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Evaluating the Association of rs6500633 Polymorphism in the SEPT12 Gene with Idiopathic Asthenozoospermia in Iranian Azeri Males: A Case-Control Study

Gholamreza Jahangirzadeh Sooreh[1], Seyed Babak Khalifehzadeh Kaleybar[2]✉

[1]Department of Molecular Biology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Pathology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

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✉**Correspondence to:**
Seyed Babak Khalifehzadeh Kaleybar, Department of Pathology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Tel: +98 144101006
Fax: +98
Email: tpourfallah@mazums.ac.ir

ABSTRACT

Introduction: *SEPT12* gene encode a testis-specific protein that play important role in terminal differentiation of germ cells. The product of this gene involved in sperm tail annulus constituent, and is essential for head-tail formation in sperm. Various polymorphisms on *SEPT12* gene are identified that are associated with impairment of sperm function as well as spermatogenesis in males with infertility. In this study we investigated correlation of rs6500633 polymorphism in the *SEPT12* gene in Iranian Azeri male with idiopathic asthenozoospermia (AZS).

Materials and Methods: We enrolled 50 men with idiopathic AZS as case group and 50 healthy men as control group from East Azerbaijan, Iran. Extraction of genomic DNA was conducted by proteinase K method from sperm samples. Genotyping was performed by tetra-primer amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS PCR).

Results: Frequency of TT, TC, and CC genotypes were 10%, 86%, and 4% in the AZS. On the other hand, the frequencies were 4%, 90%, and 6% in the healthy controls, respectively. Our study indicated no significant difference between the patient and control groups for frequency of the rs6500633 polymorphism in the *SEPT12* gene ($P > 0.05$).

Conclusion: We demonstrated no significant association between rs6500633 polymorphism in the *SEPT12* gene and AZS among Iranian Azeri men.

Keywords: Asthenozoospermia, *SEPT12* Gene, Polymorphism, Tetra-ARMS PCR

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Introduction

Infertility is an important reproduction problem in human that occur in 10-15% of couples in the world. Male infertility is responsible for approximately 50% of infertile cases (1, 2). The genetic variants and environmental toxins are considered as important causes of infertility in men that can cause to sperm dysfunction (3, 4).

Idiopathic asthenozoospermia (AZS) is one of the important spermatogenesis defects that occur 18% of men with infertility (5). This defect is a common cause of male infertility that motility of sperm is reduced (6, 7).

Septin protein is a member of cytoskeletal GTPase family that play critical role in actin and microtubule organization, vesicle trafficking, cytoskeletal remodeling, and membrane compartmentalization (8, 9). In this protein, GTP binding/hydrolysis domain is essential for septin-septin interactions that lead to integrity of Septins structure (10, 11). So far, several studies reported that the septin protein is an important cause of sperm tail annulus .

Sept12 protein is coded by *SEPT12* gene and is expressed in mature spermatozoa, spermatids, and germ cells (12, 13). Defects of this protein can cause to perturbation in head shaping of sperm and elongation of sperm tail through α- and β-tubulins organization (14). The sperm tail annulus can cause proteins confine as a diffusion barrier and provide sperm flagellum organization (15). Therefore, annulus integrity is is important for development of sperm tail and sperm motility; whereas annulus defect cause to male infertility through asthenozoospermia (16).

To our knowledge, correlation of AZS and SEPT12 rs6500633 polymorphism has not been investigated in Iranian Azeri men with infertility. Therefore, we investigated association of *SEPT12* gene rs6500633 polymorphism in infertile Iranian Azeri men with idiopathic AZS.

Materials and Methods

Study Subjects

This case-control study is consisted of 50 men with infertility (case group) who were referred to ACECR Fertility Clinic, East Azerbaijan ART Center, Tabriz, Iran, during 2017-2019. Idiopathic AZS in infertile men was diagnosed and confirmed by semen analysis. Moreover, 50 healthy men without any abnormal sperm and with previous successful fertility were collected (control group). The exclusion criteria in case group includes: abnormal karyotype, Y chromosome microdeletions, hypogonadism, orchitis, cryptorchidism, hypogonadotropic, and ejaculatory duct obstruction. The demographic information case and control group are presented in Table 1. All individuals were informed about the study and signed a consent form according to the Declaration of Helsinki ethical standards.

DNA Genotyping

Sperm sample (3 ml) received from all individuals, and genomic DNA extraction was conducted by proteinase K method. The quantity and quality of the extracted genomic DNA samples were evaluated using nanodrop instrument and electrophoresis on agarose gel, respectively. DNA genotyping was performed by tetra-primer amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS PCR) method. The used primers were as follow: Forward outer in: 5′-GAAATCACCTCGCCCGCCTC-3′; Reverse outer in: 5′-GCCCCTGGACATTGAGTTCCT-3′; Forward inner: 5′-GATGCAGTGGGTCCTCAGGTTCT-3′; Reverse inner: 5′-GATGCAGTGGGTCCTCAGGTTCC-3′. PCR amplification was performed in 25 μL total volume (12.5 μL master mix, 1 μg template DNA, and 0.5 μL each primer) as following condition: 1 cycle for initial denaturation (in 94°C for 5 minutes), 30 cycles for denaturation (94°C for 5 minutes), annealing (60°C for 45 seconds), and extension (72°C for 45 seconds), and 1 cycle for final extension (72°C for 5 minutes). The size of amplified products was determined using electrophorese on 1% agarose gel was used in order to identification of PCR products sizes (T allele: 338 bp and C allele: 233 bp) (Figure 1).

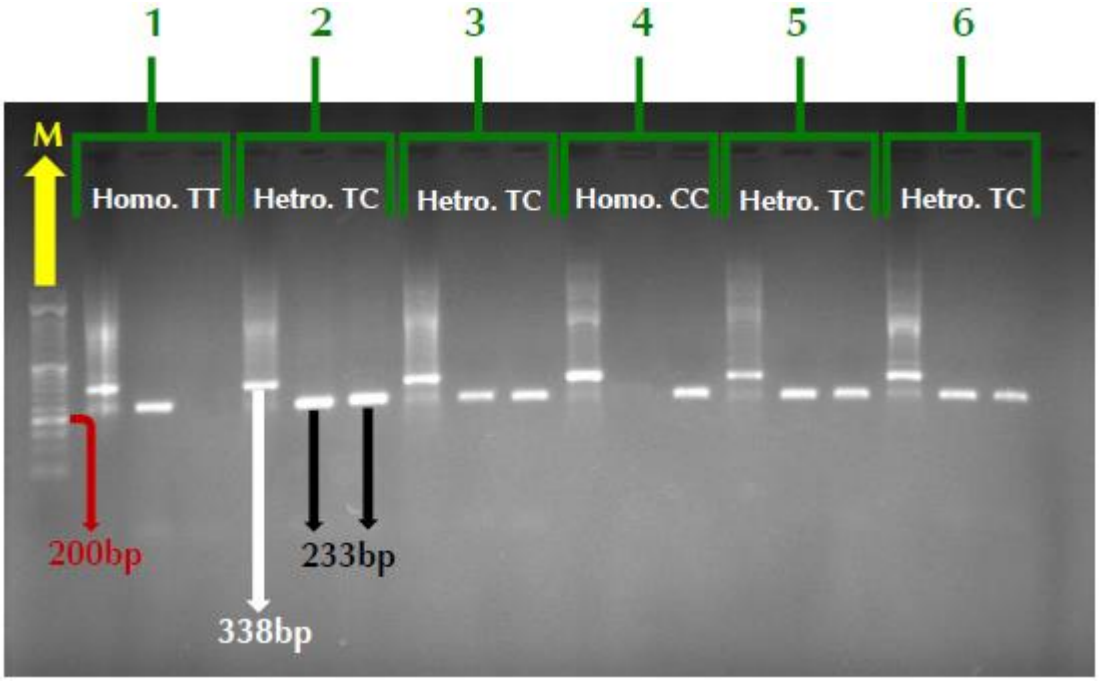

Figure 1. The PCR products of *SEPT12* gene rs6500633 polymorphism electrophoresis on 1% agarose gel.

Statistical Analysis

SPSS software version 19.0 was used for statistical analysis. The logistic regression was used to investigation of *SEPT12* rs6500633 polymorphism and AZS correlation. The chi-square ($\chi 2$) test and Fisher's exact test were used to investigation of Hardy-Weinberg equilibrium (HWE). The independent sample t-test was used to investigation of difference between demographic features between case and control group. The $P < 0.05$ was considered as statistically significant.

Results

We observed a significant difference between case and control groups in terms of family history, alcohol drinking, and semen parameters; whereas we did not find any significant difference between case and control groups in terms of tobacco smoking, body mass index (BMI), and age (Table 1).

Table 1. The clinical features and demographic variables of cases and controls.

Variables	Patients (n=50)	Controls (n=50)	P value
Age (year ± SD)	34.12 ± 3.33	36.23 ± 6.11	0.387
BMI (kg/m ± SD)	24.18 ± 4.09	23.76 ± 2.19	0.453
Tobacco smoking			
Never	29 (58%)	26 (52%)	-
Ever	21 (42%)	24 (48%)	0.122
Alcohol drinking			
Never	29 (68%)	38 (76%)	-
Ever	21 (42%)	12 (24%)	**0.001***
Family history			
Negative	41 (82%)	50 (100%)	-
Positive	9 (18%)	0 (0%)	**0.011***
Semen parameters			
Concentration ($\times 10^6$/ml)	45.9 ± 23.76	122.5 ± 41.56	**0.023***
Motility (%)	45.3 ± 21.24	79.8 ± 18.12	**0.016***
Volume (ml)	2.13 ±3.12	2.92 ± 1.77	0.832

*Statistically Significant $P < 0.05$; BMI-Body Mass Index.

We demonstrated that the genotype frequency of *SEPT12* rs6500633 polymorphism was in agreement with HWE in case and control groups ($P < 0.05$). The genotypes and allele frequencies of case and control group are presented in Table 2. The statistical analyze indicated that there is no significant association between genotypes frequency of case and control groups in all codominant, dominant, recessive, and overdominant inheritance models ($P < 0.05$). Moreover, the statistical analyze indicated that there is no significant association between alleles frequency case and control groups ($P < 0.05$).

Table 2. Genotype and allele distribution of *SEPT12* gene rs6500633 polymorphism.

Gene (polymorphism)	Inheritance models	Genotype and Allele	Patients (n=50)	Controls (n=50)	P value	OR (95% CI)
SEPT12 (rs6500633)	Codominant	TT	5 (10%)	2 (4%)	Ref	Ref =1
		TC	43 (86%)	45 (90%)	0.57	1.342 (0.222-1.934)
		CC	2 (4%)	3 (6%)	0.87	1.986 (0.633-1.575)
	Dominant	TT	20 (40%)	30 (60%)	Ref	Ref =1
		TC + CC	30 (60%)	20 (40%)	0.29	1.237 (0.564-1.975)
	Recessive	CC	0 (0%)	5 (10%)	Ref	Ref =1
		CT + TT	50 (100%)	45 (90%)	0.566	0.432 (0.691-1.346)
	Overdominant	CT	30 (60%)	15 (30%)	Ref	Ref =1
		TT + CC	20 (40%)	35 (70%)	0.46	0.554 (0.767-1.122)
		T normal	53%	49%	Ref	Ref =1
		C minor	47%	51%	0.53	1.211 (0.287-2.347)

Statistically Significant $P < 0.05$. OR: Odds Ratio. CI: Confidence Interval.

Discussion

Infertility is defined as inability of pregnancy after 12 month unprotected sexual intercourse (17). The chromosomal abnormalities and single gene polymorphisms or mutations are the main genetic basis of infertility (18). In a high proportion of patients, the main cause of infertility is remains unidentified that classifying as idiopathic infertility (19). In this study, we evaluated associations of *SEPT12* rs6500633 polymorphism and AZS in Iranian infertile male with idiopathic asthenozoospermia. Evidence suggested that high levels of seminal *SEPT12* can cause to defect in sperm motility in patients with AZS (20, 21). In addition, high seminal levels of *SEPT12*, presents a negative effect on sperm motility and spermatogenesis of infertile men (22).

Several studies have investigated association of *SEPT12* gene polymorphisms and infertility that reported contradictory results. In addition, various studies investigated association of *SEPT12* rs6500633 polymorphism and AZS in different populations and races. Several studies reported a significant association between *SEPT12* rs6500633 polymorphism and AZS (23, 24). On the other hands, some other studies reported no significant association between *SEPT12* rs6500633 polymorphism and AZS (23, 24). In a study by Rafaee et al. reported that the several polymorphisms on *SEPT12* gene were associated with males' infertility in Korean population with morphology disorders of sperm (27). In another study by Kuo et al. reported two missense mutations on *SEPT12* gene that leads to oligoasthenozoospermia and asthenoteratozoospermia through disruptive annulus and loss of *SEPT12* gene from abnormal spermatozoa annulus (28). In contrast, in our study we found no significant association between *SEPT12* rs6500633 polymorphism and AZS in Iranian Azeri population that can be due to small sample size or ethnic backgrounds (29). Moreover, reasons for difference of various studies can be due to environmental factors, other related genes, different in ethnicity, race, and geographical area (30-32).

In conclusion, we provided a more knowledge on association of AZS and *SEPT12* rs6500633 polymorphism in Iranian Azeri men with infertility. However, further studies are requared on other races and populations with larger sample sizes.

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Disclosure

The authors have nothing to disclose.

References

1. Coutton C, Satre V, Arnoult C, Ray P. Genetics of male infertility: the new players. Med Sci (Paris). 2012;28(5):497-502. doi: 10.1051/medsci/2012285014.
2. Soheilyfar S, Nikyar T, Fathi Maroufi N, Mohebi Chamkhorami F, Amini Z, Ahmadi M, et al. Association of IL-10, IL-18, and IL-33 genetic polymorphisms with recurrent pregnancy loss risk in Iranian women. Gynecol Endocrinol. 2019;35(4):342-5. doi: 10.1080/09513590.2018.1528220.
3. Nasirpour H, Azari Key Y, Kazemipur N, Majidpour M, Mahdavi S, Hajazimian S, et al. Association of rubella, cytomegalovirus, and toxoplasma infections with recurrent miscarriages in Bonab-Iran: a case-control study. Gene Cell Tissue. 2017;4(3):e60891. doi: 10.5812/gct.60891.
4. Hajizadeh YS, Emami E, Nottagh M, Amini Z, Maroufi NF, Azimian SH, et al. Effects of interleukin-1 receptor antagonist (IL-1Ra) gene 86 bp VNTR polymorphism on recurrent pregnancy loss: a case-control study. Horm Mol Biol Clin Investig. 2017;30(3):1-6. doi: 10.1515/hmbci-2017-0010.
5. Curi SM, Ariagno JI, Chenlo PH, Mendeluk GR, Pugliese MN, Sardi Segovia LM, et al. Asthenozoospermia: analysis of a large population. Arch Androl. 2003;49(5):343-9. doi: 10.1080/713828220.
6. Piomboni P, Focarelli R, Stendardi A, Ferramosca A, Zara V. The role of mitochondria in energy production for human sperm motility. Int J Androl. 2012;35(2):109-24. doi: 10.1111/j.1365-2605.2011.01218.x.
7. Poongothai JE, Gopenath TS, Manonayaki SW. Genetics of human male infertility. Singapore Med J. 2009;50(4):336-47. doi: 10.2174/1381612043453261.
8. Lin YH, Chou CK, Hung YC, Yu IS, Pan HA, Lin SW, et al. SEPT12 deficiency causes sperm nucleus damage and developmental arrest of preimplantation embryos. Fertil Steril. 2011;95(1):363-5. doi: 10.1016/j.fertnstert.2010.07.1064.
9. Hajazimian S, Maleki M, Danaei Mehrabad S, Isazadeh A. Human Wharton's jelly stem cells inhibit endometriosis through apoptosis induction. Reproduction 2020;159 (4):549-58. doi: 10.1530/REP-19-0597.
10. Kremer BE, Haystead T, Macara IG. Mammalian septins regulate microtubule stability through interaction with the microtubule-binding protein MAP4. Mol Biol Cell. 2005;16(10):4648-59. doi: 10.1091/mbc.e05-03-0267.
11. Field CM, Al-Awar O, Rosenblatt J, Wong ML, Alberts B, Mitchison TJ. A purified Drosophila septin complex forms filaments and exhibits GTPase

activity. J Cell Biol. 1996;133(3):605-16. doi: 10.1083/jcb.133.3.605.
12. Lin YH, Lin YM, Wang YY, Yu IS, Lin YW, Wang YH, et al. The expression level of septin12 is critical for spermiogenesis. Am J Pathol. 2009;174(5):1857-68. doi: 10.2353/ajpath.2009.080955.
13. Steels JD, Estey MP, Froese CD, Reynaud D, Pace-Asciak C, Trimble WS. Sept12 is a component of the mammalian sperm tail annulus. Cell Motil Cytoskeleton. 2007;64(10):794-807. doi: 10.1002/cm.20224.
14. Kuo PL, Chiang HS, Wang YY, Kuo YC, Chen MF, Yu I, et al. SEPT12-microtubule complexes are required for sperm head and tail formation. Int J Mol Sci. 2013;14(11):22102-16. doi: 10.3390/ijms141122102.
15. Cesario MM, Bartles JR. Compartmentalization, processing and redistribution of the plasma membrane protein CE9 on rodent spermatozoa. Relationship of the annulus to domain boundaries in the plasma membrane of the tail. J Cell Sci. 1994;107(2):561-70. doi: 10.1242/jcs.107.2.561.
16. Toure A, Rode B, Hunnicutt GR, Escalier D, Gacon G. Septins at the annulus of mammalian sperm. Biol Chem. 2011;392(8-9):799-803. doi: 10.1515/BC.2011.074.
17. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod. 2007;22(6):1506-12. doi: 10.1093/humrep/dem046.
18. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Samanmanesh S, Karimkhanilouei S. The effects of Factor II (rs1799963) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Riv Ital Med Lab. 2017;13(1):37-40. doi: 10.1007/s13631-017-0145-y.
19. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Moghtaran N, Maroufi NF. The effect of factor-xi (rs3756008) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Gene Cell Tissue. 2017;4(1):e43717. doi: 10.17795/gct-43717.
20. Shiralizadeh J, Barmaki H, Haiaty S, Faridvand Y, Mostafazadeh M, Mokarizadeh N, et al. The effects of high and lowdoses of folic acid on oxidation of protein levels during pregnancy: a randomized double-blind clinical trial. Horm Mol Biol Clin Investig 2017; 33:20170039. doi: 10.1515/hmbci-2017-0039.
21. Song P, Zou S, Chen T, Chen J, Wang Y, Yang J, et al. Endothelial nitric oxide synthase (eNOS) T-786C, 4a4b, and G894T polymorphisms and male infertility: study for idiopathic asthenozoospermia and meta-analysis. Biol Reprod. 2015;92(2):38-1. doi: 10.1095/biolreprod.114.123240.
22. Balercia G, Moretti S, Vignini A, Magagnini M, Mantero F, Boscaro M, et al. Role of nitric oxide concentrations on human sperm motility. J Androl. 2004;25(2):245-9. doi: 10.1002/j.1939-4640.2004.tb02784.x.
23. Yun YJ, Park JH, Song SH, Lee S. The association of 4a4b polymorphism of endothelial nitric oxide synthase (eNOS) gene with the sperm morphology in Korean infertile men. Fertil Steril. 2008;90(4):1126-31. doi: 10.1016/j.fertnstert.2007.07.1382.
24. Vučić NL, Nikolić ZZ, Vukotić VD, Tomović SM, Vuković II, Kanazir SD, et al. NOS 3 gene variants and male infertility: association of 4a/4b with oligoasthenozoospermia. Andrologia. 2018;50(1):e12817. doi: 10.1111/and.12817.
25. Safarinejad MR, Shafiei N, Safarinejad S. The role of endothelial nitric oxide synthase (eNOS) T-786C, G894T, and 4a/b gene polymorphisms in the risk of idiopathic male infertility. Mol Reprod Dev. 2010;77(8):720-7. doi: 10.1002/mrd.21210.

26. Yan L, Guo W, Wu S, Liu J, Zhang S, Shi L, et al. Genetic variants in nitric oxide synthase genes and the risk of male infertility in a Chinese population: a case-control study. PloS One. 2014;9(12):e115190. doi: 10.1371/journal.pone.0115190.
27. Rafaee A, Mohseni Meybodi A, Yaghmaei P, Hosseini SH, Sabbaghian M. Single-nucleotide polymorphism c. 474G> A in the SEPT12 gene is a predisposing factor in male infertility. Mol Reprod Dev. 2020;87(2):251-9. doi: 10.1002/mrd.23310.
28. Kuo YC, Lin YH, Chen HI, Wang YY, Chiou YW, Lin HH, Pan HA, Wu CM, Su SM, Hsu CC, Kuo PL. SEPT12 mutations cause male infertility with defective sperm annulus. Hum Mutat. 2012;33(4):710-9. doi: 10.1002/humu.22028.
29. Lin YH, Wang YY, Chen HI, Kuo YC, Chiou YW, Lin HH, Wu CM, Hsu CC, Chiang HS, Kuo PL. SEPTIN12 genetic variants confer susceptibility to teratozoospermia. PLoS One. 2012;7(3):e34011. doi: 10.1371/journal.pone.0034011.
30. Fathi Maroufi N, Aghayi E, Garshsbi H, Matin MG, Bedoustani AB, Amoudizaj FF, et al. Association of rs1946518 C/A Polymorphism in Promoter Region of Interleukin 18 Gene and Breast Cancer Risk in Iranian Women: A Case-control Study. Iran J Allergy Asthma Immunol. 2019;18(6):671-678. doi: 10.18502/ijaai.v18i6.2180.
31. Fathi Maroufi N, Gholampour Matin M, Ghanbari N, Khorrami A, Amini Z, Haj Azimian S, et al. Influence of single nucleotide polymorphism in IL-27 and IL-33 genes on breast cancer. Br J Biomed Sci. 2019;76(2):89-91. doi: 10.1080/09674845.2018.1545554.
32. Isazadeh A, Azimian SH, Tariverdi N, Rahmani SA, Esmaeili M, Karimkhanilouei S, et al. Effects of coagulation factor XIII (Val34Leu) polymorphism on recurrent pregnancy loss in Iranian Azeri women. LaboratoriumsMedizin. 2017;41(2):89-92. doi: 10.1515/labmed-2017-0012.