UDC 575.
https://doi.org/10.2298/GENSR2103167Z
Original scientific article

ASSOCIATION OF RS1294845152 IN CRISP2 WITH MALE INFERTILITY: RFLP AND DNA SEQUENCE ANALYSIS

Fahimeh ZANGANEH[a] and Masoud SHEIDAI[b]*

[a]Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran
[b]Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

Zanganeh F. and M. Sheidai (2021). *Association of rs1294845152 in CRISP2 with male infertility: RFLP and DNA sequence analysis.* - Genetika, Vol 53, No.3, 1167-1178.

Infertility is a major health human problem with 10% to 15% of world-wide occurrence. It may happen due to various reasons including physiological, environmental, social, and genetic factors. Recent genetic investigations show that hundreds of genes may act on infertility, each with minor effects. Asthenozoospermia is one of the common types of infertility in men which is determined by sperm motility reduction. CRISP2 gene is located on acrosome and tail of sperm and plays crucial role in spermatogenesis, regulation of sperm flagellum motility, acrosome reaction and gamete fusion. In general, heterogeneous results are present for association of CRISP2 gene with asthenoteratozoospermic male infertility, therefore, we carried out a starting investigation to show association of rs1294845152 of CRISP2 Gene with male infertility by RFLP analysis. We also tried to identify the known SNPs and variant nucleotides adjacent to this SNP by sequencing. The present study revealed that neighther rs1294845152, nor the neighboring sequences show any association with this kind of male infertility in the limited samples studied in Iran. In general, it seems that this part of men genetic material shows a high degree of conservation and very low level of nucleotide variability.

Key words: Association study, clustering, Nucleotide diversity, RFLP, TCS-Network.

INTRODUCTION

Infertility is a complex medical problem and is considered as an important phenomenon in human reproduction which affects 10% to 15% of couples in whole the world. It is defined as the

Corresponding author: Masoud Sheidai, Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran. E-mail: msheidai@yahoo.com

.

inability to conceive after a prolonged period (HULL *et al.*, 1985; TÜTTELMANN *et al.*, 2018; VAHEDI and SHEIDAI, 2021). Infertility may result due to various causes such as physiological, environmental, social, and genetic factors. Recent studies indicate that different molecular and genetic pathways regulate fertility and that hundreds of genes operate in these pathways (MASSART *et al.*, 2012).

Approximately half of all infertility cases are caused by factors related to the male partner (KUSZ-ZAMELCZYK *et al.*, 2012). The background of male infertility seems extremely heterogeneous including many environmental causes. In as many as 30% of individuals, the origin of infertility remains unknown (KUSZ-ZAMELCZYK *et al.*, 2012). Recently, attention has been paid to mutations causing male infertility. These mutations were identified in genes known to be responsible for male germ cell development or, for other male reproductive processes. Thousands of genes in these categories are expressed in human testes and any of them can potentially cause infertility when mutated.

Male infertility has been divided into four categories: 1- Oligospermia, 2- asthenozoospermia, 3- teratozoospermia and 4- azoospermia. Asthenozoospermia (AZS) as a cause of infertility in men is defined by absent or decreasing forward sperm motility (Progressive motility <32%). 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/or abnormal sperm morphologies (Oligoasthenoterato- and asthenoteratozoospermia) (HEIDARY *et al.*, 2019).

The cysteine-rich secretory protein (CRISP) family consists of three members called acidic epididymal glycoprotein 1 (AEG1), AEG2, and testis-specific protein 1 (TPX1), which share 16 conserved cysteine residues at their C-termini (GIESE *et al.*, 2002).

The CRISP proteins are primarily expressed in different sections of the male genital tract and are thought to mediate cell-cell interactions of male germ cells with other cells during sperm maturation or during fertilization. Therefore, their genes are of interest as candidate genes for inherited male fertility dysfunctions and as putative quantitative trait loci for male fertility traits (GIESE *et al.*, 2002).

CRISP2 gene is located on acrosome and tail of sperm and regulates ryanodine receptors Ca gating and binds to mitogen activated protein kinase11 in the acrosome and gametogenetin1 in the tail. This gene is an important gene and play crucial role in spermatogenesis, regulation of sperm flagellum motility, acrosome reaction and gamete fusion (HEIDARY *et al.*, 2019). CRISP2 (Cysteine-Rich Secretory Protein 2), is apparently related to AZS (13-15). CRISP2 is the only member of CRISP family which is expressed in the testis in an androgen-independent manner (HEIDARY *et al.*, 2019).

Agarwal et al. (2015) found CRISP2 is uniquely expressed in the spermatozoa of infertile men with unilateral varicocele and it is absent in fertile men. Moreover, JING *et al*. (2011), showed taht the expression of CRISP2 mRNA as well as CRISP2 protein were down-regulated in the asthenospermia patients, significantly lower than in the normal control group ($P < 0.05$). Therefore, it was suggested CRISP2 may serve as a potential molecular target for the research of asthenospermia. Therefore, due to importance of CRISP2 gene in male infertility, we

investigated Iranian sample males with stenospermia for rs1294845152 of CRISP2 for association with infertility for the first time. We used both RFLP (Restriction fragment length polymorphism) as well as DNA sequencing of the mentioned SNP and its adjacent sequences. We also aimed to report the SNPs and variant sequences of neighboring sequences to rs1294845152.

MATERIAL AND METHODS

Sample size determination

We used the following formula for sample size calculations (https://www.wallstreetmojo.com/sample-size-formula). where, N = Population size, Z = Critical value of the normal distribution at the required confidence level, p = Sample proportion, e = Margin of error. Therefore, we used the following data: Population size, N = 10,000, Critical value at 95% confidence level, Z = 1.96, and Margin of error, e = 5% or 0.05. Since the current conversion rate is unknown, let us assume p = 0.5. N = (10,000 * (1.96 2) *0.05*(1-0.05)/ (0.05 2)/ (10000 – 1+((1.96 2) * 0.05*(1-0.05)/ (0.05 2)))) = 72. Therefore, a sample size of 72 customers will be adequate for deriving meaningful inference in this case. However, we used 200 samples in total, 100 for each case and control persons. These samples were collected from the Infertility Clinic of Jahad-Daneshgahi, Qom (VAHEDI and SHEIDAI, 2021).

$$\text{Sample size, } n = N * \frac{\frac{Z^2 * p * (1-p)}{e^2}}{[N - 1 + \frac{Z^2 * p * (1-p)}{e^2}]}$$

DNA extraction

DNA molecules were extracted by the salting-out DNA extraction method (MOHSENI SANI *et al.*, 2018). 1% of agarose gel electrophorese was used to evaluate the quality of the extracted DNA molecules.

SNPs selection

Choosing SNP was based on PHYRE2 online software (http://www.sbg.bio.ic.ac.uk). At the first, amino acids sequence of this gene was downloaded from NCBI database and entered to this PHYRE2 software, then most important amino acids which were very sensitive to mutation and more effective in protein structure were selected. At the second step, these amino acids were investigated for existence of SNP in their codons. Finally, according to this manner, we selected rs1294845152 for association study.

PCR-RFLP procedure

Forward and reverse primers for this SNP were designed by Oligo7 software which their information are in Table 1. PCR was performed by thermos cycler system (Genetix Biotech, Australia) according to following program. Initial denaturation at 95 C° for 5 minutes, 35 cycle of denaturation at 95 C° for 45 Sec, annealing at 60 C° for 45 sec and extension at 72 C° for 45 sec. final extension was at 72 C° for 5 min. total volume of PCR reaction was 25 µl according to following amount, 4 µl of sample DNA, 0.4 µl dNTP, 0.4 µl Taq enzyme, 0.6 µl

Forward Primer, 0.6 µl Reverse Primer, 2 µl PCR Buffer, 0.7 µl MgCl2 and 16.3 µl H2O. PCR result was demonstrated by 1.5 % agarose gel electrophoresis. RFPL investigation was performed by HpyCH4 According to following mixture: 5 µl PCR product, 9 µl H2o, 2 µl Buffer and 0.5 µl restriction enzyme. Samples incubated for half an hour at 37 C°. For RFLP results we did not have to perform chi-square test as all the studied samples had GG genotype.

Table1. Primers Information

Primer name	
Forward	CCAAAGTCCTAGTGACCTAAATCC
Reverse	CCCATTGTAGTGTAAGCGATGC

DNA sequencing

For confirming PCR-RFLP results and also for performing population genetics analysis, 30 samples (15 cases and 15 controls) were selected for Sanger sequencing, then related analysis were performed on sequencing result. For sequencing result, we first aligned and cure DNA sequences. Then Kimura-2-parameters genetic distance was determined among the samples and uses it for cluster analysis (PODANI, 2000). DCA (Dentrented correspondence analysis) was used to check if the nucleotides obtain can be used for genetic screening (PODANI, 2000).

RESULTS

RFLP results

All 200 genotypes analyses had GG genotype. Therefore, the studied samples in Iran all were geno typical uniform, and that the studied SNP was present in both control as well as case samples. We therefore, conclude that, rs1294845152 of CRISP2 is not associated with asthenospermia in our study.

DNA sequencing

In order to investigate and identify adjacent nucleotides/SNPs to rs1294845152, we performed DNA sequencing in 30 randomly selected individuals. DNA segment obtained after curation was 430 nucleotides in length (Fig. 1). It showed very low level of genetic diversity: pi = 5.37375e+06, with 24 polymorphic sites and 18 parsimony-informative sites. The average genetic distance obtained among individuals was 0.0087. These nucleotide changes showed Tajima's D statistic = 1.34981e+09, p (D >= 1.34981e+09) = 0. This indicated that, nucleotide substitutions occurred randomly and mutations occurred are not in respond to selection.

DCA plot is provided in Fig. 2. It shows that several nucleotides are scattered in the plot and therefore can differentiate the studied samples.

UPGMA clustering of DNA sequence is provided in Fig. 3. It reveals that these sequences are not associated with stenospermia. Moreover, admixture of the samples from three ethnic groups of Turk, Our, and Fars in different clusters indicate that, nucleotides changes in these ethnic groups occurred at random as also evidenced by Tajima's D statistic.

Figure 1. Part of DNA sequences obtained in the studied samples after curation.

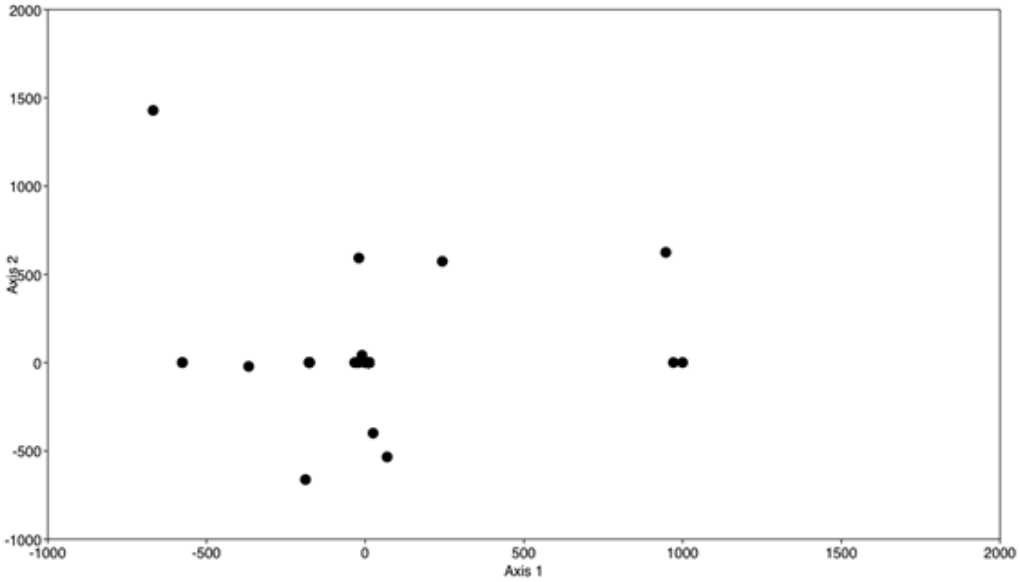

Figure 2. DCA plot od sequenced individuals, showing a good spread of many nucleotides.

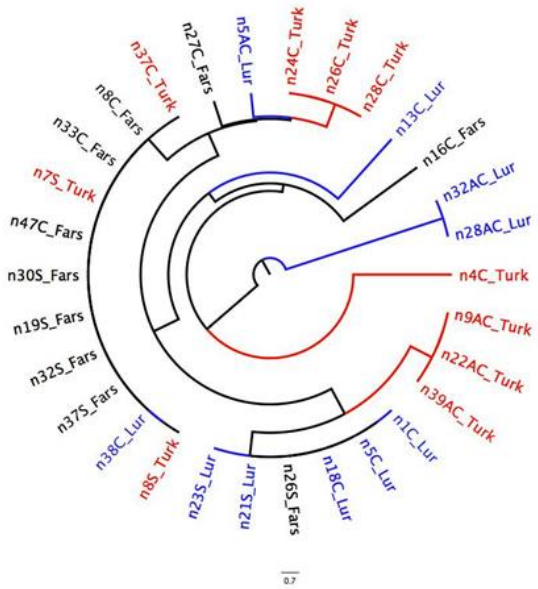

Figure 3. UPGMA dendrogram of the studied samples based on sequence data.

TCS network (Fig. 4), reveals that the number of nucleotide substitutions ranges from 1-15 among the studied samples. Therefore, in general the sequence data represent an almost conserved region of the genome in our samples.

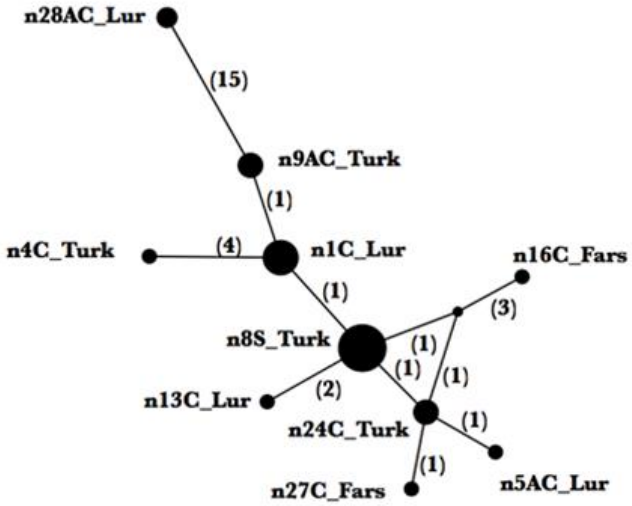

Figure 4. TCS network of the studied samples

TCS network of the studied samples indicate low level of genetic changes among the studied individuals and that the studied genome segment is of relatively conserved nature.

Detailed investigation of the adjacent nucleotides revealed the following known SNPs as well as variant nucleotides in our samples (Table 2).

Table 2 Known SNPs and variant nucleotides in the studied genomic segment reorganized

Number	SNP.	Nucleotide	Number	SNP.	Nucleotide
9	rs539358850	C/T	183	rs989587469	C/A
12	rs1324878046	G/A/C	189	rs969047837	G/A
13	rs751628883	T/A	202	rs1202915120	T/C
26	rs550967205	A/G	213	rs1440056649	C/T
27	rs755110457	T/C/G	225	rs12624	A/C/T
28	rs1028922270	G/T	236	rs373065610	G/A
43	rs1400386835	A/G	238	rs960850006	A/T
48	rs1334440307	C/T	244	rs771393009	A/G
50	rs1470866392	T/G	246	rs779132281	G/A
53	rs138757872	A/G	248	rs1246924192	T/C
54	rs1169790686	C/T	249	rs746432552	G/A
59	rs1007563898	T/C	251	rs1376501998	A/T
61	rs1474401452	C/T	253	rs772671444	C/G/T
66	rs1298879565	C/T	254	rs776309599	C/A/T
67	rs569440907	G/A	255	rs761354474	C/A
72	rs1381001779	A/G	256	rs1439834371	T/C
77	rs781434350	A/G	260	rs972280399	C/G
81	rs536401902	A/C	261	rs749710327	C/T
84	rs1016179701	T/A	262	rs1363110948	A/T
99	rs1205193744	C/G	266	rs1488423474	A/G
101	rs962303513	G/A	273	rs1164991534	C/A/T
102	rs1257861016	T/A	274	rs558511264	A/G
108	rs142795989	T/C	275	rs1191895981	C/T
110	rs1025250186	A/C	280	rs919435094	C/A
112	rs9369914	C/G/T	282	rs76267147	C/A
116	rs1380837747	T/C/G	287	rs1178266226	G/A/C
118	rs374178574	T/C	291	rs1175833252	A/T
119	rs998004932	G/A	295	rs1381092146	A/C
122	rs1345120289	A/G	302	rs577428520	T/C/G
126	rs980527724	T/C	303	rs762512665	T/C
132	rs762436093	A/G	305	rs766258708	G/A
133	rs936322053	T/C	307	rs751290579	T/G
142	rs865899371	G/A	313	rs1294845152	A/G
154	rs1167979136	T/G	314	rs1471327052	T/A
165	rs1260809703	G/A/T	315	rs759511323	A/G
166	rs1011254004	G/A	318	rs1390252325	C/A/T
167	rs1021930647	G/A	324	rs767404432	G/A
169	rs1561857407	G/A	325	rs930882229	C/T
171	rs1444888861	G/A	330	rs1242819259	C/A
176	rs1561857433	T/C	332	rs544121328	C/A
			344	rs1049784604	T/A

	347	rs1290016937	C/T
	349	rs1204488545	C/T
	352	rs1247630547	G/C
	355	rs564116939	C/T
	362	rs1463767556	A/T
	363	rs756118867	G/C
	367	rs777709973	T/C
	368	rs1198037687	A/C
	369	rs754147443	T/G
	378	rs1490009338	G/C
	384	rs757394764	C/G
	385	rs779371926	A/G/T
	386	rs910514075	G/A
	388	rs746176594	T/C
	390	rs1420025562	C/T
	392	rs772726407	G/A
	395	rs1464676020	G/A
	403	rs780653237	G/A
	405	rs747668694	T/C
	408	rs1449997676	T/G
	409	rs769500059	G/A
	414	rs772587233	C/A
	417	rs1229194782	T/A
	426	rs1561858474	G/A
	427	rs1315468385	C/T
	433	rs762716256	C/A
	434	rs369082725	A/G/T
	435	rs774235367	G/A
	439	rs1404239236	A/G
	451	rs759279881	A/G
	453	rs767445386	C/A/T
	454	rs760592389	G/A
	455	rs562593669	T/C
	463	rs1157859457	A/G
	465	rs764283148	T/C
	468	rs1038135354	G/C
	472	rs753826316	A/G
	480	rs540662348	A/G
	481	rs574616255	C/T
	487	rs1409107100	A/G
	491	rs897722005	G/A
	492	rs1215396422	T/C
	497	rs1271763385	G/A
	500	rs541657278	G/A
	501	rs1240463833	G/C
	502	rs1218154732	G/A
	503	rs1052246290	G/A
	506	rs1284663546	A/C

	507	rs892304046	G/A
	515	rs1024778658	C/A
	524	rs146056148	T/A
	530	rs73737231	C/A/T
	531	rs758149072	G/A
	544	rs551590844	T/C

DISCUSSION

Human testis specific protein 1 (TPX1) exists in the cytomembrane and cytoplasm of spermatogenic cells from pachytene spermatocytes to elongated spermtids, including pachytene spermatocytes, round spermtids and elongated spermtids. It is localized in the connecting piece, the flagellum, and the acrosome of mature human spermatozoa (DU *et al.*, 2006). It has been shown that the protein level and localization of TPX1 were altered in patients with spermatogenic arrest and in infertile men with oligoasthenoteratospermia syndrome (DU *et al.*, 2006).

JAMSAI *et al*. (2008) investigated CRISP2 variations 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 obtained 21 polymorphisms, some are previously identified, while 13 were unreported variations.

They reported the C196R polymorphism which resulted in the loss of a strictly conserved cysteine involved in intramolecular disulphide bonding. They concluded that none of the many polymorphisms identified showed a significant association with male infertility, but C196R polymorphism may compromise CRISP2 function. In present study we obtained a low level of sequence variability and all known SNPs in the studied genetic regions as well as sequence variants were not associated with asthenospermia.

Similarly, JING *et al*. (2011), studied the mRNA and protein expression levels of CRISP2 in the sperm of asthenospermia patients, and explore their relationship with sperm motility and related molecular mechanism. They used 78 adult male patients with asthenospermia and 70 controls. The relative expressions of CRISP2 mRNA and protein in the two groups was detected by RT-PCR, SYBR Green real-time PCR and Western blot.The expression of CRISP2 mRNA was down-regulated by 4.3 times and that of the CRISP2 protein by 1.71 times in the asthenospermia patients, significantly lower than in the normal control group ($P < 0.05$). Therefore, it was suggested that down-regulation of CRISP2 mRNA and protein expressions in the sperm of asthenospermia patients may be closely related with decreased sperm motility.

HEIDARY *et al*. (2019), investigated the expression level of the CRISP2, along with the other genes in the sperm of 35 men with idiopathic asthenozoospermia (AZS), as well as 35 healthy men as control. They found that CRISP2 ($p=0.03$) was significantly down--regulated in AZS men compared to the controls. Therefore, 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. According to LIU *et al*. (2015), the analysis of CRISP2 variations in asthenozoo, and/or teratozoospermia failed to find a significant association. These findings

suggest the involvement of CRISP2 in the infertility may be the result of impairing sperm motility and development of varicocele.

Similarly, ZHOU *et al*. (2015, 2016), demonstrated that miR-27b and miR27a negatively regulate CRISP2 protein expression in AZS and asthenoteratozoospermia, respectively. They showed that high miR-27b and miR-27a expression or low CRISP2 protein expression was significantly associated with low sperm motility, abnormal morphology, and infertility in asthenoteratozoospermic men. Similarly, downregulation of CRISP2 mRNA and protein or both were reported in AZS (WANG *et al*., 2004; JING *et al*., 2011) in AZS.

In general, we can state that heterogeneous results are present for association of CRISP2 gene with asthenoteratozoospermic male infertility, our study revealed that neither rs1294845152 of CRISP2 Gene, nor the neighboring sequences show any association with this kind of male infertility in the limited samples studied in Iran. In general, it seems that this part of men genetic material shows a high degree of conservation and very low level of nucleotide variability.

Received, November 30th, 2020
Accepted May 18th, 2021

REFERENCES

AGARWAL, A., R., SHARMA, D., DURAIRAJANAYAGAM, A., AYAZ, Z., CUI, B., WILLARD (2015): Major protein alterations in spermatozoa from infertile men with unilateral varicocele. Reprod. Biol. Endo. Crinol., *13*: 8.

DU, Y., X., HUANG, J., LI, Y., HU, Z., ZHOU, J., SHA (2006): Human testis specific protein 1 expression in human spermatogenesis and involvement in the pathogenesis of male infertility. Fertil. Steril., *85*(6): 1852-4.

GIESE, A., R., JUDE, H., KUIPER, T., RAUDSEPP, F., PIUMI, A., SCHAMBONY, G., GUÉRIN, B., CHOWDHARY, O., DISTL, E., TÖPFER-PETERSEN, T., LEEB (2002): Molecular characterization of the equine testis-specific protein 1 (TPX1) and acidic epididymal glycoprotein 2 (AEG2) genes encoding members of the cysteine-rich secretory protein (CRISP) family. Gene., *299*: 101-9.

HEIDARY, Z., M., ZAKI-DIZAJI, K., SALIMINEJAD, H. R., KHORRAMKHORSHID (2019): Expression Analysis of the CRISP2, CATSPER1, PATE1 and SEMG1 in the Sperm of Men with Idiopathic Asthenozoospemia. J. Reprod. Infertil., *20* (2): 70-75.

HULL, M.G.R., C.M.A., GLAZENER, N.J., KELLY, D.I., CONWAY, P.A., FOSTER, R.A., HINTON, C., COULSON, P.A., LAMBERT, E.M., WATT, K.M., DESAI (1985): Population study of causes, treatment, and outcome of infertility. BMJ., 291: 1693-1698.

JAMSAI, D., A., REILLY, S.J., SMITH, G.M., GIBBS, H.W., BAKER, R.I., MCLACHLAN, D.M., DE KRETSER, M.K., O'BRYAN (2008): Polymorphisms in the human cysteine-rich secretory protein 2 (CRISP2) gene in Australian men. Hum. Reprod., *23*(9): 2151-9.

JING, X.W., R.W., XING, Q.Z., ZHOU, Q.F., YU, W.B., GUO, S.M., CHEN, Q.J., CHU, C.Q., FENG, X.M., MAO (2011): Expressions of cysteine-rich secretory protein 2 in asthenospermia. Zhonghua. Biol. Reprod., *17*(3): 203-7.

LIU, F.J., X., LIU, J.L., HAN, Y.W., WANG, S.H., JIN, X.X., LIU (2015): Aged men share the sperm protein PATE1 defect with young asthenozoospermia patients. Hum. Reprod., *30*(4): 861-869.

MASSART, A., W., LISSENS, H., TOURNAYE, K., STOUFFS (2012): Genetic causes of spermatogenic failure. Asian J. Androl., *14*(1): 40.

MOHSENI SANI, N., M., SHEIDAI, Z., NOORMOHAMMADI, N., KALHOR, G., ATRI ROOZBAHANI (2018): Study of association the single nucleotide polymorphisms (rs6631) in 3′-UTR CGA gene with male infertility in Iranian population. Gene. Reports., *12*: 61-65.

PODANI, J. (2000): Introduction to the Exploration of Multivariate Data, Leide, Netherlands.

TÜTTELMANN, F., C., RUCKERT, A., RÖPKE (2018): Disorders of spermatogenesis Perspectives for novel genetic diagnostics after 20 years of unchanged routine. Übersichten., *30*: 12–20.

VAHEDI, M., M., SHEIDAI (2021): Association study of RS535296987 in tex14 gene in azoospermiamen: rflp and dna sequencing. Genetika, *53* (2): 729-737.

WANG, H., Z., ZHOU, M., XU, J., LI, J., XIAO, Z.Y., XU (2004): A spermatogenesis-related gene expression profile in human spermatozoa and its potential clinical applications. J. Mol. Med (Berl)., *82*(5): 317-24.

ZHOU, J.H., Q.Z., ZHOU, X.M., LYU, T., ZHU, Z.J., CHEN, M.K., CHEN (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.

ZHOU, J.H., Q.Z., ZHOU, J.K., YANG, X.M., LYU, J., BIAN, W.B., GUO (2016): MicroRNA-27a-mediated repression of cysteine-rich secretory protein 2 translation in asthenoteratozoospermic patients. Asian J. Androl., *19*(5): 591-5.

ASOCIJACIJA RS1294845152 U CRISP2 SA MUŠKOM NEPLODNOŠĆU: RFLP I ANALIZA DNK SEKVENCE

Fahimeh ZANGANEH[a] i Masoud SHEIDAI[b]*

[a]Departman za mikrobiologiju, Ogranak Qom, Islamski Azad Univerzitet, Qom, Iran
[b]Departman za biljne nauke i biotehnologiju, Faculet za prirodne nauke i biotehnologiju, Shahid Beheshti Univerzitet, Teheran, Iran

Izvod

Neplodnost je veliki zdravstveni problem ljudi sa 10% do 15% pojavljivanja širom sveta. Može se desiti iz različitih razloga uključujući fiziološke, ekološke, društvene i genetske faktore. Nedavna genetska istraživanja pokazuju da stotine gena mogu delovati na neplodnost, svaki sa manlim efektima. Astenozoospermija je jedan od uobičajenih tipova neplodnosti kod muškaraca koji se određuje smanjenjem pokretljivosti spermatozoida. CRISP2 gen se nalazi na akrozomu i repu spermatozoida i igra ključnu ulogu u spermatogenezi, regulaciji pokretljivosti flageluma sperme, reakciji akrozoma i fuziji gameta. Uopšteno govoreći, prisutni su heterogeni rezultati za povezanost gena CRISP2 sa astenoteratozoospermičnom muškom neplodnošću, stoga smo sproveli početno istraživanje da bismo pokazali povezanost rs1294845152 gena CRISP2 sa muškom neplodnošću RFLP analizom. Takođe smo pokušali da identifikujemo poznate SNP-ove i varijante nukleotida pored ovog SNP-a sekvenciranjem. Ova studija je otkrila da nijedan rs1294845152, niti susedne sekvence ne pokazuju nikakvu povezanost sa ovom vrstom muške neplodnosti u ograničenim uzorcima proučavanim u Iranu. Generalno, čini se da ovaj deo muškog genetskog materijala pokazuje visok stepen očuvanosti i veoma nizak nivo varijabilnosti nukleotida.

Primljeno 30.XI.2020.
Odobreno 18. V. 2021.