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 synopsis 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°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°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 Psp1406I 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’-TAAGGAAGTTTCTGTAATAACG-3’ | 261 | CC | 261 |
| R: 5’-GTAATTCTGTATCTTTAAGTTTGC-3’ | | 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 Psp1406I 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.](image)

**Table 2.** General information on TEX15 rs323345.

| Alleles | MAF case | HWE case | MAF control | HWE control | MAF whole population | HWE whole population | whole |
|---------|----------|----------|-------------|-------------|----------------------|----------------------|-------|
| 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   |               |                  |      |                 |         |
| CC         | 3 (1.41%)     | 1 (0.4%)         | 1.000|                 | 0.5887  |
| 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; Piekarbska 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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