=183, %)	Control group (n=183, %)	P	OR (95% CI)
rs6259				
Genotype frequency				
GG	93 (50.8)	117 (63.9)		1.00
GA	75 (41.0)	55 (30.1)	.016[*]	1.716 (1.103–2.669)
AA	15 (8.2)	11 (6.0)	.196	1.716 (0.752–3.912)
Allele frequency				
G	261 (71.3)	289 (79.0)		1.00
A	105 (28.7)	77 (21.0)	.017[*]	1.510 (1.076–2.118)
Rs727428				
Genotype frequency				
TT	73 (39.9)	61 (33.4)		1.00
TC	92 (50.3)	91 (49.7)	.459	0.845 (0.540–1.321)
CC	18 (9.8)	31 (16.9)	.034[*]	0.485 (0.248–0.951)
Allele frequency				
T	238 (65.0)	213 (58.2)		1.00
C	128 (35.0)	153 (41.8)	.057	0.749 (0.555–1.009)

CI = confidence interval, OR = odds ratio.

[*] P<.05 between the 2 groups.

Genotype distribution of rs6259 locus under different genetic modes in the 2 groups.

Genetic modes	Infertility group/ Control group	P	OR (95% CI)
Dominant mode			
GG	93/117		1.00
GA+AA	90/66	.011*	1.716 (1.129~2.607)
Overlap mode			
GG	93/117		1.00
GA	75/55	.016*	1.716 (1.103~2.669)
AA	15/11	.196	1.716 (0.752~3.912)
Recessive mode			
GG+GA	168/172		1.00
AA	15/11	.416	0.716 (0.320~1.605)
AA	15/11	.416	0.716 (0.320~1.605)

CI = confidence interval, OR = odds ratio.
* P < .05 between the 2 groups.

relation between *SHBG* gene and breast cancer in European population, Wickham et al[8] found that rs727428 locus was located in 1.1 kb away from the 3′ end of *SHBG* gene and showed strong linkage disequilibrium with rs858518 locus, which was regarded as the most important single SNP with 4.2% mutation rate, and the mutant allele was associated with decreased SHBG level. The rs6259 locus can increase SHBG level, counteracting the effect of rs727428 locus in decreasing SHBG level.

In this study, SNP of rs6259 and rs727428 loci in *SHBG* gene was analyzed by PCR-RFLP in 183 patients with male infertility that was not caused by surgical and endocrine factors, and in 183 normal men. For the rs6259 locus, the GA genotype frequency was significantly higher in the infertility group (41.0%) than in the control group (30.1%) (P = .016), and the A allele frequency was also significantly higher in infertility patient group (28.7%) than in the control group (21.0%) (P = .017). The relative risk analysis indicated that the male infertility risk increased in the men carrying GA genotype [OR = 1.716, 95% CI (1.103–2.669)] and A allele [OR = 1.510, 95% CI (1.076–2.118)], which is consistent with the study performed by Safarinejad et al.[9] Above-mentioned results suggest that rs6259 locus in *SHBG* gene is closely associated with male infertility, and the A allele in

Genotype distribution of rs727428 locus under different genetic modes in the 2 groups.

Genetic modes	Infertility group/ Control group	P	OR (95% CI)
Dominant mode			
TT	73/61		1.00
TC+CC	110/122	.193	1.327 (0.866~2.033)
Overlap mode			
TT	73/61		1.00
TC	92/91	.459	0.845 (0.540~1.321)
CC	18/31	.034*	0.485 (0.248~0.951)
Recessive mode			
TT+TC	165/152		1.00
CC	18/31	.046*	0.535 (0.278~0.996)

CI = confidence interval, OR = odds ratio.
* P < .05 between the 2 groups.

Haplotype analysis of rs6259A/G and rs727428C/T loci in *SHBG* gene in the 2 groups.

Haplotype	Infertility group (n = 183)	Control group (n = 183)	P	OR (95% CI)
A-C	22.00 (0.059)	31.00 (0.086)	.160	0.667 (0.378~1.177)
A-T	83.00 (0.228)	47.00 (0.127)	.000*	2.024 (1.367~2.997)
G-C	106.00 (0.291)	122.00 (0.332)	.227	0.824 (0.603~1.128)
G-T	155.00 (0.422)	166.00 (0.455)	.378	0.877 (0.655~1.174)

CI = confidence interval, OR = odds ratio.
* P < .05 between the 2 groups.

rs6259 locus may be a genetic risk factor of male infertility in Han population of Henan province.

Wickham et al[8] studied the relation between rs727428 locus in *SHBG* gene and polycystic ovary syndrome in American female population, and found that this locus was not associated with polycystic ovary syndrome, but was related to SHBG level. Thompson et al[10] found that rs727428 locus in *SHBG* gene was not associated with breast cancer risk, but it could decrease SHBG level in European postmenopausal women. In this study on the association of rs727428 locus with male infertility, we found that the TT, TC genotype frequencies, and allele frequencies showed no significant difference between the infertility group and the control group. However, the CC genotype frequency exhibited significant difference between the infertility group and the control group (P = .034), suggesting that CC genotype may be a protective factor of male infertility in Han population of Henan province [OR = 0.485, 95% CI (0.248–0.951)].

By genetic mode analysis of the 2 loci, rs6259 locus showed association with male infertility under dominant mode and its GA genotype was also related to male infertility under overlap mode, while under recessive model, rs6259 locus was not associated with male infertility. The rs727428 locus was associated with male infertility under recessive model, but it was not in association with male infertility under overlap or dominant models.

Both the rs6259 and rs727428 loci are located at the tail of *SHBG* gene. Their haplotypes may influence SHBG expression level by interactions with each other, so they are associated with male infertility. Here, we analyzed the haplotypes of *SHBG* rs6259A/G and rs727428C/T. Results showed that G-T haplotype was the most common type, followed by G-C haplotype, A-T haplotype, and A-C haplotype in the 2 groups. The frequency of A-T haplotype was statistically higher in the infertility group than in the control group. A-T haplotype may be a genetic risk factor of male infertility in Han population of Henan province [P = .000, OR = 2.024, 95% CI (1.367–2.997)].

In this study, there are some limitations because the subjects of this study are from a specific population. More studies from various aspects will be needed to further confirm our results and reveal the biological effects of genetic variation of *SHBG* gene on male infertility.

5. Conclusion

Our experimental data indicate that rs6259 and rs727428 loci in *SHBG* gene are related to male infertility. They are genetic risk factor and protective factor, respectively.

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