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POLYMORPHISM OF THE GENE *SHBG* rs727428 ASSOCIATED WITH MALE INFERTILITY IN A VIETNAMESE POPULATION

Thanh Thuy Nguyen[1], Dang Thuy Linh Nguyen[1] and Thuy Duong Nguyen[1,✉]

[1]*Institute of Biology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam*

✉To whom correspondence should be addressed. Email: tdnguyen@ib.ac.vn

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ABSTRACT

Infertility is a complex disease characterized by the incapability to achieve pregnancy after 24 months of regular unprotected sexual intercourse. Various studies have highlighted the role of sex hormone-related genes in contributing to the risk of male infertility. However, research on sex hormone-binding globulin (*SHBG*) – a glycoprotein involved in transporting sex hormones to target tissues, remains limited. Therefore, this study investigated whether the variant *SHBG* rs727428 is associated with idiopathic male infertility in Vietnam. A total of 287 DNA samples were extracted from the peripheral blood samples of 123 idiopathic infertile male patients and 164 healthy men with at least one biological child without seeking assisted reproductive technology (ART). The polymorphism *SHBG* rs727428 was genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. The results showed that the frequencies of the minor T allele of this polymorphism in the case, control, and total study populations were 0.33, 0.424, and 0.385, respectively. Using statistical methods, we showed that the genotype frequencies conformed to Hardy–Weinberg equilibrium (p-value > 0.05). In addition, an association between *SHBG* rs727428 and male infertility was identified in two models: the additive model (OR = 0.428; 95% CI 0.197-0.885; p-value = 0.021) and the dominant model (OR = 0.473; 95% CI 0.229-0.928; p-value = 0.029). Our results further suggested that the T allele reduced the risk of male infertility (OR = 0.680; 95% CI: 0.481-0.95; p-value = 0.028). This study enhances our understanding of the role of genetic factors in male infertility among the Vietnamese population.

Keywords: Male infertility, PCR-RFLP, rs727428, *SHBG,* Vietnam.

INTRODUCTION

Infertility is a multifaceted complication featuring the incapability to conceive pregnancy after 24 months of consistent unprotected sexual intercourse, arising from impairments within the reproductive system of either a male or female partner or both (Zegers-Hochschild *et al*., 2017). Infertility has impacted over 48 million couples globally. Male infertility constitutes half of cases of infertility and contributes to 7% of the worldwide population's infertility rates (Krausz *et al.*, 2015). It is estimated that 15%

of male infertility cases are related to genetic abnormalities, such as chromosome aberrations, loss of AZF (azoospermia factor) on the Y chromosome, and point mutation (Krausz & Riera-Escamilla, 2018). Numerous genes involved in the production and regulation of male sex hormones, including the follicle-stimulating hormone beta gene (*FSHB*) and its receptor (*FSHR*) (Hekim *et al.*, 2022; Q. Wu *et al.*, 2017), as well as the estrogen receptor α gene (*ESR1*) (Ge *et al.*, 2014; Kukuvitis *et al.*, 2002; Quy Vu *et al.*, 2023; Safarinejad *et al.*, 2010), have been linked to male infertility. Among these, the sex hormone binding globulin (*SHBG*) protein, which binds to and regulates testosterone levels in the plasma, stands out as a promising candidate for studying the association between male infertility and sex hormone-related genes.

The *SHBG* gene, located on the short arm of chromosome 17, consists of eight coding exons and encodes human sex-hormone binding globulin of 402 amino acids (SHBG) (T.-S. Wu & Hammond, 2014). While SHBG is primarily synthesized and secreted into the bloodstream by hepatocytes in the liver, it is also expressed at low levels in other tissues, such as the testis, brain, and endometrium (Hammond, 2011). In blood plasma, SHBG binds specifically to androgens and estrogens, selectively transporting these sex hormones (Hammond, 1990). As a hormone carrier, SHBG influences the metabolic clearance, biological activity, and effects of sex hormones on target tissues, including the reproductive system, bone, and muscle tissue. Furthermore, testosterone, the predominant androgen hormone in males, plays a vital role in the maintenance of normal spermatogenesis in the male reproductive system (Safarinejad *et al.*, 2011). Thus, the binding of SHBG to testosterone can modulate the bioactive testosterone levels and reduce the biological effects of sex hormones on reproductive organs, potentially impacting the normal function of the reproductive system (Laurent *et al.*, 2016). Previous studies have shown that significant variations in serum SHBG concentration among individuals can impact the actions of sex hormones (An *et al.*, 2000; Safarinejad *et al.*, 2011). Additionally, genetic influences on SHBG levels have been extensively studied, leading to the identification of genetic polymorphisms in the *SHBG* gene. Various *SHBG* variants, such as *SHBG* rs6259 and rs35785886, have been linked to idiopathic male infertility and changes in SHBG serum levels. Another variant, *SHBG* rs727428, located in 1.1 kbp downstream of the 3' end of the *SHBG* gene, has been identified (Cui *et al.*, 2017). However, research on the association between this *SHBG* variant and idiopathic infertility remains limited.

In this study, we investigated the association between *SHBG* rs727428 and male infertility in the Vietnamese population. Our study is the first to examine *SHBG* rs727428 in the Vietnamese population, contributing to the broader understanding of male infertility.

MATERIALS AND METHODS

Study subjects and sample collection

The study consisted of 287 peripheral blood samples obtained from male participants (including 123 infertile patients and 164 healthy men having at least one child without intervention of assisted reproductive technology). Selected infertile subjects were diagnosed with non-obstructive azoospermia and oligospermia (lower than 15 million sperm/mL of semen).

Furthermore, infertile patients having a chromosomal abnormality, AZF deletion, and reproductive disease (orchitis due to sexually transmitted diseases, testicular tumor, and genital trauma) were excluded from this study. All study subjects volunteered and their consent forms were given. The research was approved by the Human Research Ethics Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology (No: 9-2019/NCHG-HĐĐĐ). Two (2) mL of blood samples from all participants were collected in EDTA tubes and preserved at -80°C.

SNP genotyping

Whole genomic DNA was isolated from 287 blood samples using the Exgene Whole Blood DNA Extraction Kit (GeneAll, Korea), following the manufacturer's protocol. The quantity of extracted genomic DNA was measured by spectrophotometer, and electrophoresis of total DNA samples on 1% agarose gel was performed for quality control. All qualified DNA samples were then diluted to a final concentration of about 2.5 ng/µL and stored at -20°C.

The total genomic DNA of 287 samples was used to amplify the fragment containing the single nucleotide polymorphism *SHBG* rs727428 by polymerase chain reaction (PCR) with a specific pair of primers (Table 1). After that, to identify the genotypes of *SHBG* rs727428, PCR products of 287 samples were digested with the restriction enzyme *Hin*fI (Thermo Fisher) at 37°C in a water bath for 3 hours. The digested products were then viewed on 2.5% agarose electrophoresis gel, in which genotypes were defined based on the number of DNA bands and band size (Table 1).

Table 1. Primers used for polymerase chain reaction-restriction fragment length polymorphism.

SNP/Gene	Primer sequence (5'-3')	PCR product (bp)	PCR-RFLP	
			Genotype	Fragment size (bp)
rs727428/*SHBG*	F: GCAAAACCCCGACAGACAG R: GCAGCAAGTGGACCAAGACT	353	TT	353
			CT	150; 203; 353
			CC	150; 203

Note: SNP: Single nucleotide polymorphism

Data analysis

The genotyping results collected from the PCR-RFLP method were analyzed using R programming software version 4.2.3 (https://www.r-project.org) and Microsoft Excel (Microsoft Corp., Washington, DC, USA). Hardy-Weinberg equilibrium (HWE) of the population was examined by using the Chi-square test ($\chi 2$) (Graffelman, 2015). The assessment of the association between genotypes of *SHBG* rs727428 polymorphism and male infertility was carried out in five models: additive, dominant, recessive, co-dominant, and allele using the R package "epitools" (Aragon, 2004). The 95% Confident Interval (95%CI) and odds ratio (OR) were calculated to estimate the susceptibility of having male infertility in the presence of this

polymorphism, using the function "oddsratio" in the package. The estimation was considered to be statistically significant if the *p*-value < 0.05.

RESULTS

Genotyping distribution

The targeted DNA region containing *SHBG* rs727428 was amplified by PCR reaction with a specific pair of primers. The PCR products were then digested with the restriction enzyme *Hin*fI. Analysis on a 2.5% agarose gel revealed the following: sample 1 has one band (353 bp) corresponding with a homozygous genotype (TT); samples 2 and 3 have two bands (150 bp and 203 bp) indicating a wild-type genotype (CC); and samples 4 and 5 have three bands (150 bp, 203 bp, and 353 bp) corresponding with a heterozygous genotype (CT) (Figure 1).

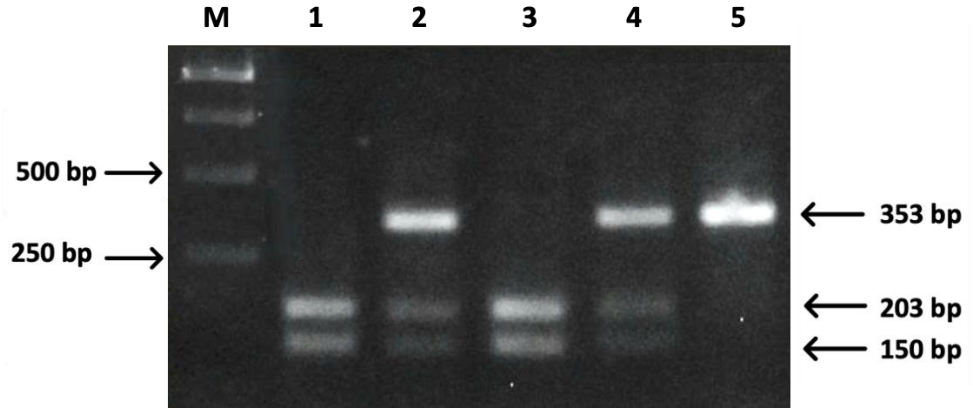

Figure 1. *Hin*fI-digested PCR products of *SHBG* rs727428 on a 2.5 % agarose gel. M: Marker 1 kbp (Thermo Fisher); 1, 3: Wild-type CC (2 bands of 203 bp and 150 bp); 2, 4: Heterozygous CT (3 bands of 353 bp, 203 bp, and 150 bp) ;5: Homozygous TT (1 band of 353 bp).

Genotype distribution and allele frequencies of the polymorphism *SHBG* rs727428 were summarized in Table 2. The Chi-squared test results revealed that the allele distribution of *SHBG* rs727428 followed the Hardy-Weinberg equilibrium in all groups of the study population with a *p*-value > 0.05 (Table 2). Furthermore, the frequencies of the minor T allele in the case, control group, and whole study population were 0.33, 0.424, and 0.385, respectively. A similar pattern was also observed in the major C allele (with 0.667, 0.576, and 0.615, respectively).

Association analysis

Four genetic models (additive, dominant, co-dominant and recessive) were statistically analyzed to understand the association between *SHBG* rs727428 and male infertility (Table 3). The results showed a significant association in additive and dominant and allele models, with *p*-values smaller than 0.05. This study revealed that the TT genotype had a protective effect against male infertility in both the additive model (OR = 0.428; *p*-value = 0.021) and the dominant one (OR = 0.473; *p*-value = 0.029). Moreover, the allele model also indicated that the T allele reduced the risk of male infertility (OR = 0.680; 95% CI: 0.481-0.959; *p*-value = 0.028). There is no association between the polymorphism and male infertility found in the recessive and co-dominant models (*p*-value > 0.05).

Table 2. Allele frequencies of polymorphism *SHBG* rs727428.

	Genotype			Allele frequency		HWE (*p*-value)
	CC	CT	TT	C	T	
Case (n = 123)	54 (55)	56 (55)	13 (13)	0.667	0.333	0.77
Control (n = 164)	58 (54)	73 (80)	33 (30)	0.576	0.424	0.26
Total (n = 287)	112 (109)	129 (137)	46 (41)	0.615	0.385	0.39

Note: n: Number of participants; HWE: Hardy-Weinberg equilibrium; genotypes were presented as observed values (expected values).

Table 3. Association of *SHBG* rs727428 with male infertility.

Gene/SNP	Model	Control (n = 164)	Case (n = 123)	OR	95% CI	*p*-value
SHBG rs727428	Additive					
	CC	58 (35.4%)	54 (43.9%)	1.000		
	CT	73 (44.5%)	56 (45.5%)	0.825	0.494 - 1.373	0.455
	TT	33 (20.1%)	13 (10.6%)	0.428	0.197 - 0.885	0.021
	Dominant					
	CC+CT	131 (79.7%)	110 (89.4%)	1.000		
	TT	33 (20.1%)	13 (10.6%)	0.473	0.229 - 0.928	0.029
	Recessive					
	CC	58 (35.4%)	54 (43.9%)	1.000		
	CT+TT	106 (64.6%)	69 (56.1%)	0.700	0.433 - 1.132	0.142
	Co-dominant					
	CC+TT	91 (55.5%)	67 (54.5%)	1.000		
	CT	73 (44.5%)	56 (45.5%)	1.042	0.650 - 1.669	0.864
	Allele					
	C	189 (57.6%)	164 (66.7%)	1.000		
	T	139 (42.4%)	82 (33.3%)	0.680	0.481 - 0.959	0.028

Note: n: Number; SNP: Single nucleotide polymorphism; OR: Odds ratio; 95% CI: 95% confidence intervals; p-value was measured using the Chi-squared test.

DISCUSSION

Human SHBG is a glycoprotein that is produced and secreted by the liver, and it binds to circulating steroid hormones (Hammond, 2011). In blood plasma, SHBG specifically attaches to androgens and estrogens, selectively transporting these sex hormones. As a carrier, SHBG impacts the metabolic clearance, biological activity, and effects of sex hormones on target tissues, including the reproductive system, bone, and

muscle tissue (Hammond, 1990). Multiple studies have indicated that SHBG concentrations are linked to male infertility, mainly by influencing sperm count and semen quality (Eriksson *et al.*, 2006; Wan *et al.*, 2021; Wickham *et al.*, 2011; Xita & Tsatsoulis, 2010). A study conducted by Chinese scientists found that interference with the *SHBG* gene in mice significantly downregulated SHBG expression in the testes, reduced sperm count, and lowered in vitro fertilization rates (Wan *et al.*, 2021). This demonstrates that *SHBG* is closely related to sperm synthesis and fertilization ability, and abnormal *SHBG* expression can lead to increased aggregation of primary spermatocytes and abnormal cells. Research on humans has also shown that infertile men have lower plasma SHBG levels and sperm counts compared to healthy men (Safarinejad *et al.*, 2011). Furthermore, SHBG levels are significantly correlated with sperm count and motility, indicating that *SHBG* influences male fertility by affecting these factors (Selva *et al.*, 2005). Consequently, low SHBG levels result in decreased sperm count and mass, increasing the risk of male infertility. Various *SHBG* variants, such as *SHBG* rs6259 and rs35785886, have been linked to idiopathic male infertility and changes in SHBG serum levels (Eriksson *et al.*, 2006; Safarinejad *et al.*, 2011; Turk *et al.*, 2008; Vanbillemont *et al.*, 2009), suggesting that these variants could contribute to anomalies in spermatogenesis.

SHBG rs727428, located 1121 bp away from the 3' end of the *SHBG* gene, was previously reported to be associated with a decrease of SHBG level (Cui *et al.*, 2017; Wickham *et al.*, 2011). Prior to our study, Cui *et al.* (2017) investigated the relationship between the *SHBG* gene variant rs727428 and male infertility, finding that this locus was associated with the condition (Cui *et al.*, 2017). A previous study involving 366 samples (183 infertile patients and 183 controls) of Han origin indicated that the wild-type CC genotype could potentially act as a protective factor against male infertility in the Han population of Henan province in the additive and recessive models (additive: OR = 0.485; 95% CI: 0.248-0.951; *p*-value = 0.034; recessive: OR = 0.535; 95% CI: 0.278-0.996; *p*-value = 0.046) (Cui *et al.*, 2017). Besides, our study found an association between *SHBG* rs727428 and male infertility in three genetic models: additive, dominant, and allele, with *p*-values less than 0.05 (0.021; 0.029; and 0.028, respectively). However, the OR values suggested that the alternative T allele and TT genotype lower the risk of male infertility (additive: OR = 0.428; 95% CI: 0.197-0.885; *p*-value = 0.021; dominant: OR = 0.473; 95% CI: 0.229-0.928; *p*-value = 0.029; allele: OR = 0.680; 95% CI: 0.481-0.959; *p*-value = 0.028). The inconsistency in results suggests that factors such as sample collection methods and environmental influences like lifestyle, ethnic diversity, and sample size might impact the outcomes. Therefore, studies should be replicated in other populations to clearly define the association of *SHBG* rs727428 with male infertility. Future research should also include larger case and control groups and comprehensive clinical data, such as semen parameters and hormone levels. Additionally, exploring other polymorphisms within the *SHBG* gene could reveal potential haplotypes associated with male infertility.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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