UDC 575.
https://doi.org/10.2298/GENSR2302689A
Original scientific article

ASSOCIATION STUDY OF RS323344 IN *TEX15* WITH NON-OBSTRUCTIVE AZOOSPERMIA IN IRANIAN POPULATION

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Aliasghar B., S. Sane, M. Sheidai, F. Koohdar, N. Kalhor (2023). *Association study of rs323344 in tex15 with non-obstructive azoospermia in Iranian population*. - Genetika, Vol 55, No.2, 689-705.

Infertility is a global health issue that affected approximately 15% of couples across the world. Genetic and environmental factors have a significant role in the manifestation of male infertility. Genetic factors contribute to 15% – 30% of male infertility. Testis expressed 15 *(TEX15)* gene plays an important role in chromosome synapsis, DNA double-strand break repair, and meiotic recombination. DNA double-strand break repair is required during homologous chromosome pairing and chromosome synapsis. The *TEX15* gene is essential for normal gametogenesis and maintenance of genome integrity. The present study was carried out to investigate the association of SNP rs323344 (p.Leu1720Val, c.5158 T>G) in the *TEX15* gene with azoospermia male infertility among the Iranian population. To conduct this case-control study, a total of 100 Iranian men, including 50 infertile cases diagnosed with non-obstructive azoospermia and 50 fertile controls from 5 different ethnics, were recruited. SNP rs323344 was genotyped using the polymerase chain reaction-restriction fragment length (PCR-RFLP) method. Furthermore, a number of samples were sequenced by the Sanger sequencing method. Variants rs323344 in *TEX15* showed the lack of statistically significant differences in genotype distributions between men diagnosed with azoospermia infertility and the fertile group in our studied samples in Iran. In general, our studied genome segment demonstrated a high degree of conservation and a low level of nucleotide variability. Our findings indicated

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that the SNP rs323344 and its neighboring nucleotides play no role in male infertility. This variant cannot be considered a biomarker for azoospermia in the Iranian population. Further studies with larger sample sizes and different ethnic backgrounds are needed to confirm the present findings.

Keywords DSB repair, male infertility, non-obstructive azoospermia, rs323344, Testis-expressed 15 (*TEX15*)

INTRODUCTION

Infertility is defined as an inability to conceive after 12 months of regular, unprotected sexual intercourse (GHIEH *et al*., 2019). Infertility studies indicate that about 15% of couples are confronted with infertility worldwide (AGARWAL *et al*., 2015). Approximately 40-50% of infertility cases can be attributed to factors associated with male infertility (BOROUJENI *et al*., 2018). Non-obstructive azoospermia is characterized as the most severe type of male infertility due to a complete absence of sperm in ejaculate caused by spermatogenesis failure (CHIBA *et al*., 2016).

Male infertility is a complex disorder with multiple factors such as immunological insufficiencies, endocrine defects, varicocele, hypogonadism, obstruction ducts, semen defects, environmental reasons, sexual behavior, infections, and genetic factors playing various roles in its manifestation (MASSART *et al*., 2012). Genetic factors involved in male infertility are manifested as chromosomal disorders (chromosomal abnormalities, Y chromosome microdeletions, gene mutations, and single nucleotide polymorphisms), mitochondrial DNA mutations, and endocrine disorders of genetic origin (SHAMSI *et al*., 2011; FERLIN *et al*., 2006).

Spermatogenesis is a highly specialized process of the mitotic division of spermatogonia, meiosis of spermatocytes, and postmeiotic differentiation of spermatids that results in the formation of functional spermatozoa (MADURO *et al*., 2002). More than 2000 genes are involved in human reproduction and controlling spermatogenesis as well as the development and maintenance of the testis (KRAUSZ *et al*., 2014). Based on studies, Testis expressed 15 *(TEX15)* is considered a potential gene linked to spermatogenic failure 25 (*SPGF25*) in the OMIM database (OMIM: 617960). Several reports demonstrated the association of *TEX15* variants with spermatogenic failure in humans and infertility (OKUTMAN *et al*., 2015; COLOMBO *et al*., 2017; WANG *et al*., 2018; CANNARELLA *et al*., 2019; ARAUJO *et al*., 2020). *TEX15* gene is located on human chromosome 8p12 with 12 exons and encodes 3,176 amino acid protein that is required for DNA double-strand breaks repair, normal chromosome synapsis, and meiotic recombination in spermatocytes during meiosis (YANG *et al*., 2008; BELLIL *et al*., 2021). *TEX15* protein is necessary for regulating the loading of DNA repair proteins RecombinaseA-like 51 (RAD51) and DNA meiotic recombinase 1 (DMC1) onto recombination sites. Its absence result in early meiotic arrest in spermatocytes before the mid-pachytene stage and a failure in meiotic recombination (HUNTER *et al*., 2001; YANG *et al*., 2008; INAGAKI *et al*., 2010). *TEX15* gene is expressed in endometrium and testis tissue and also in spermatogonia cells, early spermatocytes, and elongated, round spermatids cells. Evaluation of gene expression was obtained from the Genotype-Tissue Expression database (https://gtexportal.org/) and the Human Protein Atlas (UHLEN *et al*., 2010) (https://www.proteinatlas.org/). It was recently reported that *TEX15* is an essential executor of the mammalian PIWIL4-piRNA pathway and silences transposable

elements in male germ cells through DNA methylation. It has been supposed that *TEX15* is an essential epigenetic regulator. (YANG *et al*., 2020). In other studies, *TEX15* variants were associated with prostate cancer risk and hereditary breast cancer (LIN *et al*., 2017; MANTERE *et al*., 2017).

Given the crucial functions of the *TEX15* gene in meiosis progression (YANG *et al*., 2008) and its variants causing various SPGF phenotypes from oligozoospermia to NOA with meiotic arrest, the possibility exists that SNP rs323344 is a potential genetic factor for Non-obstructive azoospermia infertility in Iranian population. A substitution (p. Leu1720Val, c.5158 T>G) in nonsynonymous SNP rs323344 located in exon 8 leads to a change in the amino acid Leucine to Valine. The effect of this replacement and its association with infertility has been studied in men of European descent, men of Macedonian and Albanian origin, the Chinese Han population from the Anhui region, and Sichuan populations (ASTON *et al*., 2010; PLASESKI *et al*., 2012; RUAN *et al*., 2012; ZHANG *et al*., 2015).

This present study was conducted to investigate the possible association of the SNP rs323344 *TEX15* gene with azoospermia infertility among the Iranian population. Our purpose was to find out whether the variant rs323344 in the *TEX15* gene can be a risk factor for spermatogenic failure in the Iranian population. Variant rs323344 and its adjacent nucleotides in some samples were analyzed to investigate the possibility of any association in sequences with male infertility. Genotyping analysis was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Furthermore, Sanger sequencing was carried out to study DNA sequences and nucleotides surrounding the SNP rs323344 and conduct subsequent genetic analysis in some samples.

MATERIAL AND METHODS

Study Population and Sample Collection

The present study consists of 50 fertile and 50 infertile men with non-obstructive azoospermia with a range of 28-32 years old recruited from the infertility center in Qom province. Both cases and controls belong to 5 different Iranian ethnics Turk, Fars, Lor, Arab, and Kurd. Patients with abnormal karyotypes and Y chromosome microdeletions were excluded from the study. Semen analysis was conducted for all participants according to the world health organization (WHO) criteria (WORLD HEALTH ORGANIZATION, 2021; COOPER *et al*., 2010). A 2 ml blood sample was taken from each participant and the sample was collected in tubes containing anticoagulant (EDTA). Informed consent was obtained from all participants.

DNA Extraction

Genomic DNA was extracted from blood samples using the Salting-out DNA method (MILLER *et al*., 1988) The quality of DNA samples was evaluated by 1% agarose gel electrophoresis. All genomic DNA samples were stored at -20°C.

PCR Procedure

Forward and reverse primers were designed by Oligo7 software (RYCHLIK, 2007) and A Plasmid Editor (ApE) software (DAVIS and JORGENSEN, 2022). Primer information is summarized in (Table 1).

Table 1. Forward and Reverse Primers information, product size, and PCR conditions.

Primers sequence (5′–3′)	TM (°C)	Annealing temperature (°C)	Fragment length (bp)
F: 5′- AGAATCTTTTGCTTTTTCCGCTT -3′	55.30 °C	52 °C	516 bp
R: 5′- ACAGATCCTATTGAGAGACCCAA -3′	58.87 °C		

Polymerase chain reaction amplifications were carried out in a total volume of 22µl, containing 10ng of genomic DNA, 0.2 µM of forward and reverse primer, 10 µl of 2X Taq PreMix which contains PCR's components (0.4 mM of each deoxyribonucleoside triphosphate, 2 mM $MgCl_2$, and 0.2 u/µl Taq DNA polymerase, reaction buffer, and protein stabilizer) and 8 µl of double-distilled water (ddH_2O). The PCR reaction was performed in a thermal cycler (Techne Prime, United Kingdom). The thermal cycling program consisted of an initial denaturation at 94°C 5(min), followed by 30 cycles of denaturation at 94°C 40(s), annealing at 52°C 40(s), extension at 72°C 30(s) with a final extension at 72°C 5(min). Amplified fragments were separated by 2% agarose gel electrophoresis.

RFLP and DNA Sequencing

SNP genotyping was performed by Restriction Fragment Length Polymorphism (RFLP) method. Bsu36I restriction enzyme was selected using the NEBcutter V2.0 web (VINCZE *et al*., 2003) (https://nc2.neb.com/NEBcutter/). The PCR products were digested with Bsu36I restriction endonuclease enzyme (Thermo Fisher Scientific, United States) according to the manufacturer's protocols (Table 2). The reaction mixture was carried out in a total volume of 15.5 µl containing 5 µl of PCR product, 0.5 µl of Bsu36I enzyme (10 u/µl), 1 µl of Tango buffer 10X, and double-distilled water (ddH_2O) at optimal temperature. Digested products were detected on 2% agarose gel electrophoresis with 50-1500 bp DNA ladders (Sinaclon, Iran). If the recognition site of the restriction enzyme contains allele C (mutant), the PCR product will be cut. If allele A (wild) is located, the recognition site will miss. For rs323344, the expected lengths of fragments resulting from restriction digest with Bsu36I were 412 bp and 104 bp for mutant homozygote genotype (CC), 516 bp, 412 bp, and 104 bp for heterozygote genotype (AC) and 516 bp long fragment for wild homozygote genotype (AA). To validatePCR-RFLP results, conduct further genetic analysis and investigation of adjacent nucleotides (SNPs) 18 samples containing 9 azoospermia (4 samples from Turk ethnic, 5 samples from Fars ethnic) and 9 fertile control (5 samples from Turk ethnic, 4 samples from Fars ethnic) were selected for Sanger sequencing.

Table 2. Restriction enzyme information, product size, and RFLP conditions.

Enzyme	Recognition site	Recognition site number	Reaction temperature & time	Digested fragment length (bp)
Bsu36I (Eco81I)	CC ^ TNAGG	1	37 °C 12 h	Allele A: 516 bp Allele C : 412 + 104 bp

Data Processing and Analysis

The association between studied SNP and male infertility was assessed using the SNPStats web tool (SOLÉ *et al.*, 2006) (https://www.snpstats.net). It is calculated by applying the logistic regression method. Also, odds ratios (ORs) with corresponding confidence intervals (CIs) were calculated. Deviations of genotype distributions from Hardy-Weinberg equilibrium (HWE) were assessed using the exact test implemented in this web. Data on clinical characteristics were shown as mean ± SEM. The distribution normality of the variables and median, maximum, and minimum values were analyzed by the Shapiro-Wilk test. Differences in the variables' mean values among the two groups were analyzed by independent T-Test. These tests were performed by IBM SPSS statistics (SPSS Inc, Chicago, IL, United States) (Version 27). A P value < 0.05 was considered statistically significant. Sequence alignment and curation were done by MEGA7 software (KUMAR *et al.*, 2015). Kimura 2-parameters and unweighted pair group method with arithmetic mean (UPGMA) were performed by MEGA7 software. The Tajima's D test was performed for the neutral mutation hypothesis and was conducted in MEGA7 software. Haplotype groups and their relationships were identified by the Minimum Spanning network also the number of nucleotide replacements was determined by the TCS network. Both networks were constructed by PopART (Population Analysis with Reticulate Trees) software (LEIGH and BRYANT, 2015) (http://popart.otago.ac.nz). Multidimensional Scaling (MDS) method was performed using PAST software (HAMMER *et al.*, 2001). Discriminant Analysis of the Principal Component (DAPC) was run by Rstudio (http://www.rstudio.com/).

RESULTS

Clinical Characteristics

Table 3. Clinical characteristics

Parameter	Controls (N = 50)	Cases (NOA) (N = 50)	P value	Min - Max	Median	Lower reference limit WHO 2010
Age (Year)	30.46 ± 0.17	29.56 ± 0.15	0. 628	(28 - 32)	30.00	-
Semen Volume (ml)	3.56 ± 0.71	3.50 ± 0.71	0.552	(3 - 4)	4.00	1.5
Semen pH	7.43 ± 0.01	7.41 ± 0.01	0.165	(7.3 - 7.6)	7.400	>7.2
Total sperm number ($\times 10^6$ml)	120 ± 2.93	0	< 0.001*	(0 - 160)	37.50	39
Sperm concentration ($\times 10^6$/ml)	33.90 ± 0.70	0	< 0.001*	(0 - 45)	12.50	15
Total Motility (%)	58.50 ± 0.65	0	< 0.001*	(0 - 65)	25.00	40
Vitality (%)	69.30 ± 0.62	0	< 0.001*	(0 - 75)	30.00	58
Morphology (%)	5.18 ± 0.08	0	< 0.001*	(0 - 6)	2.00	4

Values are expressed as mean ± SEM.*Significant difference based on T-Test. Differences with P values <0.05 were considered significant with 95% confidential interval.

The characteristic of the study population are listed in (Table 3). There were no significant differences (P>0.05) in age, semen pH, and semen volume between the two groups. The absence of sperm in the ejaculate of azoospermic patients results in all semen parameters related to sperm being equal to zero in the NOA case. There were significantly different in total sperm number, sperm concentration, total motility, vitality, and morphology between NOA and control groups (P< 0.001).

RFLP Analysis

All 100 study samples including 50 cases and 50 controls, were genotyped for the SNP rs323344 by the RFLP technique. The genotypes of fourteen samples are depicted in (Figure 1). The allelic, genotypic frequencies and odds ratio are shown in (Table 4). P value and OR with 95% confidential interval (CIs) indicated the lack of statistically significant differences in genotype distributions between men diagnosed with NOA infertility and the control group. Therefore, variant rs323344 in the *TEX15* gene was not significantly associated with azoospermia in studied Iranian samples. The results showed the genotype distributions in case and control were not shown to significantly deviate from Hardy–Weinberg Equilibrium (P=0.091 and P=0.18 respectively). HWE details are presented in (Table 5).

Table 4. Details of allele frequencies, genotype frequencies, and Odds Ratio (OR) with 95% confidential interval (CI).

Allele/Genotype	Infertile n=50		Control n=50		All n=100		OR (95% CI)	P value
	Count	Proportion	Count	Proportion	Count	Proportion		
A	78	0.78	81	0.81	159	0.8		
C	22	0.22	19	0.19	41	0.2		
A/A	28	0.56	31	0.62	59	0.59	1.00	1
A/C	22	0.44	19	0.38	41	0.41	1.00 (0.00-NA)	
C/C	0	0	0	0	0	0		

Akaike information criterion (AIC) and Bayesian information criterion (BIC) are 76 and 175 respectively. P values <0.05 were considered significant with 95% confidential interval.

Table 5. Exact test for Hardy-Weinberg equilibrium.

	N_{11} = AA	N_{12} = AC	N_{22} = CC	N_1 = A	N_2 = C	P-value
All	59	41	0	159	41	0.011
Case	28	22	0	78	22	0.091
Control	31	19	0	81	19	0.18

The results showed the genotype distributions were in eiquilibrium (p>0.05).

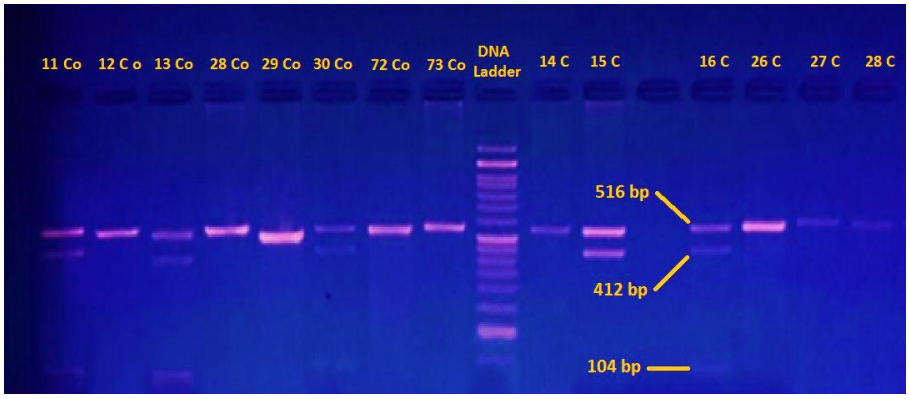

Figure **1**. RFLP-PCR results and digestion patterns by Bsu36I of case and control samples.

Sequence Analysis and Population Genetics

Genotypes of randomly selected subjects, as determined by PCR-RFLP were confirmed by Sanger sequencing. Sequence alignment and analysis were performed by the BioEdit software (HALL, 1999) (Figure 2). We examined 12 neighbor SNPs recorded in the region exon 8 in the DNA fragment. No variations were detected in these selective SNPs rs323343 (T/C), rs323345 (T/C), rs751331226 (T/C), rs1381907047 (A/G), rs1563236522 (C/T), rs148542296 (C/T), rs766094508 (G/A), rs117379409 (C/G/T), rs767613553 (G/A/C), rs755752285 (T/C), rs1807562942 (G/A), rs1309879768 (A/G) among fertile and infertile individuals in comparison with the variation in NCBI. In rs323345 C/G variation was observed instead of C/T in the 30Co-TURK sample. These variants can be good candidates for SNP association studies in future investigations in the Iranian population.

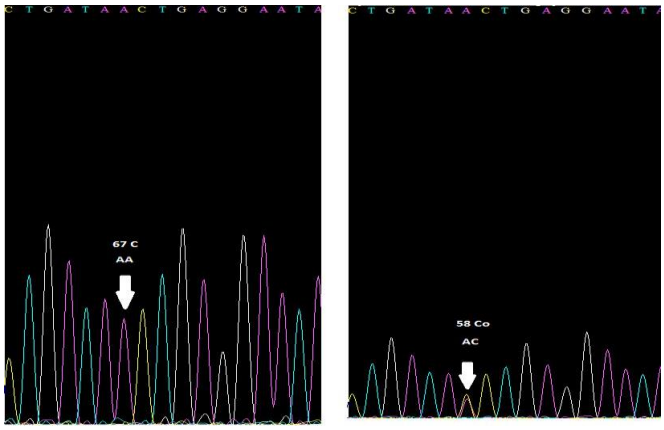

Figure **2**. DNA sequencing chromatogram analysis for Control 58 (heterozygote genotype) and Case 67 (homozygote genotype).

DNA segments obtained 469 bp nucleotides in length after alignment and curation by MEGA7 software. The preliminary statistics of these 18 sequences are: The nucleotide diversity with 18 polymorphic sites, average p-distance, and average cantor d-Jukes distance were 0.012, 0.01017, and 0.01025 respectively. Initial results indicated low genetic diversity in the studied sequences in Iranian male subjects. These nucleotide substitutions showed Tajima's D statistic = -0.926915; p (D >= -0.926915) = 0.806604. Tajima's D statistic reveals that these nucleotide substitutions occurred irrespective of selection.

We determined genetic distance based on Kimura 2 parameters among the studied subjects (Figure 3). The obtained value ranged from 0.00 to 0.05, with an average of 0.02.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. 30Co TURK																		
2. 33Co FARS	0.00																	
3. 34Co TURK	0.01	0.01																
4. 58Co FARS	0.00	0.00	0.02															
5. 72Co TURK	0.00	0.00	0.01	0.00														
6. 73Co TURK	0.00	0.00	0.01	0.00	0.00													
7. 74Co TURK	0.01	0.00	0.01	0.01	0.00	0.00												
8. 25Co FARS	0.01	0.01	0.02	0.01	0.01	0.01	0.01											
9. 57Co FARS	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01										
10. 9C TURK	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.02									
11. 11C TURK	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01								
12. 13C FARS	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.02							
13. 14C FARS	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01						
14. 23C FARS	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.02	0.00	0.01	0.01	0.01					
15. 26C TURK	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.01	0.01				
16. 32C TURK	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05			
17. 46C FARS	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.05		
18. 67C FARS	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.04	0.01	

Figure 3. Kimura 2-parameter genetic distances matrix in the studied subjects.

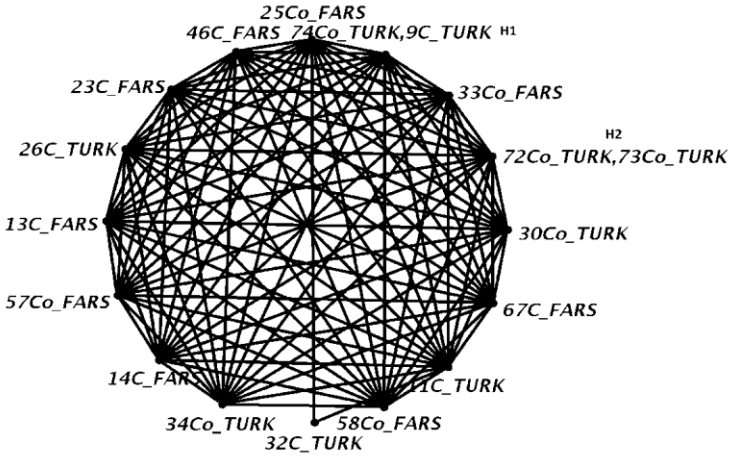

Figure 4. Minimum Spanning network

Haplotype groups are shown in the minimum spanning network (Figure 4). Network analysis revealed that the neighboring nucleotides as well as SNPs close to rs323344, play no role in male infertility as both case and control subjects were placed in a single clade. Two main haplotype groups were present (H1-H2). The number of nucleotide substitutions is provided in the TCS network (Figure 5). The number of nucleotide substitutions varied from 1 -15 among the studied samples. TCS network of the studied samples indicates a low level of genetic changes.

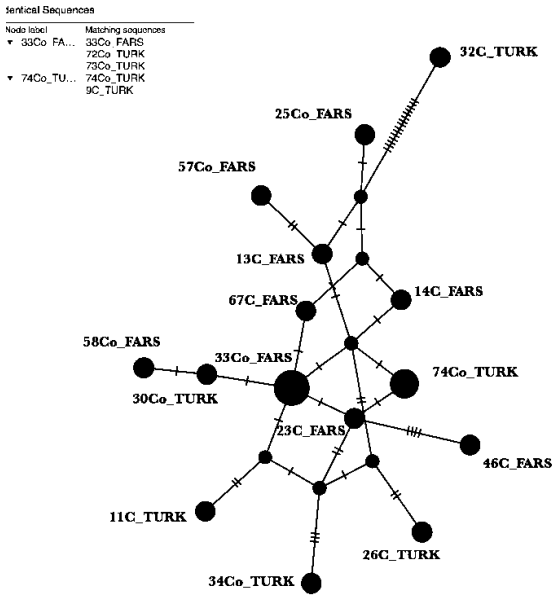

Figure 5. TCS network. The number of nucleotide substitutions is given for each branching node

UPGMA phylogenetic tree based on rs323344 sequences is presented in (Figure 6). The phylogenetic tree showed that fertile and infertile individuals were scattered in the same cluster. It reveals that these sequences were not associated with azoospermia infertility.

MDS plot is provided in (Figure 7). This plot showed that sequence distributions of case and control were scattered and the relative location of individuals was based on sequences similarity and close relationship between sequences. Therefore MDS could not differentiate the studied samples. There was no association between SNP rs323344 and adjacent SNPs in the sequence with azoospermia.

DAPC was used to identify clusters of genetically related individuals based on sequences. The bar graph were showed 5 genetic clusters (Figure 8). In our study, case and control individuals were genetically related and displayed in the same clusters. Clusters 1, 3 and 4 were common between case and control groups. The integration of clusters 3 and 4 was

observed among the control samples number 2, 5, 6. 32C_TURK with the highest number of nucleotide substitutions (15) belonged to cluster 1.

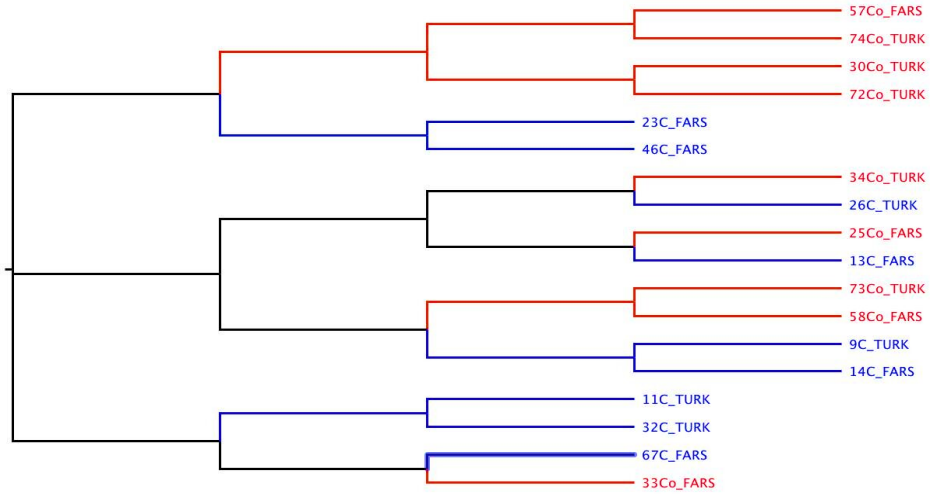

Figure 6. UPGMA dendrogram based on sequence data

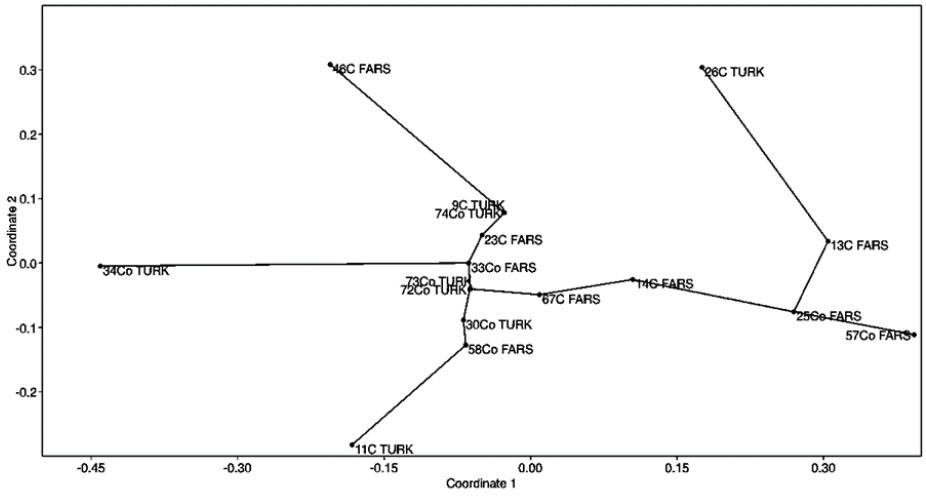

Figure 7. MDS plot

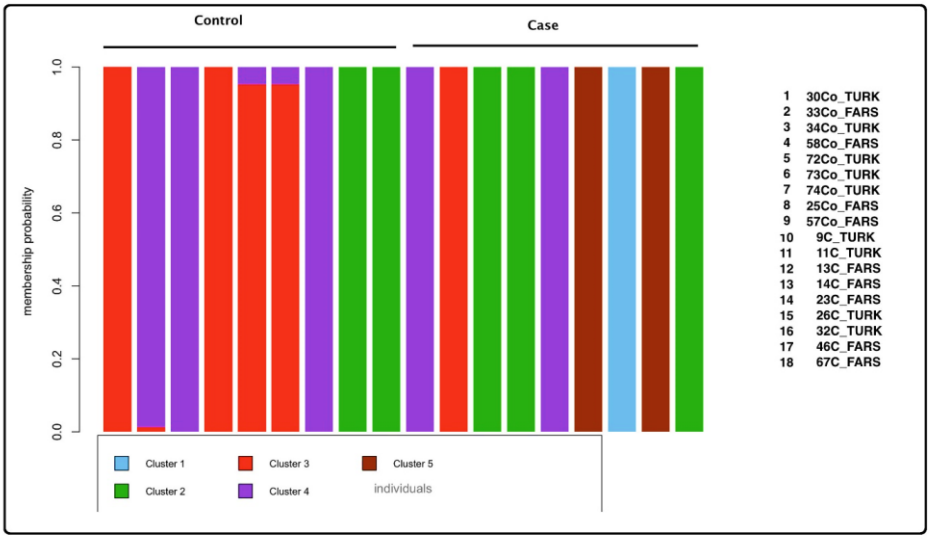

Figure 8. DAPC. Each individual was represented as a vertical bar, with colors corresponding to probabilities of assignment into the clusters.

DISCUSSION

Given the involvement and coordinated action of over 2000 genes during spermatogenesis, alterations in the expression and function of some of these genes can result in the disruption of key processes such as DNA replication, DNA double-strand break, mismatch repair, spindle formation, synapsis, recombination, and chromosome segregation. These changes can be responsible reduction or absence of functional sperm in semen and result in spermatogenic failure (KRAUSZ *et al.*, 2014; MIYAMOTO *et al.*, 2016; BOROUJENI *et al.*, 2018). Genes involved in DSB repair and chromosome synapsis play a critical role in the maintenance of genetic integrity (HARFE and JINKS-ROBERTSON, 2000; APARICIO *et al.*, 2014). The *TEX15* gene plays an essential role in chromosome synapsis, DSB repair, and meiotic recombination during meiosis (YANG *et al.*, 2008). *TEX15* gene is considered a potential target gene in conferring spermatogenic failure risk. Our aim was to assess any positive association of the rs323344 variant in the *TEX15* gene with azoospermia infertility in the Iranian population.

Results of the present case-control study showed there is no association between SNP rs323344 in the *TEX15* gene with azoospermia infertility and risk of spermatogenic failure in our studied Iranian population. Allele frequency and genotype distribution of variant rs323344 showed no significant association in azoospermic patients and fertile controls (P value=1, OR [95% CI]=1.00). In addition, various genetic analyses such as minimum spanning network, UPGMA, MDS, and DAPC on sequences revealed that there is no association between rs323344 sequence or its neighboring nucleotides with azoospermia infertility.

Several studies were conducted on different populations, providing conflicting results on the effect of the rs323344 variant on spermatogenic failure and male infertility. Among these studies, only one study reported that there was a positive association between rs323344 with azoospermia and severe oligozoospermia population of European descent (ASTON *et al*., 2010). This result is controversial. Other research studies have demonstrated there is no association between SNP rs323344 with spermatogenic failure and male infertility for populations from Macedonia and Albania, the Chinese Han population from the Anhui region, and the Sichuan populations (PLASESKI *et al*., 2012; RUAN *et al*., 2012; ZHANG *et al*., 2015). However, nonsynonymous SNP rs323344 in the coding region (exon 8) *TEX15* results in an amino acid replacement (Leu1720Val). But this structure change has no association with the risk of male infertility in Iran. These inconsistent results might be attributed to the different ethnicity and geographical origin and genetic background of the studied populations. The aforementioned studies differ in the ethnic origin of the participants. The second reason might be derived from the limited number of clinical samples taken up for analysis in studies. Differences in patient inclusion criteria are important (GURKAN *et al*., 2013). In the mentioned studies, it is observed that there are different criteria in the selection, especially the case group, based on accurate diagnosis based on abnormalities of semen parameters related to sperm concentration, morphology, and motility. Therefore, the characteristics of the participants like genetic origin, diagnosis methods, and type of male idiopathic infertility are different in each study. Another reason can be due to the multigenic origin of male infertility and effect of environmental factors on disease (MANOLIO *et al*., 2009).

According to dbSNP (http://www.ncbi.nlm.nih.gov/SNP) for SNP rs323344 major allele frequency (A) and minor allele frequency (C) in the world are 0.67 and 0.32, respectively. In this study, allele (A) and (C) frequencies were assessed at 0.8 and 0.2. It was close to the world's allele frequencies. It should be noted that the very low difference between the observed and expected MAF of our SNP likely reflects the limited number of samples (n=100), and differences related to 5 ethnics Turk, Fars, Lor, Arab, and Kurd and genetic backgrounds. Variant rs323344 had allele frequencies that are significantly different between African and other populations in the world. Differences in allele and genotype frequencies between continents have been described using data from the 1000 Genomes project. This variant has a high MAF (C): 84% in African populations and reaches (C): 32% in other populations. African populations have the lowest ancestral alleles (A) frequency and the highest genotype (CC) frequency with a calculated value of 0.722. It is possible that the difference between populations has occurred through humans migrating out of Africa to the other part of the world and the bottleneck effect. Generally, the frequencies of (AA), (AC), and (CC) genotypes worldwide are 0.558, 0.232, and 0.210 respectively. In our studied population, (AA), (AC), and (CC) genotypes frequencies were equal to 0.59, 0.41, and 0. The lowest (CC) genotype frequencies with a value of 0.004 and 0.055 belonged to East Asian and South Asian populations respectively. In East Asia and South Asia populations frequencies of (AA) and (AC) genotypes were equal to 0.863, 0.626 and 0.133, 0.319. It is not possible to accurately match the ethnicities of our research population with the reported frequencies. people from the Middle East are absent in the 1000 Genomes database. Zhang et al did not observe the (CC) genotype in Sichuan populations. In PLASESKI *et al*. (2012) study, the (CC) genotype was not observed in the azoospermia group and it was 0.012 in the

control group in Macedonians and Albanian men. In ASTON *et al*. (2010) study, the (CC) genotype frequency in the control and case was 0.04 and 0.01. Also, the (CC) genotype frequency in our study was zero.

According to nucleotide substitutions in TCS and the low level of nucleotide diversity, it seems that this studied genome segment shows a high degree of conservation. *TEX15* is a member of the Testis Expressed (*TEX*) genes family (WANG *et al*., 2001). Most of the *TEX* genes are highly conserved in mammals (BELLIL *et al*., 2021). A high degree of sequence conservation and similarity exists between human *TEX15* and other species. Male *TEX15* knockout mice are infertile due to meiotic arrest. Also, a severe reduction in testicular size has been observed in male mice while knockout female mice are fertile (YANG *et al*., 2008; BOROUJENI *et al*., 2018; BELLIL *et al*., 2021).

CONCLUSIONS

The present study demonstrated that rs323344 in the *TEX15* gene and its neighboring nucleotides were not significantly associated with Non-obstructive azoospermia in the Iranian population. The variant rs323344 in the *TEX15* gene cannot serve as a potential biomarker in spermatogenesis failure and male infertility. Further studies with large-scale clinical samples with different ethnic populations are required to confirm our findings. To achieve more power of analysis, it is necessary to perform more analysis for the neighbor SNPs (rs323343, rs323345) with infertility. Investigating the rs323344 effect on oligozoospermia infertility may prove to be beneficial. These suggestions can be effective to validate the biological role of *TEX15* polymorphism in spermatogenesis failure and infertility. Identifying key genes in the spermatogenesis process and genetic variants that raise infertility risk can contribute to prognosis, clinical diagnosis, and development of assisted reproductive technologies (ART) for infertile patients in the future.

ACKNOWLEDGMENTS

We are very thankful to the infertility center in Qom province and Shahid Beheshti University for providing the samples and laboratory respectively. We are grateful to all the patients and healthy people who participated in this research study.

Received, July 07th, 2022
Accepted May 18th, 2023

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ASOCIJATIVNO PROUČAVANJE RS323344 U *TEX15* SA NEOPSTRUKTIVNOM AZOOSPERMIJOM U IRANSKOJ POPULACIJI

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Izvod

Neplodnost je globalni zdravstveni problem koji pogađa oko 15% parova širom sveta. U ispoljavanju muške neplodnosti značajnu ulogu imaju genetski i ekološki faktori. Genetski faktori doprinose 15-30% muške neplodnosti. Testis eksprimiran 15 (*TEX15*) gen igra važnu ulogu u sinapsi hromozoma, popravci dvostrukog lanca DNK i mejotičkoj rekombinaciji. Popravka dvostrukog lanca DNK je neophodna tokom homolognog uparivanja hromozoma i sinapse hromozoma. *TEX15* gen je neophodan za normalnu gametogenezu i održavanje integriteta genoma. Ova studija je sprovedena da bi se istražila povezanost SNP rs323344 (p.Leu1720Val, c.5158 T>G) u *TEX15* genu sa azoospermijom muške neplodnosti u iranskoj populaciji. Da bi se sprovela ova studija, regrutovano je ukupno 100 iranskih muškaraca, uključujući 50 neplodnih sa dijagnozom neopstruktivne azoospermije i 50 fertilnih kontrola iz 5 različitih etničkih grupa. SNP rs323344 je genotipiziran korišćenjem metode PCR-RFLP. Pored toga, određeni broj uzoraka je sekvencioniran metodom Senger. Varijante rs323344 u *TEX15* su pokazale nedostatak statistički značajnih razlika u distribuciji genotipova između muškaraca sa dijagnozom neplodnosti azoospermije i fertilne grupe u našim proučavanim u Iranu. Generalno, naš proučavani segment genoma pokazao je visok stepen očuvanosti i nizak nivo varijabilnosti nukleotida. Naši nalazi su pokazali da SNP rs323344 i njegovi susedni nukleotidi ne igraju nikakvu ulogu u muškoj neplodnosti. Ova varijanta se ne može smatrati biomarkerom za azoospermiju u iranskoj populaciji. Potrebne su dalje studije sa većim uzorcima i različitim etničkim poreklom da bi se potvrdili sadašnji nalazi.

Primljeno 07.VII.2022.
Odobreno 18. V. 2023.