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ASSOCIATION OF *TEX15* rs142485241 WITH MALE INFERTILITY IN 429 VIETNAMESE INDIVIDUALS

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ABSTRACT

Male infertility is a reproductive disease caused by various factors, including environmental factors and genetic defects. Thousands of genes have been identified to cause and associate with male infertility, such as *TEX15*. Our study aimed to identify the association between the polymorphism *TEX15* rs142485241 and male infertility. Total DNAs were extracted from the whole blood of 429 unrelated Vietnamese individuals, including 202 healthy controls and 227 patients with male infertility. The genotypes and alleles of the polymorphism were determined by the PCR-RFLP method. The data were analyzed by statistical methods to assess the association of *TEX15* rs142485241 with male infertility. The results showed that the distribution of genotypes of the polymorphism followed the Hardy-Weinberg equilibrium (p-value > 0.05). However, no association was established between the polymorphism *TEX15* rs142485241 and male infertility in the three models (additive, dominant, and recessive) (p-value > 0.05). This study would contribute to the knowledge about the association of *TEX15* with male infertility in the Vietnamese population.

Keywords: Male infertility, PCR-RFLP, rs142485241, *TEX15*, Vietnam.

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INTRODUCTION

Male infertility is accountable for 40% to 50% of infertility complications and can be determined by environmental elements, while many are attributed to genetic factors (Hirsh, 2003; Brugh & Lipshultz, 2004). Thousands of genes have been reported to participate in spermatogenesis, where spermatogenic failure can be caused by any changes in the expression of these genes and lead to male infertility (Zhou et al., 2009). Therefore, identifying genetic polymorphisms in spermatogenesis will shed light on the mechanisms underlying male infertility. So far, single nucleotide polymorphism microarrays have successfully determined a few genes, such as *SPATA16*, *DPY19L2*, *DNAH1*, and *TEX15*, involved in male infertility (Dam et al., 2007; Koscinski et al., 2011; Plaseski et al., 2012; Ben Khelifa et al., 2014).

Testis-expressed 15 (*TEX15*) (NC_000008.11) gene is located in human chromosome 8p12 and is only expressed in germ cells (Wang et al., 2001). *TEX15* transcript is present in spermatogonia, early spermatocytes, and down-regulated in pachytene spermatocytes (Yang et al., 2008). Furthermore, it is vastly expressed in post-meiotic germ cells, showing that this gene might play a role in different developmental stages during spermatogenesis (Wang et al., 2005). The absence of the *TEX15* gene in male mice resulted in significantly reduced testis size and a completely lacking of germ cells (Yang et al., 2008). Knockout of *TEX15* leads to early meiotic arrest and disruption of chromosomal synapsis during male meiosis (Yang et al., 2008).

Previous studies have successfully demonstrated the association of polymorphisms in the *TEX15* gene, including rs323343, rs323344, rs323345, rs323346 and rs323347, with spermatogenetic failure in different cohorts (Aston et al., 2010; Ruan et al., 2012). However, there has not been any study on polymorphism *TEX15* rs142485241 regarding male infertility to the best of our knowledge. Therefore, we conducted a case-control association study of polymorphism *TEX15* rs142485241 (NC_000008.11:g.30844125C>G) in the Vietnamese population to determine the relationship between this polymorphism and reproductive defect in males.

MATERIALS AND METHODS

Study participants and collection of blood samples

A total of 227 infertile patients, including idiopathic non-obstructive azoospermia (NOA) and oligospermia (< 15 million sperms/mL) men, were recruited from several hospitals in Ha Noi, 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 202 healthy men who naturally conceived at least one child. All participants that met the requirements above gave informed consent for the blood collection. The Institutional Review Board approved the study of the Institute of Genome Research, Vietnam Academy of Science and Technology. Blood samples (2 mL) were collected from the patients in EDTA-coated tubes and subsequently stored at -20 ^{o}C.

Methods

SNP genotyping

Genomic DNA was extracted from whole blood samples of participants using Gene JET Whole Blood Genomic DNA Purification Kit (Thermo Fisher). To assess DNA quality, genomic DNA was measured by both electrophoresis and spectrophotometry. DNA samples were then diluted to the final concentration (~2.5 ng/μL) and stored at -20 ^{o}C. For polymorphism *TEX15* rs142485241 genotyping, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was employed 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 restriction enzymes *Pfe*I to identify the genotypes of *TEX15* rs142485241 (Table 1).

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

Primer sequence	PCR product length (bp)	PCR-RFLP	
		Genotype	Fragment (bp)
F: 5'-CGCCTATTTTACCTGCCCAC- 3' R: 5'-GCTTCGCAATGTTGGTTCAC-3'	431	CC	170, 261
		CG	170, 261, 431
		GG	431

Statistical analysis

Data collected from the methods mentioned were statistically analyzed using Microsoft Excel (Microsoft Corp., Washington, DC, 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. 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 *p*-value < 0.05.

RESULTS

Genetic analysis of *TEX15* polymorphism

The targeted DNA region containing *TEX15* rs142485241 was amplified using specific primers. Electrophoresis on agarose gel 1% showed specific, sharp, and bright DNA bands with the desired molecular weight (data not shown). After that, PCR products were digested with *Pfe*I to determine the genotypes of *TEX15* rs142485241 (Fig. 1).

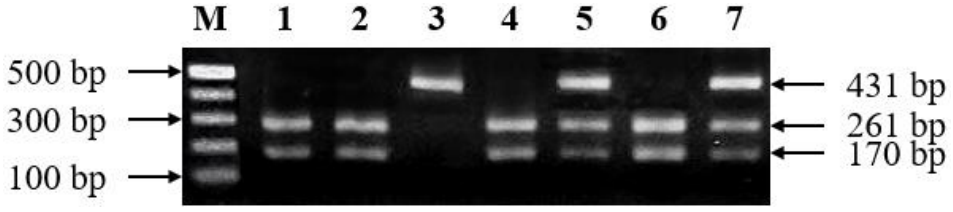

Figure 1. Restriction enzyme-digested PCR products on agarose gel 1.5%. M: Marker; 100 bp; 1, 2, 4, 6: Wildtype CC (2 bands of 170 bp and 261 bp); 5, 7: Heterozygous CG (3 bands of 170 bp, 261 bp, and 431 bp); 3: Homozygous GG (1 band of 431 bp)

A total of 429 samples (227 cases and 202 controls) were genotyped for polymorphism *TEX15* rs142485241. The minor allele frequencies (MAF) in the case, control, and the overall population were 0.052, 0.049, and 0.051, respectively (Table 2). The distribution of the polymorphism *TEX15* rs142485241 was confirmed to follow HWE using the Chi-square test in case, control, and the overall population (*p*-values > 0.05).

Table 2. General information on the studied single nucleotide polymorphism

Allele	MAF case	HWE case	MAF control	HWE control	MAF whole population	HWE whole population
C > G	0.052	1	0.049	0.391	0.051	1

Association of *TEX15* rs142485241 with male infertility

To identify the association of polymorphism rs142485241 with male infertility, we performed statistical analysis in three test models: additive, dominant, and recessive (Table 3). *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 statistical significance. In conclusion, genotypes (CC/CG/GG) and alleles (C/G) of *TEX15* rs142485241 were not correlated with male infertility in the studied population in all test models.

Table 3. Association of *TEX15* rs142485241 with male infertility

Test model	Case (n = 227)	Control (n = 202)	OR	95% CI	*p*-value
Additive					0.677
CC	203 (89.42%)	183 (90.59%)	1.000		
CG	24(10.58%)	18 (8.91%)	0.834	0.431–1.586	0.574
GG	0 (0.00%)	1 (0.50%)	2.081	0.167–65.742	0.505
Recessive					
CC + CG	227 (100%)	201 (99.50%)	1.000	0.170–66.898	0.495
GG	0 (0.00%)	1 (0.50%)	2.119		
Dominant					
GG + CG	24 (11.82%)	19 (9.40%)	0.888	0.471–1.656	0.706
CC	203 (88.18%)	183 (90.60%)	1.000		
Allele					
C	215 (94.71%)	192 (95.04%)	1.000		
G	12 (5.29%)	10 (4.96%)	0.935	0.382–2.238	0.874

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

DISCUSSION

Spermatogenesis is a complicated process involving a series of highly regulated genes which the knockout of such genes might cause severe spermatogenic failure, leading to infertility. Wang et al showed that the *TEX15* gene was expressed in mouse spermatogonia (Wang et al., 2001). Through the study in mice, the finding of Yang et al demonstrated that *TEX15* was essential for DNA double-strand break repair, chromosomal synapsis, and meiotic recombination during meiosis. Thus *TEX15* gene was hypothesized as a potential gene causing impaired spermatogenesis in its absence (Yang et al., 2008). *TEX15* encodes a 2789-amino-acid serine-rich protein (TEX15) predominantly expressed in the testis. In mice, knockout of the orthologous gene (*TEX15*) results in sterile male mice while female mice are still fertile. Loss-of-function mutations in *TEX15* cause early meiotic arrest in spermatocytes before the mid-pachytene stage, leading to spermatogenic failure associated with the most severe forms of male infertility (non-obstructive azoospermia - NOA). In a study of the Turkish population, Okutman et al. revealed that a homozygous *TEX15* nonsense mutation (c.2130T>G, p.Tyr710*) caused NOA in two male brothers and SO in one male brother (Okutman et al., 2015). All affected males had their testicular size reduced to more than half of the average normal size, especially one of the NOA-affected males who experienced maturation arrest at the primary spermatocyte stage. Recently, two novel *TEX15* mutations (p.Lys807* and p.Ser1014Leu*fs**5) were identified to change the protein structure, resulting in a truncated protein missing two domains (Pfam PF15326), and are likely to terminate its function (Colombo et al., 2017).

Increasing studies showed that single nucleotide polymorphisms might be an important factor in male infertility susceptibility (Matzuk & Lamb, 2008). In a Han Chinese cohort study, *TEX15* rs323346 and *TEX15* rs323347 were significantly associated with the likelihood of bearing impaired spermatogenesis. Besides that, SNPs rs323344 and rs323345 have not been associated in Han Chinese, Macedonia and Albania populations but were shown to associate with NOA, severe oligozoospermia (SO), and moderate oligozoospermia in a European descent population (Aston et al., 2010; Plaseski et al., 2012; Ruan et al., 2012). The *TEX15* rs142485241, a missense variant p.Q1631H, 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. In this study, we established the relationship between *TEX15* rs142485241 and male infertility in the Vietnamese population. The distribution of genotypes of the polymorphism was in accordance with the Hardy-Weinberg equilibrium (p-value > 0.05). However, the study did not find any correlation between *TEX15* rs142485241 and male infertility in the three models (additive, dominant, and recessive) (p-value > 0.05).

CONCLUSION

Here, we studied the relationship of *TEX15* rs142485241 with male infertility in a Vietnamese cohort using the PCR-RFLP method. The results showed no association between the polymorphism genotypes and male infertility. This study contributes to the knowledge of genetic effects on male infertility in the Vietnamese population.

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