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THE ASSOCIATION OF *TEX15* HAPLOTYPE WITH MALE INFERTILITY IN VIETNAMESE INDIVIDUALS

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SUMMARY

Infertility is a global concern that affects 15% of couples, and roughly half of those cases are male-specific. Among the genetic factors that contributed heavily to male infertility, *TEX15* (testis-expressed gene 15) has been studied across multiple cohorts worldwide and identified to relate to meiotic recombination failure and DNA repair system malfunction. To assess the relationship between male infertility and *TEX15* in a Vietnamese cohort, we performed a case-control association study of polymorphism *TEX15* rs323345 and a further analysis of haplotypes of *TEX15* rs323345 and *TEX15* rs142485241. A total of 420 unrelated Vietnamese males, including 212 infertile patients and 208 healthy controls, were recruited for the present study. The genotype and allele frequencies of the polymorphism *TEX15* rs323345 were determined by PCR-RFLP method. The results showed that the distribution of genotypes of this polymorphism followed Hardy-Weinberg equilibrium (p-value > 0.05), but the association between the polymorphism *TEX15* rs323345 and male infertility was not significantly different in all three models (additive, dominant, and recessive) (p-values > 0.05). However, haplotype analysis revealed that haplotype GT of the two variants (rs323345 and rs142485241) of the *TEX15* gene was correlated with an increased risk of male infertility (p = 0.023, OR = 1.937, 95% CI = 1.085-3.456). This study demonstrated that haplotype analysis could unveil potential associations in genes that could normally be unnoticed in an individual SNP analysis.

Keywords: Male infertility, PCR-RFLP, rs323345, *TEX15*, Vietnam.

INTRODUCTION

It is reported that 15% of couples having unprotected intercourse are affected by infertility (male and/or female) (Agarwal *et al.*, 2015). Although the prevalence of male-related sterility is difficult to gauge correctly, roughly half of those cases are attributed to male factors (Agarwal *et al.*, 2015; Boroujeni *et al.*, 2018). There are various factors in the spermatogenetic failure including genetic factors of 4000 genes (Ruan *et al.*, 2012). Numerous chromosomal abnormalities, Y-chromosome micro-deletions, and single nucleotide polymorphisms (SNPs) have been associated with male infertility risk (Ghadirkhomi *et al.*, 2022). Genetic polymorphism is also associated with increased susceptibility to non-obstructive azoospermia (NOA) and oligozoospermia. Therefore, studies on genetic polymorphism can be used to gain insight into the etiology of male infertility (Tüttelmann *et al.*, 2007).

TEX15 (testis-expressed gene 15, MIM*605795) encodes a 3176 amino acid protein in humans and is located in chromosome 8p12 (Yang *et al.*, 2008). *TEX15* is expressed in spermatogonia and early spermatocytes and is downregulated in pachytene spermatocytes (Colombo *et al.*, 2017). In *Tex15*-absent mice, males experienced significantly reduced testis size and a lack of germ cells, whereas females were unaffected and stayed fertile (Yang *et al.*, 2008). During spermatogenesis, TEX15 is required for normal chromosome synapsis and meiotic recombination in germ cells (Yang *et al.*, 2008). Thus the absence of such protein can lead to meiotic arrest (Yang *et al.*, 2008).

TEX15 rs323345, a missense variant p.N1694S, is a studied SNP across multiple populations that have been linked with inconsistent results. This SNP is predicted to have a deleterious effect using *in silico* prediction tools, including PPH2-var, PPH2-div, SIFT, and PROVEAN, indicating a promising candidate for an association study. To contribute to the knowledge of the effect of *TEX15* variants on male infertility, we conducted a case-control association study of polymorphism *TEX15* rs323345 (NC_000008.11:g.30845086T>C) in Vietnamese individuals and further analysis of haplotypes of SNPs *TEX15* rs323345 and *TEX15* rs142485241 (unpublished data) in a Vietnamese cohort. To the best of our knowledge, this is the first study of *TEX15* rs323345 in a Vietnamese population.

MATERIALS AND METHODS

Study participants and collection of blood samples

A total of 420 Vietnamese individuals, including 212 infertile patients and 208 healthy controls, were recruited. The infertile group involved idiopathic NOA and oligospermia (< 15 million sperms/mL) in men from several hospitals in northern Vietnam. Patients with azoospermia factor (AZF) region disorders, abnormal karyotype, and medical history of fertility-affecting diseases such as mumps and sexually transmitted diseases were excluded from the study. The control group included 208 healthy men with at least one child naturally. All participants that met the requirements above gave informed consent for the blood collection. The study was approved by the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology. Blood samples (2 mL) were collected from the study subjects in EDTA-coated tubes and stored at -20^{o}C.

SNP genotyping

Genomic DNA was extracted from whole blood samples of participants using Gene JET Whole Blood Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). DNA quality was assessed by measuring genomic DNA using both electrophoresis and spectrophotometry. DNA samples were then diluted to the final concentration (~2.5 ng/μL) and stored at -20^{o}C. Next, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was employed to genotype the polymorphism *TEX15* rs323345 using specific pairs of primers (Table 1). The primers were designed by Primer blast and checked for dimerization on the IDT website (https://www.idtdna.com/pages). After that, the PCR products were digested with the restriction enzyme *Psp*1406I to identify the genotypes of *TEX15* rs323345 (Table 1).

Statistical analysis

Data were statistically analyzed using Microsoft Excel (Microsoft Corp., USA) and R version 4.1.2 (R Core Team, 2020). Hardy-Weinberg equilibrium (HWE) of the population was calculated using the Chi-square test ($\chi 2$) of package "Hardy Weinberg" (Graffelman, 2015). The correlation between polymorphisms and male infertility was assessed using package "epitools" (Aragon, 2020) under three test models: additive, dominant, and recessive. Haplotype analysis was performed using SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) (Shi *et al.*, 2005). An odds ratio with a confidence interval of 95% was calculated to estimate the association. All the statistical tests were two-sided. The estimation was considered to be statistically significant if the p-value < 0.05.

Table 1. List of primers used for PCR-RFLP.

Primer sequence	PCR product length (bp)	PCR-RFLP	
		Genotype	Fragment (bp)
F: 5'-TAAGGAAGTTTCCTGTAATAACG-3' R: 5'-GTAATTCTGTATCTTTAAGTTGC-3'	261	CC	261
		CT	261, 238, 23
		TT	238, 23

RESULTS

Genetic analysis of *TEX15* rs323345 polymorphism

The desired DNA region containing *TEX15* rs323345 was amplified using the specific primers. Electrophoresis on agarose gel 1% showed specific, sharp, and bright DNA bands with the appropriate molecular weight (data not shown). After that, PCR products were digested with *Psp*1406I to determine the genotypes of *TEX15* rs323345 (Figure 1). The band of 23 bp could not be seen on the agarose gel due to its small molecular weight.

A total of 420 study subjects, including 212 cases and 208 controls, were genotyped for the polymorphism *TEX15* rs323345. The minor allele frequencies (MAF) in the case, control, and the overall population were 0.108, 0.100, and 0.104, respectively (Table 2).

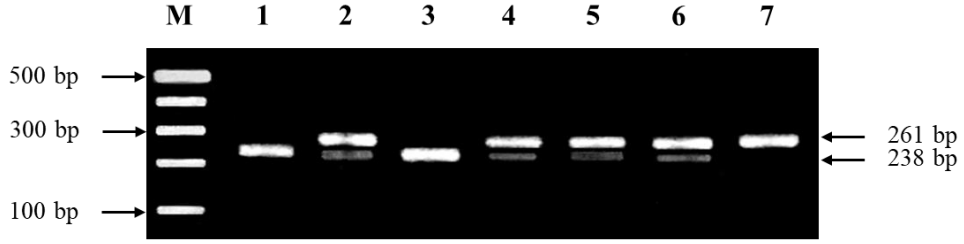

Figure 1. Restriction enzyme-digested PCR products on agarose gel 3%. M: Marker 100 bp. 1, 3: Wildtype TT; 2, 4, 5, 6: Heterozygous TC; 7: Homozygous CC.

Table 2. General information on *TEX15* rs323345.

Alleles	MAF case	HWE case	MAF control	HWE control	MAF whole population	HWE whole population
T>C	0.108	0.711	0.100	0.703	0.104	0.478

Note: HWE: Hardy-Weinberg equilibrium; MAF: Minor allele frequency.

Association of *TEX15* rs323345 with male infertility

Statistical analysis was performed in three test models: additive, dominant, and recessive, to identify the association of the polymorphism rs323345 with male infertility (Table 3). The p-values obtained from analysis of the correlation between the identified genotypes with male infertility in three models (additive, dominant, recessive) and alleles were higher than 0.05, indicating no significant difference between the patient group and the control group. In conclusion, genotypes (TT/TC/CC) and alleles (T/C) of *TEX15* rs323345 were not correlated with

male infertility in the studied population in all test models (p-values > 0.05).

TEX15 haplotypes and risk of male infertility

Combining with the result of our polymorphism *TEX15* rs142485241 (unpublished data), the association of the haplotypes of two variants, rs323345 and rs142485241, with male infertility was analyzed (Table 4). The haplotype GT exhibited significantly increased risk of male infertility (p = 0.023, OR = 1.937, 95% CI = 1.085-3.456).

Table 3. Association of *TEX15* rs323345 with male infertility.

Test model	Case (n = 212)	Control (n = 208)	OR	95% CI	p-value
Additive					0.5887
CC	3 (1.41%)	1 (0.4%)	1.000		
CT	39 (18.39%)	41 (19.71%)	2.871	0.318-84.911	0.305
TT	170 (80.2%)	166 (80.25%)	2.684	0.308-77.559	0.331
Dominant					
CC + CT	42 (19.81%)	42 (20.19%)	1.000		
TT	170 (80.19%)	166 (79.81%)	0.976	0.603-1.579	0.922
Recessive					
TT + TC	209 (98.59%)	207 (99.6%)	2.723	0.314-78.582	0.324
CC	3 (1.41%)	1 (0.4%)	1.000		
Allele					
C	190 (89.21%)	187 (89.48%)	1.000		
T	23 (10.79%)	22 (10.52%)	0.972	0.519-1.815	0.927

Note: n: Number of participants; OR: Odds ratio; 95% CI: 95% confidence interval of odds ratio; p-value measured by Chi-square test.

Table 4. Haplotype analysis of *TEX15* rs323345 and *TEX15* rs142485241.

Haplotype	Frequency		p-value	OR	95% CI
	Case n (%)	Control n (%)			
CC*	35 (8.6)	34 (8.5)	0.898	1.033	0.630-1.693
CT*	330 (81.3)	343 (86.7)	0.093	0.718	0.487-1.058
GT*	35 (8.6)	19 (4.7)	0.023	1.937	1.085-3.456
GC	6 (1.5)	1 (0.25)	UA	UA	UA

Note: n: Number of participants; OR: Odds ratio; 95% CI: 95% confidence interval of odds ratio; p-value measured by Pearson test; *: Haplotypes could be compared. UA: unattainable.

DISCUSSION

The synaptonemal complex (SC) is a large protein structure needed for synapsis, and the incorrect assemblage of this complex resulted in maturation arrest and infertility (Page *et al.*, 2003). Previous studies revealed that the *Tex* genes are needed in chromosomal synapsis and meiotic recombination (Adelman *et al.*, 2008; Yang *et al.*, 2008; Boroujeni *et al.*, 2018). More specifically, *TEX15* (*Tex15*'s human ortholog) is abundantly expressed in various stages of

spermatogenesis, indicating its vital role in that process (Wang *et al.*, 2005; Yang *et al.*, 2008). Variants of the *TEX15* gene, including rs323342, rs323344, rs323345, rs323346, rs323347, rs142485241, and rs12114073, have been reported in multiple populations worldwide (Aston *et al.*, 2010; Plaseski *et al.*, 2012; Ruan *et al.*, 2012). Among these variants, *TEX15* rs323345 was studied to investigate its association with the risk of male infertility in three different cohorts, indicating conflicting results. This polymorphism was associated with non-obstructive azoospermia (NOA), severe oligozoospermia (SO), and moderate oligozoospermia in the European descent population (Aston *et al.*, 2010). However, there was no association between *TEX15* rs323345 and male infertility in Macedonia, Albania, and Han Chinese (Plaseski *et al.*, 2012; Ruan *et al.*, 2012). In our study, we established the relationship between *TEX15* rs323345 and male infertility in the Vietnamese population. The distribution of genotypes of this polymorphism followed Hardy-Weinberg equilibrium (p-value > 0.05). However, the study did not find any correlation between *TEX15* rs323345 and male infertility in the three models (additive, dominant, and recessive) (p-values > 0.05). This inconsistency might be explained by the different environmental and genetic backgrounds of various ethnic populations.

In addition to genotype analysis, haplotype analysis can also be used to examine the potential combination effects of candidate variants in correlation with male infertility (Ruan *et al.*, 2012; Jahantigh *et al.*, 2017; Piekarska *et al.*, 2022). Therefore, we employed haplotype analysis to investigate the haplotype effects of *TEX15* rs323345 and *TEX15* rs142485241 (unpublished data). It is noteworthy that in the four possible haplotypes analyzed, the frequency of haplotype GT was significantly different between the case group and the control group (p = 0.023, OR = 1.937, 95% CI = 1.085-3.456). Therefore, further studies should be implemented to determine the association of the haplotype GT with male infertility in different populations, as well as the mechanism of this effect.

CONCLUSION

In this study, the minor allele frequency of *TEX15* rs323345 was identified to be 0.104 in a Vietnamese cohort using PCR-RFLP method. The distribution of the genotypes of rs323345 followed Hardy-Weinberg equilibrium (p-value > 0.05), but no association between this polymorphism and male infertility was found. However, our results provided evidence of the association of *TEX15* haplotype GT with male infertility. Further investigation should be performed to obtain more information on the association of *TEX15* variants with male infertility in the Vietnamese population.

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