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A PRELIMINARY REPORT ON POPULATION GENETICS ANALYSIS OF TWO CRISP2 GENE' SNPS IN RELATION TO ASTHENOSPERMIA INFERTILITY: COMPUTATIONAL AND PHYLOGENETIC APPROACHES

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Asthenospermia (AZS) is a cause of infertility in men in which the motility of the sperm is reduced. CRISP2 genes' SNPs may be involved in this type of infertility and reach on association studies for these SNPs are going on in different parts of the world. The present study was conducted to investigate association between two SNPs of gene CRISP2 namely, rs1301440109, and rs-760204944, with asthenospermia in Iranian samples. For this study, 83 infertile men suffered from asthenospermia and 40 fertile males with normal karyotype and semen analysis were studied, by using tera-arms method. We performed different computation methods on both genetic data, sequences, as well as clinical features related to sperm characteristics. Genotypig of the studied samples showed the presence of two different alleles in both SNPs studio. The frequency of the genotypes and the alleles obtained are presented, with T alleles as the most frequent allele in both SNPs studied. Logistic regression showed only a significant association between rs1301440109 and infertility. Sequencing of the target SNPs along with their neighboring sequences, compared to the ancestral sequences, showed a significant sequence divergence for the indigenous individuals with positive significant Tajimas' D statistic showing adaptive change in these sequences for the rs 1301440109. Linear discriminant analysis (LDA), differentiated the case and control individuals in the rs1301440109,

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based on clinical features studied. Redundancy analysis (RDA), did not produce a significant association between the genotypes obtained and geographical variables.

Key words: Asthenospermia, Iran, Logistic regression, RDA, sequence divergence, SNPs

INTRODUCTION

Asthenospermia (AZS) is a cause of infertility in men in which the motility of the sperm is reduced. It is defined as a condition with <40% sperm motility or less than 32% with progressive motility.

The human CRISP2 gene contains 10 exons and spans over 21 kb on chromosome 6p21 (http://www.genenames.org/data/hgnc_data.php?hgnc_id=12024;http://www.ncbi.nlm.nih.gov/sites/entrez). So far, a few genes have been associated with AZS; however, in most of the cases, its molecular etiology is unclear (HEIDARY *et al*. 2019). However, down-regulation of CRISP2 is reported to be associated with AZS, which could be suggested as the potential candidate genes for the development of a diagnostic marker or potentially for more studies for treatment of AZS (HEIDARY *et al*. 2019).

Low sperm motility is a frequent accompaniment of oligospermia and is often also associated with a mixed picture of morphologic defects suggesting defective spermiogenesis. Asthenospermia may also be associated with sperm autoimmunity. The causes of other motility defects of moderate degree are unidentified (JAMESON *et al*. 1996; HEIDARY *et al*. 2019).

AZS could be seen as a pure isolated condition or could be coupled with additional sperm abnormalities. isolated form of azs is considered as one of the causes of infertility in men, approximately accounts for 20% of infertile men and in more than 60% of cases this condition is associated with decreased number of sperm (oligoasthenozoospermia) and abnormal sperm morphologies (oligoasthenoterato- and asthenoteratozoospermia) (HEIDARY *et al*. 2019).

Differentially expressed genes including CATSPER1 (Cation Channel Sperm Associated) in mouse sperm, and PATE1 (Prostate and Testis Expressed), SEMG1 (Semenogelin 1), and CRISP2 (Cysteine-Rich Secretory Protein), in human are related to AZS, and CRISP2 is the only member of CRISP family which is expressed in the testis in an androgen-independent manner (HEIDARY *et al.* 2018).

CRISP2 protein is located in the acrosome and the outer dense fibers of the sperm tail (KRATZSCHMAR *et al.* 1996). This protein may be secreted from the acrosome during the acrosome reaction or be implicated in sperm-egg fusion (26) and it may modulate sperm flagellar motility. CATSPER1 is a voltage-gated Ca2+-permeable channel specifically expressed in the sperm flagellum especially on the plasma membrane of sperm tail and in a murine model of AZS, up-regulation of CATSPER1 increased the sperm intracellular Ca2+ concentration, sperm concentration, and percentages of sperm activity and overall sperm motility. It seems that CATSPER1 is essential for sperm motility and hyperactivation through regulation of calcium concentration (MIZUKI *et al.* 1992, HAENDLER *et al*. 1997, LUO *et al*. 2013, YU *et al.* 2014, ZHOU *et al.* 2015).

Iran has a high rate of infertility (20-25%), and a limited study are yet concerned with genetics and ethnic groups involved in this matter (MOGHBELINEJAD *et al.* 2018). Therefore, the present study was carried out to investigate association of a few SNPs in CRISP2 gene which is

involved in Asteospermia infertility. Here we report association two SNPs which were selected randomly and also take into account association between geographical variables of ethnic groups of patients and both clinical features studied, the genotypes obtained and azoospermia itself. This is a first report of this kind of landscape genetics from the country.

We used different computational methods like redundancy analysis (RDA), and logistic regression, in this study.

MATERIALS AND METHODS

DNA samples

Eighty- three infertile men with normal karyotype suffered from astheospermia and 40 fertile males with normal karyotype and semen analysis based on World Health Organization were participated in current study. The Blood samples were collected from Jihad of Qom University (informed consent was obtained from all participate). The standard salting out method was used for extraction of genomic DNA from blood samples. Quality of DNA samples were examined by 1% agarose gel electrophorese.

Tetra ARMS-PCR

PCR reaction was carried out in total volume 25 µl containing 4 µl of DNA samples, 21 µl Master Mix (H2O, Buffer 10X, MgCl2 100 mM, dNTP-Mix 40 mM, Forward primer, Reverse primer and Taq polymerase). Then, PCR was performed in thermo cycler system (Genetix Biotech, Australia). The PCR fragments were separated by 1% agarose gel electrophoresis and visualized by DNA power load.

Clinical features studied

The following characteristics were studied in selected cases and controls (Table 1).

Table1. Clinical features of the studied samples.

No	case/ control	Ethnic	Age	Sperm /Ml	Volume	Sperm number	Morohology	Mobility	PH	Viability	clasA	clasB	clasC
1	control	Tork	34	25	4	100	5	70	7.2	80	10	35	25
2	control	Fars	33	30	4	120	6	65	7.3	75	5	35	25
3	control	Fars	33	35	4	140	6	60	7.3	65	10	30	20
4	control	Fars	35	35	3	105	5	65	7.2	65	5	30	30
5	control	Tork	34	30	5	150	7	55	7.3	70	5	25	25
6	control	Kord	28	35	4	140	5	65	7.2	75	10	30	25
7	control	Arab	33	35	4	140	5	65	7.3	70	10	25	30
8	control	Fars	32	35	3	105	4	70	7.2	75	5	25	40
9	control	Tork	32	35	4	140	5	65	7.2	70	10	35	20
10	control	Fars	34	30	5	150	5	55	7.4	65	10	25	20
11	control	Fars	35	35	4	140	7	65	7.2	70	5	30	30
12	control	Kord	32	30	3	90	7	60	7.2	70	10	35	15
13	control	Tork	31	30	3	90	5	55	7.2	70	5	30	20
14	control	Fars	29	30	4	120	5	60	7.4	70	10	30	20

15	control	Fars	29	35	3	105	6	70	7.2	65	10	30	30
16	control	Fars	29	30	4	120	5	70	7.3	65	5	30	35
17	control	Lor	28	35	3	105	7	55	7.4	75	10	35	10
18	control	Fars	30	35	3	105	7	75	7.3	70	5	30	40
19	control	Fars	32	30	3	90	5	65	7.3	65	10	30	25
20	control	Fars	30	25	2	50	5	70	7.2	70	10	30	30
21	control	Fars	32	40	3	120	5	70	7.5	65	5	35	30
22	control	Fars	32	30	3	90	7	75	7.3	65	10	30	35
23	control	Gilaki	31	30	4	120	7	55	7.4	75	10	30	15
24	control	Lor	33	30	4	120	5	70	7.3	75	10	20	40
25	control	Fars	32	35	4	140	5	55	7.2	65	5	25	25
26	control	Fars	33	30	3	90	5	65	7.3	65	10	30	25
27	control	Tork	33	35	3	105	5	55	7.2	65	5	25	25
28	control	Lor	30	35	4	140	5	65	7.1	65	5	30	30
29	control	Fars	32	25	3	75	5	65	7.3	65	10	25	30
30	control	Tork	33	35	3	105	5	55	7.3	65	10	30	15
31	control	Fars	32	35	3	105	5	65	7.3	75	10	35	20
32	control	Tork	32	35	3	105	6	70	7.2	65	10	25	35
33	control	Fars	34	35	3	105	6	65	7.3	70	5	25	35
34	control	Tork	28	30	4	120	5	55	7.2	70	10	30	15
35	control	Lor	29	35	4	140	7	65	7.3	70	5	35	25
36	control	Lor	33	35	3	105	7	65	7.3	70	5	35	25
37	control	Fars	32	35	3	105	5	55	7.3	65	5	25	25
38	control	Fars	33	30	3	90	5	65	7.3	65	10	30	25
39	control	Tork	32	35	3	105	6	65	7.2	65	10	25	35
40	control	Lor	30	35	4	105	6	65	7.1	65	5	30	35
41	case	Fars	32	30	5	150	6	30	7.4	75	5	10	15
42	case	Fars	28	25	5	125	5	25	7.2	70	0	10	15
43	case	Fars	32	25	3	75	4	25	7.3	65	5	5	15
44	case	Fars	33	35	3	105	5	30	7.3	65	5	10	15
45	case	Arab	29	30	4	120	4	25	7.3	65	0	15	10
46	case	Lor	29	25	5	125	4	25	7.4	60	0	10	15
47	case	Fars	29	35	3	105	6	35	7.3	65	5	15	15
48	case	Tork	34	25	4	100	4	25	7.4	65	5	10	10
49	case	Tork	28	20	3	60	6	35	7.2	65	0	10	25
50	case	Arab	30	30	3	90	4	30	7.3	60	0	15	15
51	case	Fars	35	25	3	75	4	20	7.4	65	0	5	15
52	case	Tork	30	20	4	80	4	30	7.3	65	0	10	20
53	case	Tork	30	30	4	120	4	25	7.4	65	0	10	15
54	case	Fars	30	25	4	100	5	30	7.3	60	0	10	20
55	case	Fars	31	30	3	90	4	25	7.3	60	0	15	10

56	case	Tork	30	30	3	90	4	25	7.2	65	0	5	20
57	case	Tork	31	30	4	120	5	25	7.4	70	5	10	10
58	case	Tork	30	30	3	90	5	25	7.3	70	0	10	15
59	case	Arab	32	40	3	120	5	25	7.2	65	0	15	10
60	case	Fars	31	25	3	75	5	30	7.3	60	5	10	15
61	case	Tork	32	30	4	120	5	35	7.3	65	0	10	25
62	case	Fars	31	30	3	90	4	30	7.1	65	5	10	15
63	case	Fars	33	35	5	175	7	35	7.3	70	0	10	25
64	case	Fars	32	30	3	90	4	25	7.2	70	5	10	10
65	case	Fars	33	30	5	150	7	30	7.2	70	0	15	15
66	case	Fars	31	30	3	90	5	30	7.4	70	0	10	20
67	case	Fars	28	30	3	90	5	30	7.2	60	0	15	15
68	case	Fars	31	30	3	90	4	25	7.5	60	0	15	10
69	case	Kord	33	25	4	100	4	25	7.3	60	5	15	5
70	case	Lor	31	30	4	120	5	25	7.4	65	5	10	10
71	case	Fars	32	35	3	105	5	30	7.4	65	5	15	10
72	case	Fars	34	25	3	75	5	25	7.2	70	0	10	15
73	case	Tork	31	25	3	75	4	30	7.3	70	0	5	25
74	case	Fars	30	25	4	100	4	35	7.3	65	5	10	20
75	case	Gilaki	31	30	4	120	4	30	7.3	65	0	5	25
76	case	Fars	31	25	3	75	5	25	7.3	60	0	10	15
77	case	Kord	28	25	5	125	4	35	7.2	65	0	10	25
78	case	Tork	30	25	4	100	4	25	7.2	70	5	5	15
79	case	Fars	32	25	3	75	4	25	7.2	60	5	15	5
80	case	Fars	30	25	4	100	4	25	7.2	70	0	10	15
81	case	Fars	29	30	3	90	4	30	7.4	65	0	15	15
82	case	Lor	30	30	3	90	5	30	7.2	60	5	10	15
83	case	Fars	33	30	3	90	5	25	7.3	65	5	10	10
84	case	Kord	30	30	3	90	4	30	7.2	60	5	15	10
85	case	Fars	32	25	3	75	4	25	7.2	65	0	15	10
86	case	Tork	31	25	3	75	5	30	7.1	70	0	15	15
87	case	Tork	30	20	3	60	4	30	7.2	70	0	10	20
88	case	Lor	29	25	3	75	5	20	7.4	60	0	10	10
89	case	Fars	30	30	3	90	5	30	7.2	70	5	20	5
90	case	Gilaki	28	25	3	75	4	25	7.2	60	0	15	10
91	case	Fars	28	25	4	100	4	25	7.2	65	5	10	10
92	case	Tork	34	30	3	90	7	30	7.3	65	5	10	15
93	case	Lor	34	30	4	120	5	20	7.3	60	5	10	5
94	case	Kord	28	30	3	90	5	35	7.3	65	0	15	20
95	case	Kord	30	30	3	90	4	20	7.2	70	0	15	5
96	case	Tork	32	25	4	100	5	35	7.2	65	0	10	25

97	case	Lor	33	25	3	75	5	30	7.3	60	0	15	15
98	case	Tork	32	35	3	105	5	25	7.3	70	0	10	15
99	case	Lor	32	25	3	75	5	30	7.3	60	5	15	10
100	case	Fars	29	30	3	90	5	30	7.3	65	0	15	15
101	case	Fars	30	35	3	105	5	20	7.4	60	0	15	5
102	case	Tork	30	25	3	75	5	25	7.2	65	5	5	15
103	case	Fars	30	30	3	90	5	30	7.3	60	5	15	10
104	case	Fars	32	30	3	90	4	25	7.2	65	0	5	20
105	case	Kord	32	35	4	140	5	25	7.3	75	5	15	5
106	case	Tork	30	35	4	140	6	30	7.4	65	0	10	20
107	case	Tork	30	30	3	90	4	30	7.2	65	0	10	20
108	case	Fars	29	35	3	105	4	25	7.2	65	0	10	15
109	case	Fars	30	30	3	90	4	30	7.3	60	0	10	20
110	case	Fars	29	30	3	90	5	30	7.3	65	0	15	15
111	case	Tork	33	30	4	120	6	35	7.3	65	0	15	20
112	case	Kord	31	25	3	75	4	30	7.3	60	0	10	20
113	case	Fars	32	30	3	90	5	20	7.4	60	0	10	10
114	case	Fars	33	25	3	75	5	25	7.3	65	5	15	5
115	case	Fars	30	20	5	100	4	30	7.3	60	0	10	20
116	case	Fars	30	30	3	90	4	35	7.2	60	5	5	25
117	case	Tork	30	25	3	75	4	25	7.2	70	0	10	15
118	case	Fars	29	25	4	100	4	20	7.3	60	0	5	15
119	case	Fars	30	25	3	75	4	25	7.2	60	0	10	15
120	case	Tork	31	30	4	120	4	25	7.3	65	0	5	20
121	case	Fars	30	25	4	100	5	35	7.3	65	0	10	25
122	case	Fars	30	30	4	120	4	25	7.3	65	0	10	15
123	case	Fars	30	25	4	125	5	25	7.3	60	0	10	15

Data analyses

Genotyping and association studies

The frequency of different genotypes was estimated and compared with available data from the other parts of the world. We used logistic regression to study association between the SNPs and Asthenospermia. For this we used SNP-STAT software online. Moreover, RDA was used to investigate association between geographical variables and the genotypes as well as the infertility versus fertility of the studied people.

SNP sequencing and phylogenetic analyses

We sequenced a random samples of control as well as case individuals and carried out a preliminary phylogenetic analysis of these data along with ancestral sequence of the same SNPs from ensemble data center (https://asia.ensembl.org).

We aligned the sequences (the SNP and neighboring sequences) by Muscle program and after thriving and removing the gaps, carried out clustering by unweighted paired group using average method (UPGMA), as well as maximum likelihood method (ML), followed by 100 times bootstrapping.

Genetic distance between the studied samples were performed based on Kimora 2-parameters. Similarly, Tajimas, D test of neutrality was performed both within the Iranian samples sequenced, as well as between indigenous samples and ancestral sequences. These analyses were carried out in MEGA 11 software.

To reveal molecular significant change in DNA content, AMOVA (Analysis of molecular variance), was performed between the indigenous samples and ancestral sequences. These analyses were carried out in Adegenet and pegas packages of R 4.2.2.

Clinical features analyses

We used the linear discriminant analysis (LDA), to differentiate the case and control individuals as well as the genotypes (heterozygote versus homozygote individuals), based on clinical features studied. We used principal components analysis (PCA), to compare clinical features among control and infertile individuals and identify the most variable features among these groups. Moreover, we used RDA analysis to study association between geographical variables of the individuals from ethnic areas with clinical features.

RESULTS

Genotyping, allele frequency and association studies

Rs1301440109

In total, for rs1301440109, we obtained 23% heterozygote (TC), and 77% homozygote (TT) individuals. These values were 0.34%, and 66%, in asthenospermia individuals, respectively (Table 2).

Table 2. The genotypes' frequency in control and case individuals studied.

SNP Rs1301440109 genotype frequencies (n=123)						
	All subjects		Status=case		Status=control	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
T/C	28	0.23	28	0.34	0	0
T/T	95	0.77	55	0.66	40	100

Table 3. The allele frequencies obtained in the studied samples.

SNP2 Rs1301440109 genotype frequencies (n=123)						
	All subjects		Status=case		Status=control	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
T	218	0.89	138	0.83	80	1
C	28	0.11	28	0.17	0	0

Similarly, the frequency of different alleles observed are provided in Table 2. Allele T comprised 0.89 of total alleles in the studied population, while allele C formed 0.11% in total. These values in the infertile samples were 0.83, and 0.17, respectively, while the T allele was observed in all the studied control individuals (100% presence) (Table 3).

The Hardy-Weinberg test for equilibrium, did not produce a significant difference in total number of the individuals studied, as well as for both the case and control samples ($p>0.1$). Therefore, the studied samples are not deviated from a normal population.

Logistic regression analysis of the haplotype association for the rs 1301440109, is provided in Table 4. A significant association ($p<0.0001$), was observed between the studied SNP and infertility. This result and the frequency of the alleles presented before, suggest the role of allele T in causing the infertility in causing infertility of the studied samples.

Table 4. Logistic regression result of rs1301440109 and the infertility.

SNP2 Rs1301440109 association with response status (n=123, crude analysis)						
Model	Genotype	Status= case	Status=control	OR (95% CI)	P-VALUE	AIC BIC
---	T/T	55 (66.3%)	40 (100%)	1.00	<0.0001	133.3138.9
	C/T	28 (33.7%)	0 (0%)	0.00 (0.00-NA)		

Rs760204944

We obtained only 6 heterozygote individuals (TC), for the rs760204944, in total samples studied, with 3 persons and only comprising 0.02% in the control as well as infertile individuals (Table 4). These values for homozygotes (TT), were 120 persons in total with 96%, and 100% frequency in the case and control individuals (Table 5).

Table 5. The genotype frequencies in control and case individuals studied for rs760204944.

SNP Rs760204944 genotype frequencies (n=123)						
	All subjects		Status=case		Status=control	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
T/C	3	0.02	3	0.04	0	0
T/T	120	0.98	80	0.96	40	1

The frequency of different alleles for rs-760204944. are provided in Table 5. The allele T comprised 0.99 of total alleles in the studied population, while allele C formed 0.01 in total. These values in the infertile samples were 0.98, and 0.02, respectively, while the T allele was observed in all the studied control individuals (100% presence) (Table 6).

T*able 6. The frequency of different alleles in rs760204944, in the studied samples*

SNP Rs760204944 genotype frequencies (n=123)						
	All subjects		Status=case		Status=control	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
T	243	0.99	163	0.98	80	1
C	3	0.01	3	0.02	0	0

The Hardy-Weinberg test for equilibrium, did not produce a significant difference in total number of the individuals studied, as well as for both the case and control samples ($p>0.1$). Therefore, the studied samples are not deviated from a normal population.

Logistic regression analysis of the haplotype association for the rs-760204944, did not produce A significant association ($p = 0.12$), (Table 7), and therefore, the result obtains suggest that this rs is not causing the infertility in case Samples studied.

Table 7. Logistic regression analysis result of rs760204944, and infertility in the studied samples.

SNP2 Rs760204944 association with response status (n=123, crude analysis)					
Model Genotype Status= case Status=control OR (95% CI) P-VALUE AIC BIC					
--- T/T	80 (96.4%)	40 (100%)	1.00	0.12	156.8162.4
C/T	3 (3.6%)	0 (0%)	0.00 (0.00-NA)		

Phylogenetic analyses

The final sequences length obtained for rs1301440109 SNP and the neighboring sequences was 113 bp. The nucleotide diversity(pi) within these sequences was 0.195, with number of segregating sites =51, and number of parsimony-informative sites = 42. The final sequences length obtained for rs 760204944 SNP and the neighboring sequences was 372 bp. The Nucleotide diversity of the studied sequences (pi), was 0.230, with the number of segregating sites = 167, and the number of parsimony-informative sites = 160.

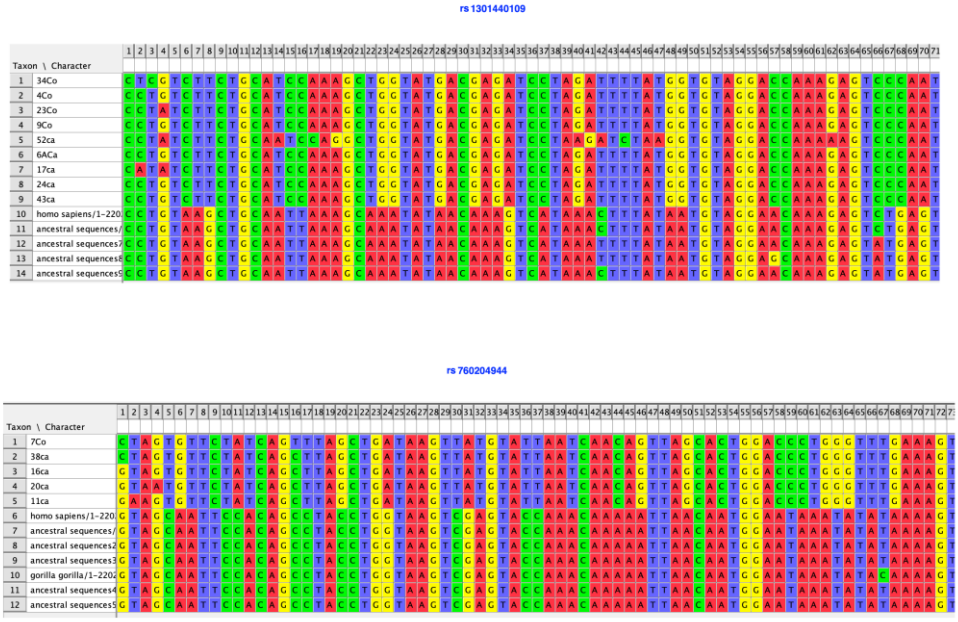

Fig. 1. Representative sequences obtained after alignment and thrumming from both of the studied SNPs.

Both UPGMA and ML methods of phylogenetic tree construction produced similar results in both SNPs sequence data (Fig. 2). These phylogenetic trees reveal that the sequences obtained for both studied SNPs and neighboring sequences differ from the ancestral sequences as they comprise two different separate clusters. Moreover, AMOVA test for DNA sequences produced significant difference ($p<0.01$), between these two cluster groups. Therefore, these results indicate that the genetic regions (sequences), of the indigenous samples have undergone significant changes during population divergence. However, fertile and infertile individuals did not differ significantly in their sequence content.

This is interesting to cite that Tajimas' D test of neutrality produced significant positive D value for both the studied SNPs and between the studied samples (D = 1.65, with p value = 0.05, and D = 2.57, with p value = 0.001, for rs1301440109, and rs 760204944, respectively). These values suggest that the above said sequences changes were of adaptive type. However, Tajimas'D statistics among the samples studied in Iran, was negative (D= -1.65, and -1.54, respectively), and were not significant (P>0.05). These results indicate that the sequence changes within limited Iranian samples studied are not adaptive.

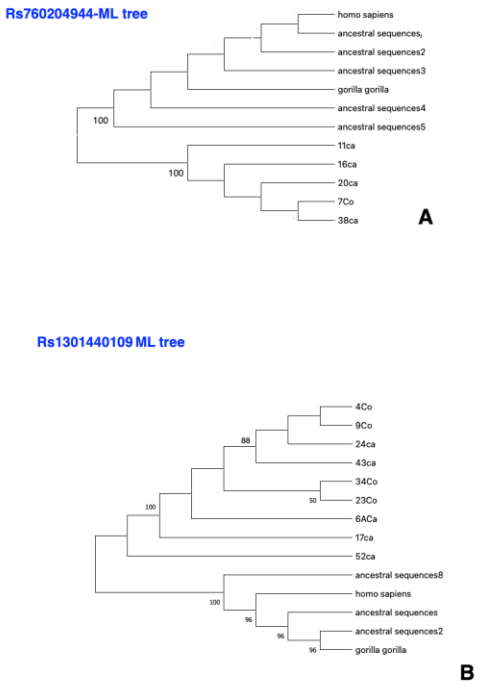

Fig. 2. ML phylogenetic tree of the sequences obtained in both rs1301440109, and rs760204944, showing significant sequence divergence in indigenous samples studied.

Clinical features analyses

LDA analysis of clinical features between case and control samples, as well as the genotypes obtained in bot studied SNPs, are presented in Fig. 3. The separation of case and control individuals based on clinical features in rs1301440109, is in accord with the logistics analysis of the same SNP, showing significant association with infertility (Fig. 3, A). However, the genotypes (heterozygotes versus homozygotes), are not differentiated based on the same features (Fig. 3. B). This is in accord with the effect of allele T, in causing infertility, as this allele is present in both genotypes studied.

The same plots (Fig. 3. C, and D), concerned with rs760204944, revealed that neither case and control individual, nor the genotypes studied differ in their clinical features studied. This is again in a chord with the non-significant association between rs760204944 and asthenospermia.

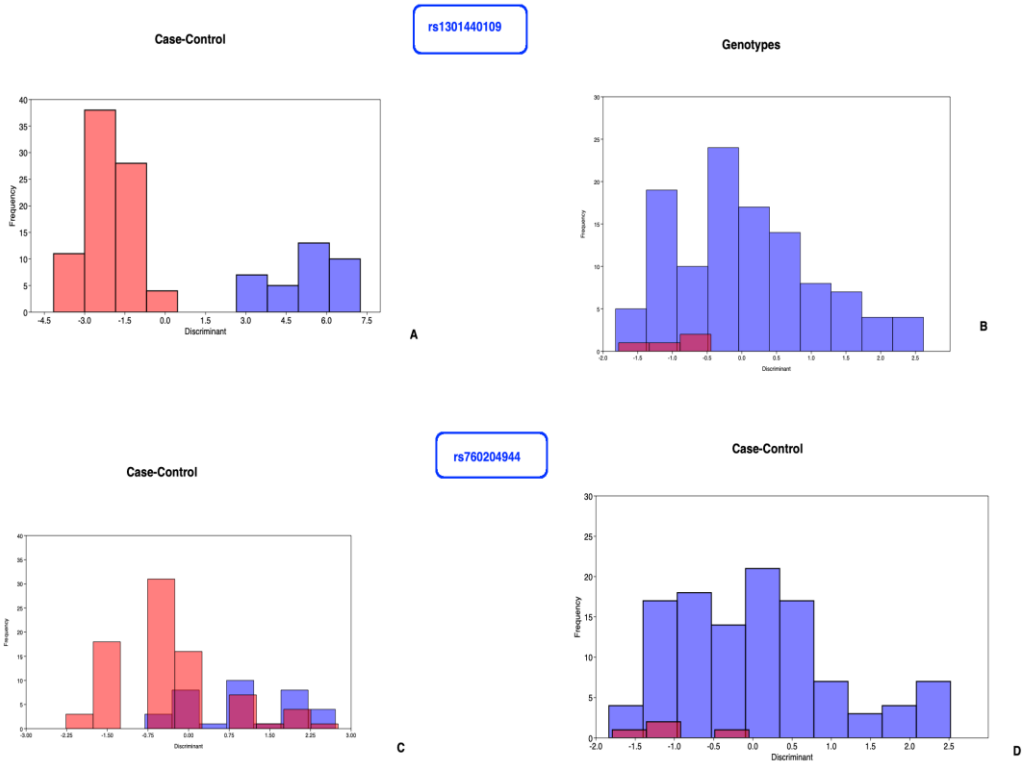

Fig. 3. LDA plots of case versus control individuals (A, and C), as well as genotypes (heterozygotes versus homozygotes, B, and D), in the studied SNPs.

PCA-Biplot of the clinical features in both studied SNPs are presented in Fig. 4. A, and B. In rs1301440109, two clinical features related to sperm characteristics, viz. sperm mobility, and total sperm number, are differentiating features Among the case and control individuals (Fig. 4. A). However, in rs 760204944, with no separation between case and control individuals, there are also no differencing clinical features identified (Fig. 4. B).

RDA analysis of clinical features between case and control samples, as well as the genotypes obtained in bot studied SNPs, did not produce significant association ($p>0.05$), between geographical variables (Latitude, longitude and altitude) and clinical features (Fig. 5, A, and B). Therefore, although the clinical features related to the sperm characteristics did differ between case and control samples studied, they were not under the influence of geographical areas in which the samples were taken.

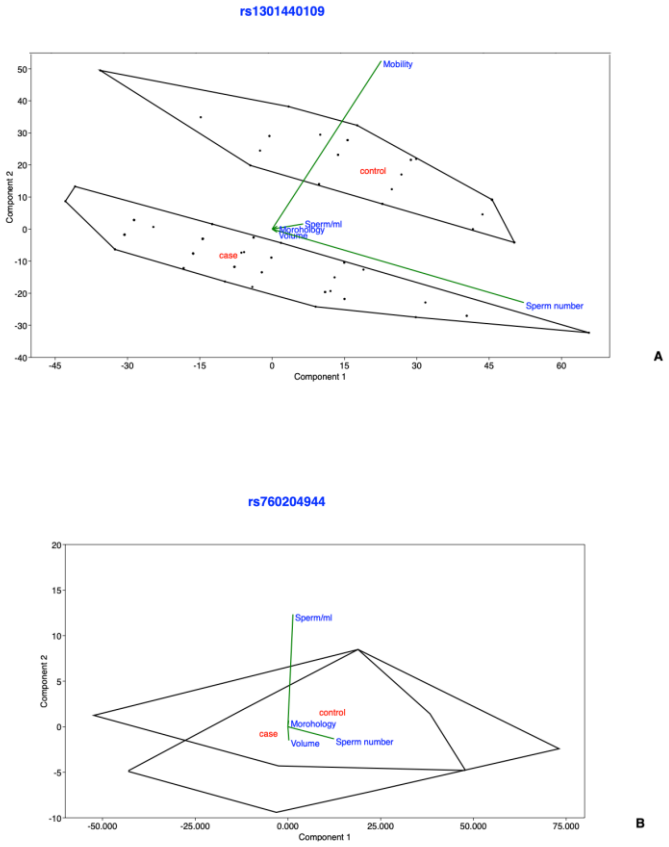

Fig. 4. PCA biplot of clinical features among case and control individuals showing the most variable features in the studied SNPs.

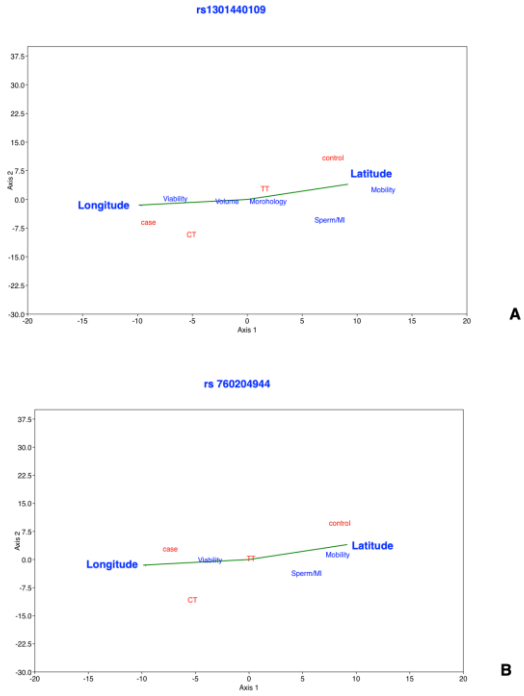

Fig. 5. RDA plots (A, and B), of the samples studied based on case versus control, and the heterozygotes versus homozygotes, and geographical variables.

DISCUSSION

Genotype and allele frequencies

The present study reports the genotyping and allele frequencies of two SNPs namely, rs1301440109, and rs760204944, from the gene CRISP2 in a sample of Iranian population.

In both SNPs, two types of alleles T and C, have been reported in the world as evidenced in ensemble site of SNP information. The allele T is considered to be ancestral and the allele C with its highest population frequency is MAF = < 0.01. Almost different populations studied world-wide have the same frequency as we also reported in present study. The present study, revealed a significant association between rs1301440109 and asthenospermia, while rs760204944, did not show a significant association.

AZS, a cause of infertility in men, could be caused by dysfunction of energy metabolism or structural defects in the sperm-tail proteins and the sperm motility proteins. Despite the advances in etiology of male infertility, the molecular mechanisms that impair sperm motility in most cases are unclear. The mRNA expression analysis of four candidate genes including CRISP2, CATSPER1, SEMG1, and PATE1 in the sperm of men with AZS and control

groups showed that down-regulation of CRISP2 (p=0.036) and up-regulation of SEMG1 (p= 0.03), are associated with AZS (JAMSAI, *et al*. 2008).

JAMSAI, *et al*. (2008), studied CRISP2 variations and their contribution to male infertility. They screened coding and flanking intronic regions in 92 infertile men with asthenozoo- and/or teratozoospermia and 176 control men using denaturing HPLC and sequencing. They identified 21 polymorphisms, with three SNPs namely, L59V, M176I and C196R, which resulted in amino acid substitutions. The C196R substitution resulted in the loss of CRISP2–GGN1 binding, and therefore, these authors concluded that, the C196R polymorphism may compromise CRISP2 function (JAMSAI, *et al.* 2008).

In present study we reported sequence difference between both control and case individuals studied which contained the SNP and its neigh ring sequences. We also showed a significant difference in sequence content between indigenous samples studied and ancestral sequences, as evidenced by AMOVA. However, fertile and infertile individuals did not differ significantly in their sequence content. These sequences show adaptive change between the same two groups by TAJIMAS' D statistics and no negative and non-adaptive sequence change among indigenous individuals (both fertile and infertile).

In a similar study, JAMSAI, *et al*. (2008), reported several polymorphisms within the non-coding region of the CRISP2 gene, but their frequency was not significant between infertile and fertile men.NACHMAN *et al.* (1998), sequenced 11,365 bp from introns of seven X-linked genes in 10 humans, one chimpanzee, and one orangutan and reported the average value for pi as low as 0.063% with Standard error = 0.036%. Among the studied loci, pi value varied by over one order of magnitude. These authors suggested that the joint effects of selection and linkage are important in shaping patterns of nucleotide variation in human.

We reported a preliminary analysis of association between geographical variables and the genotype and allele frequency in the case and control individuals, as well as association with clinical features. We obtained no significant association in RDA analysis.

Reports in many regions of the world, show the presence of isolation by distance (IBD), between human genetic differentiation and geographic distance. Therefore, with an increase in geographical distance, an increase in magnitude of genetic differentiation (see for example, BRADBURD and RALPH 2019; BATTEY *et al*. 2019);

However, due to the complexities of geography and history, this relationship varies across the globe, for example, ROSENBERG *et al.* (2002), study by using micro-satellite markers produced a broad geographic clustering, suggested that the human fine-scale genetic variation is better characterized by discrete clusters or continuous clines.

DENG *et al.* (2014), conducted a genome-wide study using over 900,000 single nucleotide polymorphisms (SNPs) in four major Malaysian ethnic groups and made comparisons of 17 world-wide populations. They reported that Peninsular Malaysia has greater genetic diversity corresponding to its role as a contact zone of both early and recent human migrations in Asi, and concluded that a long isolation period, subsequent gene flow and local adaptations have jointly shaped the genetic architectures of MEGs, and this study provides insight into the peopling and human migration history in Southeast Asia.

At present time, it seems that landscape genetic studies in human contain powerful methods and are capable of modeling population structure and allowing for spatial heterogeneity.

These studies indicated that the geographic patterns in human genetic diversity carry footprints of population history and provide insights for genetic medicine which can be used across human populations (PETER, *et al*. 2020).

In conclusion, we report genotyping and allele frequency in two SNPs of CRISP2 gene which is a candidate gene for infertility due to sperm improper mobility. Here, we report a significant association between rs1301440109, and infertility, while rs760204944 is not significantly associated. We also reported sequence divergence in indigenous samples studied in compare to available ancestral sequences and that these changes may be of adaptive nature. The genotypes studied were not associated with geographical variables.

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REFERENCES

BRADBURD, GS, PL.RALPH (2019): Spatial population genetics: it's about time. Annu Rev Ecol Evol Syst. *50*:427–449.

BATTEY, CJ, PL RALPH, AD.KERN (2019): Space is the place: effects of continuous spatial structure on analysis of population genetic data. bioRxiv. 659235.

DENG, L, BP HOH, D LU, R FU, ME PHIPPS, S LI, AR NUR-SHAFAWATI, WI HATIN, E ISMAIL, SS MOKHTAR, L JIN, BA ZILFALIL, CR MARSHALL, SW SCHERER, F AL-MULLA, S XU (2014): The population genomic landscape of human genetic structure, admixture history and local adaptation in Peninsular Malaysia. Hum Genet Sep. *133*(9):1169-85.

EMISON, ES, AS MCCALLION, CS KASHUK, RT BUSH, E GRICE, S LIN, *et al.* (2005): A common sex-dependent mutation in a RET enhancer underlies Hirschsprung disease risk. Nature. *434*(7035):857.

GARRIGAN, D, SB KINGAN, MM PILKINGTON, JA WILDER, MP COX, H SOODYALL, *et al.* (2007): Inferring human population sizes, divergence times and rates of gene flow from mitochondrial, X and Y chromosome resequencing data. Genetics. *177*(4):2195-207.

HAENDLER, B, UF HABENICHT, U SCHWIDETZKY, I SCHUTTKE, WD.SCHLEUNING (1997): Differential androgen regulation of the murine genes for cysteine-rich secretory proteins (CRISP). Eur J Biochem. *250*(2):440-6.

HARRIS, EE, J.HEY (1999): X chromosome evidence for ancient human histories. PNAS. *96*(6):3320-4.

HEIDARY, Z, K SALIMINEJAD, M ZAKI-DIZAJI, HR.KHORRAM KHORSHID (2018): Genetic aspects of idiopathic asthenozoospermia as a cause of male infertility. Hum Fertil (Camb). 1-10.

HEIDARY, Z, M ZAKI-DIZAJI, K SALIMI NEJAD, HR.KHORRAM KHORSHID (2019): Expression Analysis of the CRISP2, CATSPER1, PATE1 and SEMG1 in the Sperm of Men with Idiopathic Asthenozoospermia. J Reprod Infertil. *20*(2):70-75.

HULL, M, C GLAZENER, N KELLY, D CONWAY, P FOSTER, R HINTON, *et al*. (1985): Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed). *291*(6510):1693-7.

JARVI, K, K LO, A FISCHER, J GRANTMYRE, A ZINI, V CHOW, *et al*. (2010): CUA Guideline: The workup of azoospermic males. Can Urol Assoc J. *4*(3):163-7.

JAMESON, J L, DM DE KRETSER, AB GROSSMAN *et al.* (1996): Endocrinology: Adult and Pediatric, Seventh Edition. Elsevier Inc.

JAMSAI D, A REILLY, SJ.SMITH *et al*. (2008): Polymorphisms in the human cysteine-rich secretory protein, 2 (CRISP2) gene in Australian men. Hum Reprod. *23* (9): 2151–2159.

KAESSMANN, H, F HEIßIG, A VON HAESELER, S.PÄÄBO (1999): DNA sequence variation in a non-coding region of low recombination on the human X chromosome. Nat Genet. 22(1):78.

KRATZSCHMAR, J, B HAENDLER, U EBERSPAECHER, D ROOSTERMAN, P DONNER, WD.SCHLEUNING (1996): The human cysteine-rich secretory protein (CRISP) family. Primary structure and tissue distribution of CRISP-1, CRISP-2 and CRISP-3. Eur J Biochem. *236*(3): 827-36.

LIU, FJ, X LIU, JL HAN, YW WANG, SH JIN, XX LIU, *et al*. (2015): Aged men share the sperm protein PATE1 defect with young asthenozoospermia patients. Hum Reprod. *30*(4):861-9.

LUO, S, J JIA, H HU, W MA, Y JIAO, J.DONG (2013): A Chinese herbal decoction can increase the intracellular Ca2+ concentration and CatSper1 expression in mouse sperm tails. Mol Med Rep. *7*(1):195-200.

LUNTER, G, CP PONTING, J.HEIN (2006): Genome-wide identification of human functional DNA using a neutral indel model. PLoS Comput. Biol. *2*(1): e5.

MIZUKI, N, DE SARAPATA, JA GARCIA-SANZ, M. KASAHARA (1992): The mouse male germ cell-specific gene Tpx-1: molecular structure, mode of expression in spermatogenesis, and sequence similarity to two non-mammalian genes. Mamm Genome. 3(5):274-80.

MOGHBELINEJAD, S, H MOZDARANI, P GHORAEIAN, R.ASADI (2018): Basic and clinical genetic studies on male infertility in Iran during 2000-2016: A review. Int J Reprod Biomed. *16*(3):131-148.

NACHMAN, MW, VL BAUER, SL CROWELL, CF. AQUADRO (1998): DNA variability and recombination rates at X-linked loci in humans. Genetics. *150*(3):1133-41.

PETER, B M, D PETKOVA, J. NOVEMBRE (2020): Genetic Landscapes Reveal How Human Genetic Diversity Aligns with Geography. Mol Biol Evol. *37*: 943–951.

REIJO, R, T-Y LEE, P SALO, R ALAGAPPAN, LG BROWN, M ROSENBERG, *et al*. (1995): Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA–binding protein gene. Nat. Genet. *10*(4):383.

ROSENBERG, NA, JK PRITCHARD, JL WEBER, HM CANN, KK KIDD, LA ZHIVOTOVSKY, MW. FELDMAN (2002): Genetic structure of human populations. Science. *298*(5602): 2381–2385.

TOURNAYE, H, C STAESSEN, I LIEBAERS, E VAN ASSCHE, P DEVROEY, M BONDUELLE, *et al.(*1996): Genetics: Testicular sperm recovery in nine 47, XXY Klinefelter patients. Hum Reprod.*11*(8):1644-9.

YANG, F, R DE LA FUENTE, NA LEU, C BAUMANN, KJ MCLAUGHLIN, PJ.WANG (2006): Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. JCB. *173*(4):497-507.

YANG, F, K GELL, GW VAN DER HEIJDEN, S ECKARDT, NA LEU, DC PAGE, *et al.* (2008): Meiotic failure in male mice lacking an X-linked factor. Genes Dev. *22*(5):682-91.

YANG, F, S SILBER, NA LEU, RD OATES, JD MARSZALEK, H SKALETSKY, *et al*. (2015): TEX11 is mutated in infertile men with azoospermia and regulates genome-wide recombination rates in mouse. EMBO Mol Med. *7*(9):1198-210.

YATSENKO, AN, AP GEORGIADIS, A RÖPKE, AJ BERMAN, T JAFFE, M OLSZEWSKA, *et al.* (2015): X-linked TEX11 mutations, meiotic arrest, and azoospermia in infertile men. NEJM *372*(22):2097-107.

YU, Y-H, F-P SIAO, LC-L HSU, PH.YEN (2012): TEX11 modulates germ cell proliferation by competing with estrogen receptor β for the binding to HPIP. Mol Endocrinol. *26*(4):630-42.

YU, Q, Q ZHOU, Q WEI, J LI, C FENG, X. MAO (2014): SEMG1 may be the candidate gene for idiopathic asthenozoospermia. Andrologia. *46*(2):158-66.

ZHENG, K, F YANG, PJ.WANG (2010): Regulation of male fertility by X-linked genes. J. Androl. *31*(1):79-85.

ZHANG, S, Y BIAN, L LI, K SUN, Q ZHAO, L ZHA, *et al.* (2015): Population genetic study of 34 X-Chromosome markers in 5 main ethnic groups of China. Sci Rep. *5:*17711.

ZHANG, X, M DING, X DING, T LI, H.CHEN (2015): Six polymorphisms in genes involved in DNA double-strand break repair and chromosome synapsis: association with male infertility. Syst Biol Reprod Med *61*(4):187-93.

ZHOU, JH, QZ ZHOU, XM LYU, T ZHU, ZJ CHEN, MK CHEN, *et al*. (2015): The expression of cysteine-rich secretory protein 2 (CRISP2) and its specific regulator miR-27b in the spermatozoa of patients with asthenozoospermia. Biol Reprod. *92*(1):28.

PRELIMINARNI IZVEŠTAJ O POPULACIONO GENETIČKOJ ANALIZI DVA CRISP2 GENA SNP-a U VEZI SA ASTENOSPERMIJA NEPLODNOSTI: RAČUNARSKI I FILOGENETSKI PRISTUPI

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Izvod

Astenospermija (AZS) je uzrok neplodnosti kod muškaraca kod koje je smanjena pokretljivost spermatozoida. SNP-ovi CRISP2 gena mogu biti uključeni u ovu vrstu neplodnosti i brojne studije asocijacije za ove SNP-ove se sprovode u različitim delovima sveta. Ova studija je sprovedena da bi se ispitala povezanost između dva SNP-a gena CRISP2, naime, rs1301440109 i rs-760204944, sa astenospermijom u iranskim uzorcima. Za ovu studiju, 83 neplodna muškarca sa dijagnozom astenospermije i 40 plodnih muškaraca sa normalnim kariotipom i analizom sperme je proučavano korišćenjem tera-arms metode. Sprovedene su različite metode izračunavanja na genetskim podacima, sekvencama, kao i na kliničkim karakteristikama vezanim za karakteristike sperme. Genotipizacija proučavanih uzoraka pokazala je prisustvo dva različita alela u oba SNP-a. Prikazana je učestalost dobijenih genotipova i alela, pri čemu su T aleli najčešći aleli u oba proučavana SNP-a. Logistička regresija je pokazala samo značajnu povezanost između rs1301440109 i neplodnosti. Sekvenciranje ciljnih SNP-ova zajedno sa njihovim susednim sekvencama, u poređenju sa predačkim sekvencama, pokazalo je značajnu divergenciju sekvenci za autohtone jedinke sa pozitivnom značajnom Tadžimasovom D statistikom koja pokazuje adaptivnu promenu u ovim sekvencama za rs 1301440109. Linearna diskriminantna analiza (LDA) je razlikovala jedinke slučaja i kontrolne jedinke u rs1301440109, na osnovu proučavanih kliničkih karakteristika. Analiza redundantnosti (RDA) nije pokazala značajnu povezanost između dobijenih genotipova i geografskih varijabli.

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