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HAPLOTYPE OF *TEX15* SINGLE NUCLEOTIDE VARIANTS ASSOCIATED WITH MALE INFERTILITY IN 401 VIETNAMESE INDIVIDUALS

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ABSTRACT

Spermatogenesis is a process of cell differentiation to produce fertilized sperm. Testis-expressed 15 (*TEX15*), an important gene in spermatogenesis, has been reported to be linked with male infertility in various populations. This study aimed to assess the association of single nucleotide polymorphism (SNP) *TEX15* rs323347 with male infertile individuals in a Vietnamese cohort and further analyze the haplotypes of *TEX15* rs323346 and *TEX15* rs323347. A total of 401 unrelated males, including 202 male infertility patients and 199 healthy controls were genotyped for *TEX15* rs323347 using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Using statistical methods, the results showed that the allele frequencies of *TEX15* rs323347 were in agreement with Hardy-Weinberg equilibrium (HWE) (p-value > 0.05), but its genotype frequencies were not significantly different between male infertility patient and control groups (p-values > 0.05). However, the CA haplotype of the two variants (rs323346 and rs323347) increased the risk of male infertility (p = 0.046, OR = 2.547, 95% CI = 0.982–6.602). Thus, this study would enrich the knowledge of the impact of genetic factors on male infertility in the Vietnamese population.

Keywords: Haplotype, male infertility, PCR-RFLP, rs323347, *TEX15*, Vietnamese.

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INTRODUCTION

Infertility is a global health problem that affects millions of people of reproductive age around the world. In the male reproductive system, infertility is often related to problems with ejaculation, such as a low sperm count (oligospermia) or a complete absence of sperm (azoospermia), or abnormalities in sperm morphology (teratozoospermia) and motility (asthenozoospermia), which are common causes of infertility cases (WHO. Infertility., 2020). Sperm quality is a major indicator of male fertility, and any factor that affects sperm quality can affect male fertility (Kumar & Singh, 2022). Spermatogenesis (male germ cell development) is a process of cell differentiation that ensures the production of healthy sperm that can fertilize an egg to form a zygote (Linn et al., 2021). This process is very complicated, starting with spermatogonium undergoing mitosis to produce primary spermatocytes, followed by two meiosis-producing spermatids, and finally maturation of spermatids to mature spermatozoa (sperm) (Hess & Renato de Franca, 2008). This process is controlled by at least 2000 genes, and any change in the expression or function of these genes can lead to spermatogenesis failure, sperm functionality defects, and male infertility (Krausz & Riera-Escamilla, 2018). Among these genes, the *TEX* gene family is notable, with most of the genes in this family being expressed in the testis and playing an important role in the reproductive pathway and different stages of spermatogenesis (Bellil et al., 2021). These genes are associated with many different pathways and functions in testis cells, germ cells (from spermatogonia to spermatids), Sertoli cells, and Leydig cells, not only in human cells but also in mouse cells (Bellil et al., 2021). Previous studies demonstrated that polymorphisms in the *TEX* genes are associated with azoospermia and/or infertility.

The *TEX15* gene (testis-expressed gene 15, MIM*605795) is located on chromosome 8 (8p12), contains 11 exons, and is expressed in only the testis and ovary (Wang et al., 2001). *TEX15* transcript is found in spermatogonia, early spermatocytes, plentiful in postmeiotic germ cells, and also regulated in pachytene spermatocytes (the third stage of prophase 1 of meiosis) (Wang et al., 2005; Loriot et al., 2003), suggesting that this gene is a role in different stages of spermatogenesis (Yang et al., 2008). Previous studies have shown that knockout of the *TEX15* gene in male mice leads to an early meiotic arrest and a complete lack of germ cells (Yang et al., 2008). Protein *TEX15* is essential for chromosome synapsis in males and meiotic recombination in spermatocytes (Yang et al., 2008).

Recently, multiple mutations in the *TEX15* gene have been found in male infertility patients (Okutman et al., 2015; Colombo et al., 2017; Wang et al., 2018), confirming that *TEX15* plays an important role in spermatogenesis and its defects may be responsible for male infertility. The polymorphism *TEX15* rs323347, a missense variant (NM_001350162.2:c.1459T>C, p.C487R), has been studied in male infertility populations such as Europe or China (Aston et al., 2010; Ruan et al., 2012), however, the results of studies between populations are not consistent. Therefore, to understand the impact of the polymorphism in *TEX15* on male infertility in Vietnamese individuals, we conducted a case-control study of *TEX15* rs323347 in the Vietnamese population and further analysis of haplotypes of *TEX15* rs323346 and *TEX15* rs323347.

MATERIALS AND METHODS

Study participants

A total of 401 Vietnamese men, including 202 individuals diagnosed with male infertility and 199 healthy males who had at least one child naturally were collected for the study. Semen analysis was performed to confirm that the selected infertile patients all had idiopathic non-obstructive azoospermia (NOA) and oligospermia (< 15 million sperm/ml). Other infertility cases such as abnormal karyotype, azoospermia factor (AZF) region disorders, and suffering from diseases affecting fertility were excluded from the study. The study was approved by the

Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology. All subjects gave informed consent before the blood collection.

Extraction of peripheral blood DNA

Total DNA was extracted and purified from 401 blood samples using GeneJET Whole Blood Genomic DNA Purification (Thermo Fisher Scientific, USA) following the instructions of the manufacturer. The quantity and quality of the extracted DNA were checked using spectrophotometry and electrophoresis in 1% agarose gel. All DNA samples were then diluted to the final concentration (~2.5 ng/µL) and stored at -20 ^{o}C.

SNP genotyping

To determine the genotypes of *TEX15* rs323347, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used with specific primers (Table 1). The primers were designed by Primer blast and checked for dimerization (https://www.idtdna.com/pages). PCR products of rs323347 were then digested with restriction enzyme *Mun*I (Thermo Fisher). The digestion reaction mixture was incubated at 37 ^{o}C in a water bath for 4-6 hours and then electrophoresed on 3% agarose gel. Based on the size and number of DNA bands appearing on the gel, the genotypes of *TEX15* rs323347 were determined.

Table 1. List of primers used for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) amplification

Gene	Primer sequence	PCR product (bp)	PCR-RFLP	
			Genotype	Fragment size (bp)
TEX15 rs323347	F:5'-ATTACCGGACTCCTGTTGGGCT-3' R:5'-CCTCAGAAGTTGTCCCTGGCAAT-3'	275	AA	24; 251
			AG	24; 251; 275
			GG	275

Statistical analysis

Statistical analysis was performed using the specialized statistical software R.4.1.3 (R Core Team, 2021). The chi-square test ($\chi 2$) was used to test whether the study subjects follow the Hardy-Weinberg equilibrium (HWE) (Graffel-man, 2015). The analysis of the correlation between polymorphism rs323347 and male infertility was performed using the "epitools" package (Aragon et al., 2020). Association was estimated by calculating the odds ratio (OR) with 95% confidence intervals and *p*-value < 0.05 then the estimation was considered to be statistically significant. Haplotype association was performed using SHesis software (Shi & He, 2005; Li et al., 2009).

RESULTS

Genotype identification of *TEX15* rs323347

Specific DNA fragments containing *TEX15* rs323347 were amplified using PCR, and the electrophoresis results showed bright, sharp product bands with expected molecular weight (data not shown). The PCR products were then digested with restriction enzyme *Mun*I to determine the SNP's genotypes. Electrophoresis results showed six representative samples on 3% agarose gel (Fig. 1). The band of 24 bp could not be seen on the 3% agarose gel due to its small molecular weight.

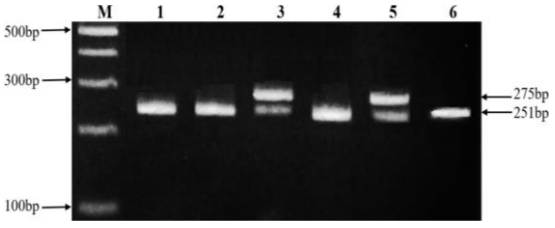

Figure 1. Restriction enzyme-digested PCR products on 3% agarose gel
Note: M: Marker 100 bp; *Mun*I-digested PCR products of *TEX15* rs323347.
1, 2, 4, and 6: Wildtype AA; 3 and 5: Heterozygous AG

A total of 401 samples were genotyped for *TEX15* rs323347 (Table 2). The minor allele frequencies of *TEX15* rs323347 were 0.101, 0.106, and 0.103 in case, control, and whole population, respectively. The genotype distribution of this polymorphism was in accordance with Hardy Weinberg equilibrium (*p*-values > 0.05).

Table 2. General information on the polymorphism *TEX15* rs323347

	Genotypes		Allele frequencies		HWE (*p*-value)
	AA	AG	A	G	
Case (n = 202)	162	40	0.899	0.101	0.118
Control (n = 199)	157	42	0.894	0.106	0.09
Total (n = 401)	319	82	0.897	0.103	

Note: n: Number of participants; HWE: Hardy-Weinberg Equilibrium.

Association analysis between *TEX15* rs323347 and male infertility

To analyze the correlation between the polymorphism and male infertility, statistical analyses were performed on the dominant model and allele forms because the GG genotype was not present in the studied population (Table 3). Obtained *p*-values were 0.746 and 0.76 in the model dominant and allele forms, respectively and both higher than 0.05, indicating no association of *TEX15* rs323347 (AA/AG/GG) with male infertility in Vietnamese population.

Table 3. Association of *TEX15* rs323347 with male infertility

	Test model	Cases (n = 202)	Controls (n = 199)	OR	95% CI	*p*-Value
TEX15 rs323347	Dominant					
	AA	80.2%	78.9%	1.000		
	AG	19.8%	21.1%	1.083	0.665–1.765	0.746
	Alleles					
	A	89.9%	89.4%	1.000		
	G	10.1%	10.6%	1.073	0.678–1.701	0.760

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

***TEX15* haplotypes and risk of male infertility**

The association of the haplotypes of two variants, rs323347 and rs323346 (Phuong Anh et al., 2023) with male infertility was analyzed by SHEsis (Table 4). The CA haplotype exhibited a significantly increased risk of male infertility (p = 0.046, OR = 2.547, 95% CI = 0.982–6.602).

Table 4. Haplotype analysis of *TEX15* rs323347 and *TEX15* rs323346

Haplotype	Frequency		*p*-value	OR	95% CI
	Case n (%)	Control n (%)			
CA*	15 (3.8%)	6 (1.5%)	0.046	2.547	0.982–6.602
CG*	35 (8.6%)	39 (9.8%)	0.581	0.874	0.541–1.412
TA*	349 (86.3%)	350 (87.9%)	0.619	0.897	0.584–1.378
TG	5 (1.3%)	3 (0.8%)	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

Spermatogenesis is a complex process of cell differentiation that is important for male reproduction and its regulation involves the expression of a large number of genes (Hermann et al., 2018). Among these genes, the *TEX15* gene is considered one of the potential target genes for the risk of spermatogenesis failure. TEX15 protein contributes to the regulation of meiosis in germ cells in both the testis and ovary (Wang et al., 2001). There was a failure of chromosomal synapsis in *TEX15*-deficient spermatocytes, suggesting the role of the *TEX15* gene in meiotic recombination and chromosomal synapsis in males (Yang et al., 2008). Therefore, mutations in the *TEX* 15 gene cause meiotic recombination failure, leading to spermatogenesis disruption. To date, several *TEX15* SNPs, including rs323346 and rs323347, have been reported to be associated with male infertility in many populations around the world (Ruan et al., 2012; Aston et al., 2010; Zhang et al., 2015; Plaseski et al., 2012; Ghadirkhomi et al., 2022). In a previous study, rs323347 was found to be associated with the risk of spermatogenic failure in the Chinese Han population (*P*=0.046), with 309 cases (199 cases of nonobstructive azoospermia and 110 cases of severe oligozoospermia) and 377 controls (Ruan et al., 2012). However, no association was found between rs323347 and male infertility in the European population (Aston et al., 2010). In this study, *TEX15* rs323347 was not associated with male infertility in the Vietnamese population. The correlation of this polymorphism is inconsistent in different populations possibly due to the limited number of samples, the genetic and ethnic differences or gene interactions with other factors such as nutrients, environment and ethnicity (Ruan et al., 2012; Zhang et al., 2015).

Haplotype analysis is a valuable tool for detecting possible associations between a gene that cannot be seen with individual SNP evaluation (Ruan et al., 2012; Jahantigh et al., 2017; Piekarska et al., 2021). In the Chinese Han population, two haplotypes of two variants rs323347 and rs323346 were reported to be strongly associated with spermatogenesis (Ruan et al., 2012). The AT haplotype reduced the risk of being severe oligozoospermia (p = 0.040) (Ruan et al., 2012). On the other hand, the GC haplotype increased the risk of being severe oligozoospermia (p = 0.040) (Ruan et al., 2012). The polymorphism rs323346 has been previously studied in this Vietnamese population but did not show an association with male infertility (Phuong Anh et al., 2023). In this study, the haplotypes analysis of *TEX15* SNPs (rs323346 and rs323347) showed that the CA haplotype increased the risk of male infertility in the Vietnamese population (p = 0.046, OR = 2.547, 95% CI = 0.982–6.602). However further studies are needed to determine the mechanism of this effect on male infertility.

CONCLUSION

In this study, the minor allele of *TEX15* rs323347 was identified to be 0.103 in 401 Vietnamese men, including 202 men with male infertility and 199 controls. The genotypic distribution of the polymorphism followed the Hardy-Weinberg equilibrium but no correlation between *TEX15* rs323347 and the risk of male infertility was found. However, the CA haplotype of two *TEX15* variants (rs323346 and rs323347) might affect on male infertility in the Vietnamese population (p = 0.046). This is the first study on genotype and allele frequencies of the *TEX15* rs323347 in Vietnamese. The findings of this study provide useful information on *TEX15* haplotype associated with male infertility in the Vietnamese population.

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