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Association study between rs10966811 single nucleotide polymorphism of TUSC1 gene with oligospermia infertility in Iranian men

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Abstract

Worldwide, 15% of couples are affected by infertility, with genetic factors accounting for about 10% of infertility causes. Single nucleotide polymorphisms can play preeminent role in this regard because variation in a nucleotide can cause diversity in the gene product and thus can act as a pathogenic mutation and lead to be infertile. Among the genes that can play a role in male infertility, the role of TUSC1 gene in oligospermia and azoospermia in men is also known and therefore to investigate and find out which single nucleotide polymorphisms in this gene lead to infertilityIn this study, the association of rs10966811 polymorphism in TUSC1 gene with oligospermia in Iranian men was investigated. Tetra-ARMS-PCR method was used for this study and during that, it was found that the two groups of patients and controls are genotypically different. In this way, three types of AA, AG and GG genotypes were seen among the samples, the frequency of which was 0.5, 0.5 and 0 in the control samples, respectively, and 0.02, 0.47 and 0.51 in the patient samples. In terms of HardyWeinberg equilibrium, the oligosperm group is in equilibrium but the control group is not in equilibrium. Also, the type of heritability of these genotypes based on the trait of sperm motility, regression analysis was significant and its heritability was also significant in Dominant and Overdominant conditions. Also, two types of ethnicities were examined from the samples, which were genotypically differentiated within the ethnicities, but there was no distinction between the two ethnicities. The study of the association of this polymorphism with infertility in the Iranian male population was performed for the first time.

Keywords: Single nucleotide polymorphism, TUSC1 gene, Tetra ARMS PCR, infertility, Oligospermia.

Introduction

Worldwide, 15% of couples are affected by infertility, with genetic factors accounting for about 10% of the causes of infertility(1). Generally infertility is defined in a couple after one year try for baby and especially in case of the woman age is less than 35 years old. This is reduced to 6 months for women over 35(2). According to global statistics, 15 to 20 couples in the world have infertility problems, which is also true in Iran(3).Numerous factors can play role in causing infertility in individuals; these include lifestyles such as alcohol and tobacco use, genetic factors, anatomical disorders of the male and female genital tract, acquired causes such as a history of surgery and pelvic adhesions, environmental causes such as work and exposure to toxic substances, etc.(4).

Among the genetic factors and genes involved in infertility, single nucleotide polymorphisms (SNPs) are among the most widely considered today. SNPs are used as markers in genes to find the association of gene sequences with specific diseases and traits. In fact, a specific type of single nucleotide polymorphism in a gene can be associated with infertility, and by identifying them, the genes involved in infertility can be identified(5). By examining the presence or absence of a specific single nucleotide polymorphism in the genes of infertile specimens, its association between SNP and infertility and finally the association of the gene is determined(6). Polymorphism is the presence of two or more distinct genetic forms (alleles or allelic variants) in a population, the rarest of which are not conserved in frequency by mutation alone. Conventionally, a polymorphic locus is a locus that has at least two alleles, each with a frequency of more than 1%. Gene sequences can be different in a single nucleotide, resulting in diversity and polymorphism in the genes (7). SNPs are considered as a major genetic source of phenotypic change within a species and serve as a genetic marker in identifying the relationship between shape and a gene and causing a trait or disease (8, 9).

The TUSC1 gene, also known as CCDC89B and TSG-9, is a suppressor tumor located in the short arm of chromosome 9 (9p21 1)(10). Researches show that this gene is highly expressed in testicular tissue while its expression is low in muscles, lungs, spleen and colon. Since this gene is a tumor suppressor, its lack of heterozygosity (LOH) causes Non-small-cell lung cancer (NSCLC). Also, the study of its single nucleotide polymorphisms as a result of GWAS studies has revealed that a number of variants of TUSC1 are involved in the development of cancers of the esophagus, stomach, pancreas, kidney, breast and bladder. Improper methylation can also cause liver cancer (11).

In addition to the major role that TUSC1 plays in the development of various types of cancer, according to recent research, a number of single nucleotide polymorphisms in TUSC1 may be associated with infertility in men and cause a variety of male infertility such as oligospermia and

Although the role of this gene in the process of spermatogenesis has not yet been determined, a type of transcription product has been found in the testis called TUSC1-S(12, 13). The polymorphism studied in this study is rs10966811, which is in the intergenic region between the two genes at 445.4 kb downstream of TUSC1 and 687.8 kb upstream of IZUMO3. This polymorphism can contain A / G nucleotide. Since single nucleotide polymorphisms play a major role in causing mutations and genetic diseases, knowing them as factors involved in causing diseases can be essential for finding treatment methods and knowing the causes of infertility. It can also help in conducting research to identify other genes involved in infertility. The purpose of this research was to identify and introduce the single nucleotide polymorphism rs10966811 in the TUSC1 gene, which can be involved in the development of oligospermia.

Also, in this project, the genetics of the population of Iranian ethnic groups in the TUSC1 gene and its association with the TUSC1 gene were investigated.

Material and method

In this study, samples related to people with oligospermia that were collected from the infertility center of Qom province were used. These patient samples were collected based on semen analysis and after confirming the existence of the disease and people with oligospermia, blood samples were taken from the subjects for genetic studies after receiving written consent. The samples included 100 fertile samples that were considered as control and 50 samples of patients with oligospermia whose disease was confirmed after semen analysis. According to the criteria described in the fourth edition of WHO guidelines (1999), patients with Oligozoospermia was defined as a sperm concentration of less than 20 × 106/mL.

SNP selection

According to the previous studies around the TUSC1 and its role in infertility, the rs10966811 was known as an effective polymorphism in occurrence of oligospermia. The first research on the effect of the TUSC1 gene on infertility began in 2017 during a larger study. This study, conducted by Gu¨lu¨m Kosova et al., Identified 41 infertility-related polymorphisms for both family size and birth rate through the Genome Wide Association Study (GWAS). The study looked at 123 Chicagoans whose semen had previously been analyzed. In this study, 10 common parameters of semen were analyzed. For example, low sperm count, poor testicular function, or endocrine malformations. Nine parameters were associated with a number of single nucleotide polymorphisms, and the likelihood of these traits increased if these polymorphisms were present. SNP rs10966811 of the TUSC1 gene is one of the effective polymorphisms known in this study.

Genotyping

Genomic DNA was extracted from the peripheral blood samples by salting out method. In next step the quality and quantity of extracted DNA was evaluated by 1.5 % electrophoresis gel. In this study the Investigation and identification of the single nucleotide polymorphism is done by ARMS PCR method. In this method there are 4 primers including 2 outer and 2 inner primers (table.1) which are designed by primer1 app and are specified for SNP rs10966811 sequences. The PCR was done in 25 ul containing of 5ul DNA, 0.7 ul primers which are mixed before, 7 ul distilled water and finally 14 ul master mix. The resulting mix is placed in thermocycler device by Biorad Co. the PCR program is available in table.2

After PCR, we have to take the resulting products on 1.5% agarose gel and see the result.

Table1: primers of ARMS PCR

Primer	Sequence
Inner forward	AAAAGAAACCCCGTATTTATTGTCAATTACTCTACA
Inner reverse	GTTGCCTGTGAAGTAGTGTTAAAACAC
outer forward	CCCAAGTGGTTACTCATTAAGACTGTAC
outer reverse	AATATTTGCTATAATGAGGCAAAGTGAA

Table2. PCR program

Temperature	Time	cylce
95^{0}C	5min	1
94^{0}C	30Sec	32
60^{0}C	30Sec	32
72^{0}C	30Sec	32
72^{0}C	5min	1

Statistical analysis

Logistic regression was performed for clinical the traits and oligospermia in the studied individuals as performed in R package. Ver.4.1. Association between the SNP and infertility was determined by logistic regression as performed in SNP-STAT program. PCoA (Principal coordinate analysis) and UPGMA (Unweighted paired group using average) dendrograms were sed to differentiate control versus infertile individuals.

Results

Information and specifications of collected samples

oligosperm samples and 50 control samples were collected from the infertility center of Qom, from which a total of 95 DNA samples were extracted and then PCR was performed using the Tetra-ARMS method. The information of the examined samples is attached.

Statistical and bioinformatic data analysis

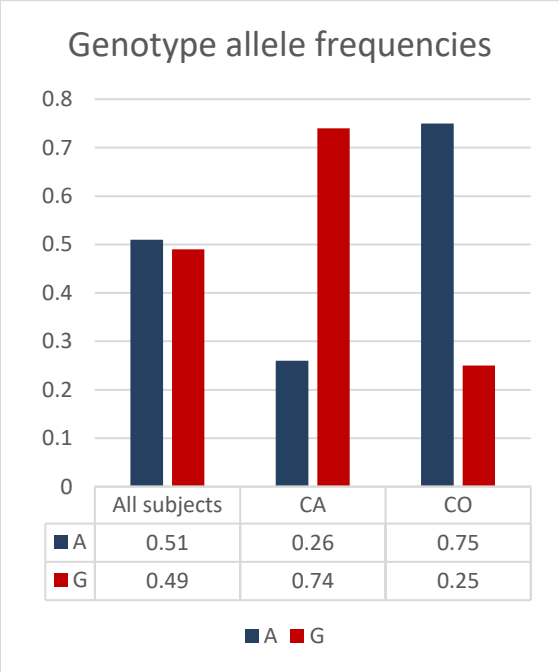
Statistical analysis of alleles and genotypes in the desired polymorphism

SNP Stat software was used to investigate the relationship between genotypes observed in patient and control samples, and the relationship between each trait and the observed genotypes, as well as the relationship between the two ethnicities and the maximum number of people in both groups of samples, was investigated.

At first, the distribution of alleles among people was checked, the table of which is as described in Table 3:

Table 3: Statistical analysis of alleles

Genotype allele frequencies(n=95)						
	All Subjects		Group=CA		Group=CO	
Allele	Count	Proportion	Count	Proportion	Count	Proportion
A	96	0.51	24	0.26	72	0.75
G	94	0.49	70	0.74	24	0.25



Graph 1 Statistical comparison graph of alleles.

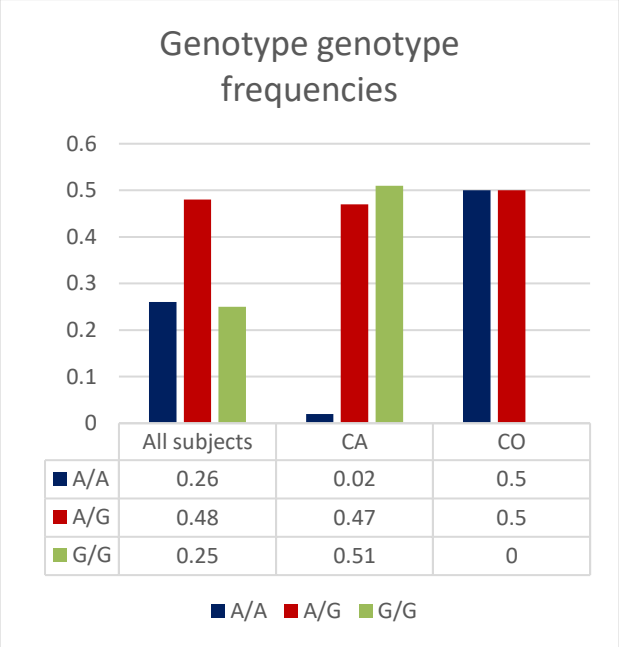
According to the above table, in general, 95 samples from both patient and control groups were examined, and in the control group, the ratio of G allele is 0.75 and A allele is 0.25. Also, among oligosperm maples, the ratio of A allele is 0.26 and G allele is 0.74.

Table 4 statistical comparison of genotypes

Genotype allele frequencies(n=95)						
	All Subjects		Group=CA		Group=CO	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
A/A	25	0.26	1	0.02	24	0.5
A/G	46	0.48	22	0.47	24	0.5
G/G	24	0.25	24	0.51	0	0

In addition to allelic analysis, the genotypes were also checked, the table of which is as described in Table 2-4:

According to the above table, three types of genotypes A/A, A/G and G/G were observed in people, whose diagram is as follows:



Graph 2, statistical comparison graph of genotypes

Table 5: Checking the significance of sperm motility trait

Genotype association with response Group)n=95,adjusted by mobility)							
Model	Genotype	Group=CA	Group=CO	OR(95% CI)	P-value	AIC	BIC
Codominant	A/A	1(2.1%)	24(50%)	1.00	0.15	11.8	**22**
	A/G	22(46.8%)	24(50%)	0.00(0.0-NA)			
	G/G	24(51.1%)	0(0%)	0.00(0.0-NA)			
Dominant	A/A	1(2.1%)	24(50%)	1.00	0.051	9.8	**17.5**
	A/G-G/G	46(97.9%)	24(50%)	0.00(0.0-NA)			
Recessive	A/A-A/G	23(48.9%)	48(100%)	100	1	13.6	**21.3**
	G/G	24(51.1%)	0(0%)	0.15(0.0 0 NA)			
Overdominant	A/A-G/G	25(53.2%)	24(50%)	1.00	0.051	9.8	**17.5**
	A/G	2(46.8%)	24(50%)	0.00(0.0-NA)			
Log-additive	---	---	---	0.00(0.0-NA)	0.051	9.8	**17.5**

Also, based on the determined genotypes, the inheritance modes were checked and they are given in the table below, and it was found that dominant and overdominant inheritance are significant.

Analysis of traits effective in infertility

Each of the effective traits in infertility was analyzed separately by SNPstat database and the results are as follows.

Table 6 Examination of sperm volume per milliliter between sick and healthy people

Covariate: Volume Type: quantitative

	n	missing	Unique	mean	.05	.10	.25	.50	.75	.90	.95
All Subjects	95	0	3	3.31							
Group=CA	47	0	2	3.3							
Group=CO	48	0	3	3.31							

Table 7: Examination of total sperm volume between sick and healthy people

Covariate: Total Type: quantitative

	n	missing	Unique	mean	.05	.10	.25	.50	.75	.90	.95
All Subjects	95	0	21	65.91	15	20	30	50	100	120	140
Group=CA	47	0	9	27.36	15	15	22	30	30	40	40
Group=CO	48	0	12	103.65	60	73.5	87.5	100	120	140	160

Lowest: 12,15,18,20,4 highest:105,120,140,160,180

According to Table 7 the minimum and maximum values of total sperm volume in milliliters are specified.

Table 8: Examination of total sperm motility between sick and healthy people

Covariate: Mobility Type: quantitative

	n	missing	Unique	mean	.05	.10	.25	.50	.75	.90
All Subjects	95	0	9	49.16	35	35	40	50	60	60
Group=CA	47	0	5	40	35	35	35	40	45	45
Group=CO	48	0	5	58.12	50	55	55	60	60	65

Lowest: 30,35,40,45,50 highest: 50,55,60,65,70

According to Table 4-7, the minimum and maximum sperm motility values are determined.

Table 9 Overall survival between sick and healthy people

Covariate: Viability Type: quantitative

	n	missing	Unique	mean	.05	.10	.25	.50	.75	.90	.95
All Subjects	95	0	8	60.21	50	50	55	60	65	70	75
Group=CA	47	0	5	53.94	50	50	50	55	55	60	63.5
Group=CO	48	0	7	66.35	56.75	60	60	65	70	75	75

Lowest: 45,50,55,60,65 highest:60,65,70,75,80

Table 10, study of frequency of alleles among people of Fars ethnicity

Genotype allele frequencies(n=53)

	All Subjects		Group=CA		Group=CO	
Allele	**Count**	**Proportion**	**Count**	**Proportion**	**Count**	**Proportion**
A	56	0.53	18	0.32	38	0.76
G	50	0.47	38	0.68	12	0.24

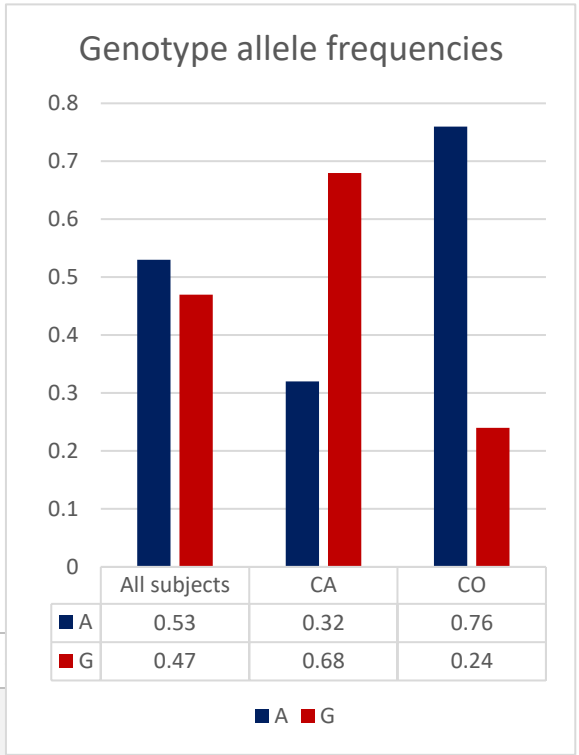


Figure 3 comparative graph of frequency of alleles among people of Fars ethnicity

As can be seen in Table 4-8, among the group of control samples, the ratio of allele G is equal to 0.24 and allele A is equal to 0.76. Also, among oligosperm samples, the ratio of allele A is 0.32 and the ratio of allele G is 0.68.

Comparison of the frequency of genotypes

Table 11 Studying the frequency of genotypes among people of Fars ethnicity

Genotype genotype frequencies(n=53)						
	All Subjects		Group=CA		Group=CO	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
A/A	14	0.26	1	0.04	13	0.52
A/G	28	0.53	16	0.57	12	0.48
G/G	11	0.21	11	0.39	0	0

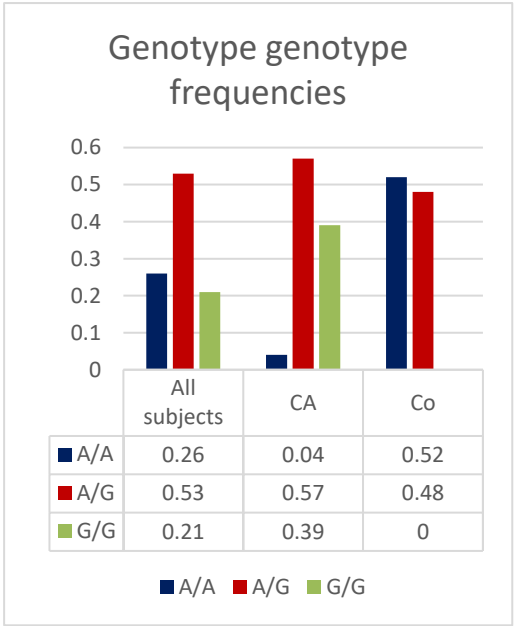


Figure 4 Comparative graph of the frequency of genotypes among people of Fars ethnicity

Table 12. The significance level of genotypes in Fars ethnicity

Genotype association with response Group)n=95,adjusted by mobility)							
Model AIC BIC	Genotype	Group=CA	Group=CO	OR(95% CI)		P-value	
Codominant	A/A	1(3.6%)	13(52%)	1.00	<0.0001	51.4	**57.4**
	A/G	16(57.1%)	12(48%)	**0.06(0.01-0.5)**			
	G/G	11(39.3%)	0(0%)	0.00(0.00-NA)			
Dominant	A/A	1(3.6%)	13(52%)	1.00	<0.0001	59.3	**63.3**
	A/G-G/G	27(96.4%)	12(48%)	**0.03(0.00-0.29)**			
Recessive	A/A-A/G	17(60.7%)	25(100%)	1.00	<0.0001	60.7	**64.6**
	G/G	11(39.3%)	0(0%)	0.00(0.00-NA)			
Overdominant	A/A-G/G	12.(42.9%)	13(52%)	1.00	0.51	76.9	**80.8**
	A/G	16(57.1%)	12(48%)	0.69(0.23-2.05)			
Log-additive	---	---	---	**0.04(0.00-0.29)**	<0.0001	50.2	**54.1**

Also, in another analysis, the significance level of genotypes in the population was checked, which is shown in the table below (Table 4-11).

From Table 12, it is clear that all types of genotypes are significant in this population.

Turkish ethnicity

Also, in addition to Persian ethnicity, allelic and genotypic frequencies and genotypes analysis were performed in Turkish ethnicity, the tables of which are given below. Table 13 shows the ratio of frequency of alleles in both patient and control groups.

Table 13. Allele frequency ratio in both patient and control groups

Genotype allele frequencies(n=25)						
	All Subjects		Group=CA		Group=CO	
Allele	Count	Proportion	Count	Proportion	Count	Proportion
A	27	0.54	4	0.2	23	0.77
G	23	0.46	16	0.8	7	0.23

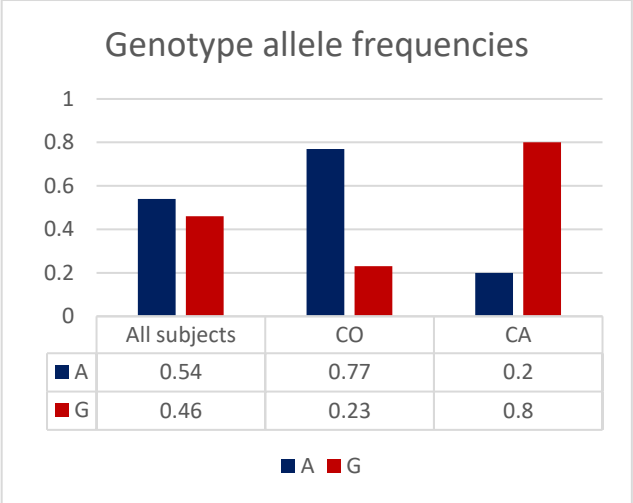


Chart 5 Comparative graph of frequency of alleles in two patient and control groups

Table 14 also shows the frequency values of genotypes in two groups, healthy and sick, and its graph is as follows:

Table 14 frequency values of genotypes in two healthy and diseased groups

Genotype genotype frequencies(n=25)						
	All Subjects		Group=CA		Group=CO	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
A/A	8	0.32	0	0	8	0.53
A/G	11	0.44	4	0.4	7	0.47
G/G	6	0.24	6	0.6	0	0

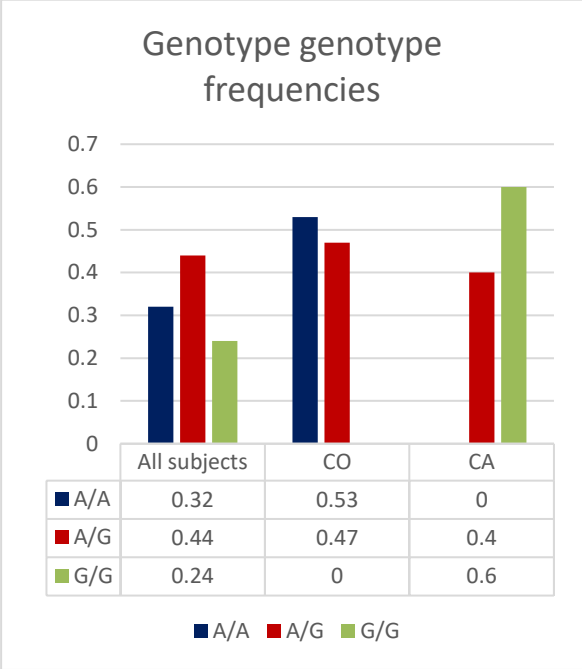


Chart 6 Comparative graph of the frequency of genotypes in two healthy and diseased groups

Table 15: Examination of the significance of genotypes in Turkish people

Genotype association with response Group)n=95,adjusted by mobility)							
Model	**Genotype**	**Group=CA**	**Group=CO**	**OR(95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	A/A	0(0%)	8(83%)	1.00	1e-04	20.4	**24.1**
	A/G	4(40%)	7(46.7%)	0.00(0.00-NA)			
	G/G	6(60%)	0(0%)	0.00(0.00-NA)			
Dominant	A/A	0(0%)	8(53%)	1.00	0.0011	27	**29.5**
	A/G-G/G	10(100%)	7(46.7%)	0.00(0.00-NA)			
Recessive	A/A-A/G	4(40%)	15(100%)	1.00	2e-04	23.6	**26**
	G/G	60(60%)	0(0%)	0.00(0.00-NA)			
Overdominant	A/A-G/G	6(60%)	8(53.3%)	1.00	0.74	37.5	**40**
	A/G	4(40%)	7(46.7%)	1.31(0.26-6.64)			
Log-additive	---	---	---	0.00(0.00NA)	<0.0001	18.4	**20.9**

Also, in another analysis, the significance level of genotypes in the population was checked, which is shown in the table below (15).

From this table, it is clear that all types of genotypes are significant in this population.

Principal component analysis: PCA

This method has two important functions: The first function of this method is that it determines the factors directly and without estimating commonalities from the correlation matrix. In this method, in order to explain the maximum amount of variance of the variables, their linear combination is estimated. In this way, the first component explains the most variance of the variables. Then, the second component explains the highest degree of residual variance in the variables after the first component, and so on until the end.

Another function of PCA principal component analysis is that it transforms a set of measured variables into a set of orthogonal linear combinations with the maximum amount of variance.

In this study, using R-package software, this analysis was done to check the separation of patient and control samples, whose diagram is shown below. This diagram confirms the results of Fst and shows that the patient and healthy samples They are completely separate.

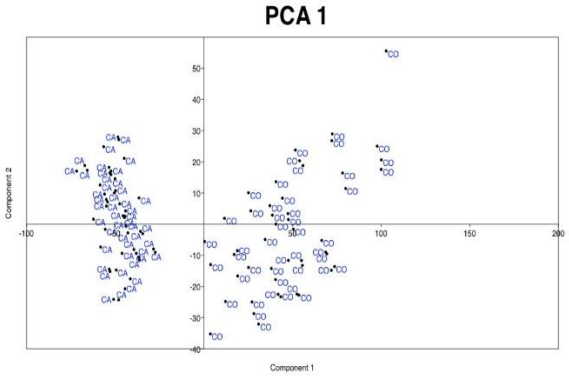


Chart 7: Graph related to principal component analysis between sick and healthy samples

According to the graph obtained, it was found that the patient and control groups are completely separate, and there is some diversity in the control group, while the patient sample group has no diversity and is completely similar. (Chart 4-7).

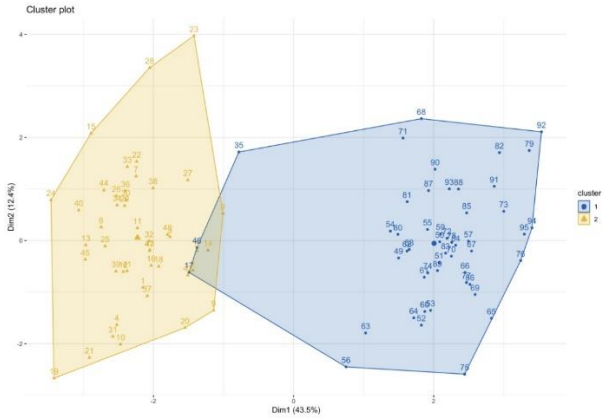


Figure 8: Separation analysis of sick and healthy groups based on clustering

The above diagram, which is a clustering pilot and drawn by R-Package software, also confirms the PCA diagram and shows this separation in a clustered form.

In addition to examining the genetic differentiation between the control and patient populations in two ethnicities, it is also possible to calculate the degree of differentiation between the first and third ethnicities, which is as follows:

Persian ethnicity: P: 0.455 q: 0.54

Turkish ethnicity: p: 0.48 q: 0.515

Persian: 2pq= 2(0.455)(0.54)= 0.4914

Turkish: 2pq= 2(0.48)(0.515) = 0.4944

$H_S = \frac{0.4914 + 0.4944}{2}$ =0.4929

P= 0.455 + 0.48 / 2= 0.4675

q= 0.54+0.515 / 2 = 0.5275

H_T = 2 (0.4675)(0.5275)= 0.4932

Fst = 0.4932 – 0.4929 / 0.4932 = 0.00006

As can be seen, the amount of Fst between Persian and Turkish populations is very low and little difference is observed between these two populations. According to the calculations that were done to check the genetic differentiation between ethnicities and also sick and healthy people in the same ethnicity, it was found that healthy and sick people are genetically different, but people between ethnicities do not show any difference.

Analyzing the separation of individuals and ethnicities by PCA method

In another analysis, the samples were analyzed based on their ethnicity using the PCA method, whose diagram is as follows: (Figure 4-9).

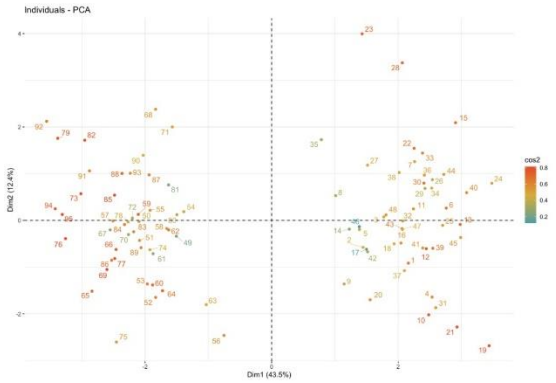


Chart 9 PCA analysis of samples based on ethnic groups

In this graph, it is clear that the samples based on different ethnicities are not related to each other and do not show any separation from each other. The above graph confirms the Fst results between the ethnicities and shows that the ethnicities do not have any genetic differences. In addition, the ethnic groups were also grouped, which did not show any separation according to the diagram below.

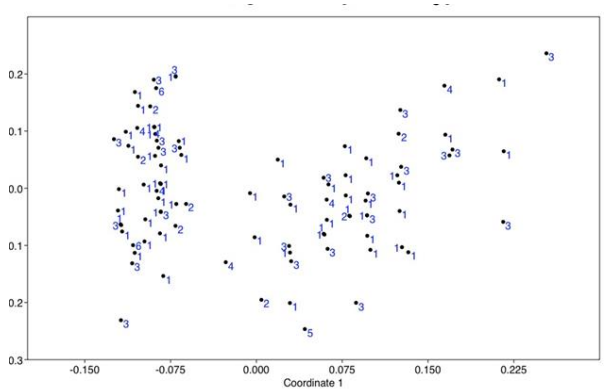


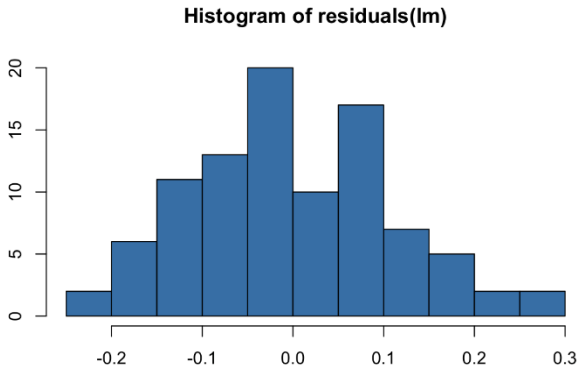
Chart 10 Grouping of ethnic separation

Regression

Table 16 multivariate logistic regression analysis of traits

Intercept	**2.609619**	**1.056722**	**-2.470**	**0.015505 ***
Sperm.NO	0.031529	0.006858	4.597	**1.46e-05 *****
Volume	0.076850	0.048829	1.574	**0.119187**
Total. No	-0.003431	0.001901	-1.805	**0.074596**
Mobility	0.013951	0.002228	6.262	**1.45e-08 *****
pH	0.209142	0.141095	1.482	**0.141920**
Viability	0.001772	0.002275	0.779	**0.438361**
ClassA	0.027208	0.006723	4.047	**0.000113 *****
Class B	0.001517	0.003096	0.490	**0.625297**
Class C	NA	NA	NA	**NA**

Multiple regressions were performed to investigate the relationship between the studied variables and infertility. In this way, all traits were checked one by one using R-package software and their significance or non-significance was checked with infertility and each trait along with their coefficient is given in Table 4-11.



The above table shows shows that 3 variables, sperm count, sperm movement, and class A are significant (star sign). As a result, these variables have an effect on the infertility of the studied people.

Importance of variables

Sperm.NO.	4.5972839
Volume	1.5738808
Total.No.	1.8048669
Mobility	6.2618978
pH	1.4822823
Viability	0.7785861
clasA	4.0471707
clasB	0.4901203

For example, the graph below shows the difference in sperm motility in the sick and healthy groups.

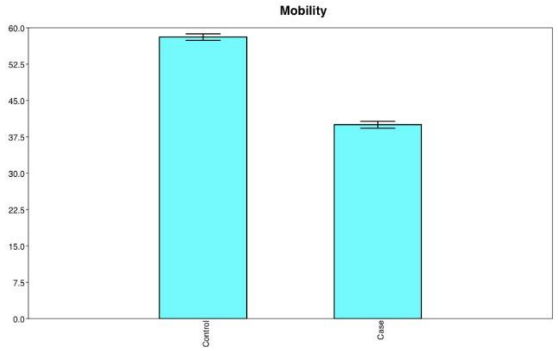


Diagram 11 multivariate logistic regression analysis of mobility attribute in sick and healthy samples

According to diagram 11, the amount of sperm movement is lower in the group of patient samples, and this shows the significance of this trait between these two groups.

After analyzing the significance of the variables, the regression formula was extracted and the conclusion was made.

Infertility = -2.60 + (0.03*Sperm.NO.) + (0.013*Mobility) + (0.027*clasA) ± e

From this formula and model, it can be predicted that the people in whom these 3 variables were measured and for Examples of values below are radars, how likely they are to be infertile.

The probability of a person being infertile [1] -1.588542 = -(-1.58) = 1.58٪

Correctness of time regression assumptions. It is confirmed that:

Residuals have a normal distribution. (Chart 12).

Chart 12: Chart of Residuals

Checking the appropriateness of the regression model

1. Calculation of the value of R2 (Squared R), which is the power of two coefficients of determination. This value varies from zero to 1, and a value of 1 or close to 1 indicates a linear relationship between the variables and the variable. The answer is (infertility).

2. Miscalculation of residual standard error (Residual Standard Error):

This value shows the dispersion of observations from the regression line, and the lower its value, the better and stronger the regression line explains the data, and the obtained model is a strong model.

Residuals

Residual standard error: 0.116 on 86 degrees of freedom

Multiple R-squared: 0.9512,

Adjusted R-squared: 0.9467

F-statistic: 209.7 on 8 and 86 DF, p-value: < 2.2e-16

The obtained model is a strong model, because the error value of the residuals is very low (0.11), the power of R is very strong (0.95), and the F test value is very high and the test is significant (0.0001). As a result, the obtained regression model is very suitable.

According to another type of PCA analysis, which is called PCA-Biplot, the separation of patient and control

group samples was done based on traits. This analysis was done by R-Package software. (Chart 13).

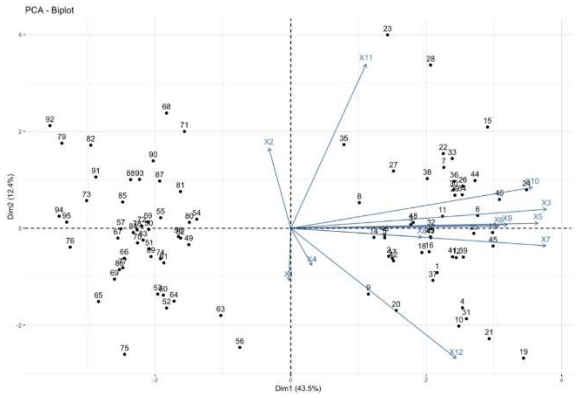


Chart 13: Separation of samples from the control and control groups based on traits

As shown in the graph, some traits caused more separation between the patient and control samples. For example, traits 3, 5, 7, 10, 9, 8, which respectively include smoking, volume, morphology, motility, pH and viability of sperms, have a greater effect on the separation of these two groups and put the patient group on the same side. As a result, this diagram confirms the results of the regression analysis.

In another type of this analysis, called Variable-PCA, the most varied traits have been examined, which means that the traits that cause diversity between the two groups were examined, and its diagram is given below: (Figure 14)

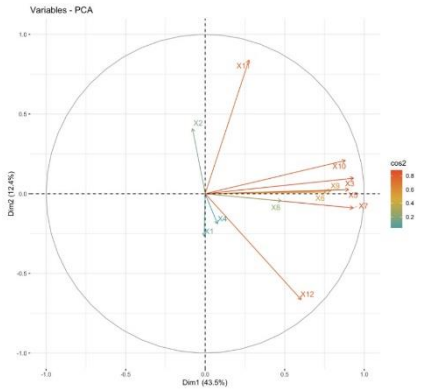


Figure 14: Variable-PCA analysis

According to this diagram, the lines that are placed around show the traits that cause diversity in the control and patient groups. which include traits number 1 and 2, which express the traits of age and ethnicity of people, respectively.

As it can be seen, the ethnicities are scattered among each other and no separation is observed among them, so it can be admitted that the SNP under investigation has no effect on the separation of ethnicities from each other.

Analysis by Heat-Map method

In this type of analysis, traits that are shown with a bolder color are more effective in creating distinction between people. (Chart 15)

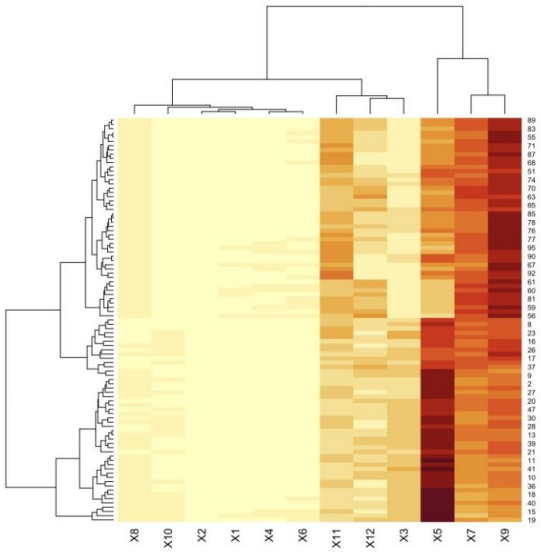


Diagram 15: Heat map examination of traits and their effect on infertility

According to the diagram above, attributes 9, 5, and 7 have a greater effect in separating people and placing them in a group. In general, the attributes that have caused people to be placed in a cluster have a greater role in separating them from each other.

UPGMA method

This method is one of the most common clustering methods and is based on the average distance of populations. In this method, the length of the clusters is drawn at the same level, and the length of the two clusters is considered equal based on the molecular clock model. In this study, UPGMA and PCA classification charts were drawn to separate the boundaries between patient and control samples, and both charts confirm each other and the patient and control groups are completely separate.

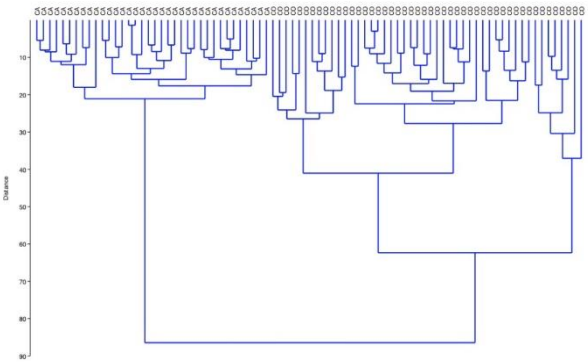


Diagram 16: Dendrogram of the separation of sick and healthy groups

Discussion

In this study it was demonstrated that the rs10966811 has significant association with the azoospermia in Iranian population.rs10966811 is located In the intragenic region between the two genes is 445.4 kb downstream of TUSC1 and 687.8 kb upstream of the IZUMO3 gene.Therefore, we speculate that TUSC1 or IZUMO3 may be contribute to spermatogenesis, and assessed whether TUSC1 and IZUMO3 polymorphisms are related to male infertility. In this study, it was found that the trait of sperm motility is different in sick and healthy individuals and is significant in the dominant and over dominant states. An association with oligozoospermia has been found with rs12376894, which is located 445.4 kb downstream of TUSC1.(14). The expression of genes both nearby and distant to SNPs in noncoding regions (intronic, intergenic, etc.) has been shown to be influenced by SNPs in noncoding regions (15). Liu et al. reported that rs10129954 is strongly associated with idiopathic male infertility (asthenozoospermia, oligozoospermia, and oligoasthenozoospermia) in Chinese Han people (16). It has been shown that EPSTI1-rs12870438 and PSAT1-rs7867029 are both involved in severe oligospermia, while USP8-rs7174015 may contribute to nonobstructive azoospermia susceptibility.There was also an association between the minor allele of TUSC1-rs10966811 and a higher likelihood of TESE success for hypospermatogenesis-Nonobstructive azoospermia subphenotype.(17). The status of this polymorphism was studied between two groups of Iranian ethnic groups, including Turks and Persians, and it was found that in both ethnicities, genotypes are significant in dominant and codominant states. The Fst value in Persian ethnicity is equal to 1.8074 and 0.32281 in Turkish ethnicity. According to the FST test in Fars ethnicity, the two groups of oligosperm and healthy showed a high difference with each other. Totally based on Principal component analysis (PCA) method the case and control group are different from each other. Genetic variants of this type might result in varying phenotypes related to male fertility, depending on the individual's genetic background. The concept of idiopathic male infertility is based on the notion that it is a complex disease. It can lead to mild outcomes (such as a slight reduction in sperm counts or low birth rates) to more severe conditions like severe oligospermia, which includes nonobstructive azoospermia, which supports the notion.(18).

Conclusion

In conclusion the study of the association of this polymorphism with infertility in the Iranian male population was performed for the first time and during it, it was found that the two groups of patients and controls are genotypically different. In studies conducted in Japan, genotypes showed significance in the recessive state, while this significance was not observed among the Iranian population.

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