

New single nucleotide polymorphism G5508A in the *SEPT12* gene may be associated with idiopathic male infertility in Iranian men

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Abstract

Background: Male infertility is a multifactorial disorder, which affects approximately 10% of couples at childbearing age with substantial clinical and social impact. Genetic factors are associated with the susceptibility to spermatogenic impairment in humans. Recently, *SEPT12* is reported as a critical gene for spermatogenesis. This gene encodes a testis specific member of Septin proteins, a family of polymerizing GTP-binding proteins. *SEPT12* in association with other Septins is an essential annulus component in mature sperm. So, it is hypothesized that genetic alterations of *SEPT12* may be concerned in male infertility.

Objective: The objective of this research is exploration of new single nucleotide polymorphism G5508A in the *SEPT12* gene association with idiopathic male infertility in Iranian men.

Materials and Methods: In this case control study, 67 infertile men and 100 normal controls were analyzed for genetic alterations in the active site coding region of *SEPT12*, using polymerase chain reaction sequencing technique. Fisher exact test was used for statistical analysis and $p < 0.05$ was considered as statistically significant.

Results: Genotype analysis indicated that G5508A polymorphic *SEPT12* alleles were distributed in three peaks of frequency in both control and diseases groups. Categorization of the alleles into (GG), (GA), (AA) types revealed a significant difference between infertile patients (azoospermic and asthenospermic) and normal controls ($p = 0.005$).

Conclusion: According to our finding we suggest that G5508A polymorphism in *SEPT12* gene can affect spermatogenesis in men, the opinion needs more investigation in different populations.

Key words: Male infertility, Polymorphism, *SEPT12*, Septin, Spermatogenesis.

Introduction

Subfertility is one of the major clinical, social and economic concern. In up to 55% of couples seeking medical attention, the male partner is diagnosed with spermatogenic failure, defined as one or more semen parameters falling below the world health organization (WHO) cut-off for normozoospermia (1). In the most severe forms of infertility with male factor azoospermia defined as complete absence of sperm from ejaculate, and asthenospermia means having defects in sperm motility (2). The etiology of spermatogenesis failure

includes genetic abnormalities (3), infectious, and environmental causes (4).

Spermatogenesis is governed by the parallel and serial actions of thousands of genes, alterations in any of them or their expression may cause male infertility (5-10). In reality, only a handful of genetic alterations have clearly been shown to cause spermatogenic failure (11).

Recently, a number of reports have linked altered expression of Septin genes to a range of diseases, including male infertility (12, 13). Since 1997, 14 members of Septin proteins have been characterized in humans (*SEPT1-SEPT14*), some of which are tissue-specific. All of the 14 genome-mapped human Septin

genes encode a highly conserved central GTP binding/hydrolysis domain which is very critical in GTPase signaling properties, as well as oligomerization between Septins and other filamentous proteins. This functional domain consists of three distinct amino acid sequence elements, G1, G3 and G4, which share sequence identity to the well-characterized Ras GTPase family (14).

Among all septins, *SEPT12* is dominantly expressed in testis tissue of adults, with an established essential role in annulus structure of mature sperm (15). Recently, it has been shown by investigators that high levels of *SEPT12* mRNA is observable exclusively in the testis of sexually mature males (human and mouse), while this mRNA was not detectable in men with sterility resulting from inability to produce mature spermatozoa. So, it has been considered that *SEPT12* is crucial for the process of spermatogenesis in mammals (16).

With this in mind, in the current study we tried to monitor genetic variations of *SEPT12* gene in an Iranian population of infertile men with non-obstructive azoospermia and asthenospermia, in order to find any relationship between genetic alterations in the *SEPT12* gene and some cases of idiopathic male infertility.

Materials and methods

Specimen

The study population in this case-control report is consisted of 67 infertile men (50 azoospermia and 17 asthenospermia) who were referred to Royan Institute, Tehran, Iran, during 2012-2013. Also, 100 normozoospermic men with female factor subfertility were analyzed as control (Table I). Using power analysis and sample size (PASS, 2011), the power of the study was calculated 0.78. Patients with hypogonadotropic hypogonadism, cryptorchidism, orchitis, ejaculatory duct obstruction, and men with microdeletions of the long arm of the Y-chromosome or karyotype abnormalities were excluded from the study. The classification of men into the normozoospermic and azoospermic groups was according to WHO criteria (2). Informed consent was obtained from all individuals enrolled in the study.

Genomic DNA was extracted from peripheral blood specimens using a QIAamp

DNA minikit (Qiagen Germany), according to the instructions provided by the manufacturer.

Polymerase Chain Reaction (PCR)-Sequencing analysis

For genetic analysis, the coding region of G1, G3 and G4 functional domains of *SEPT12* including exons 2-3 and their interval intron, and also exon 6 and its exon-intron boundaries were screened by polymerase chain reaction (PCR)-directed sequencing, using the specific primers designed by Primer3 software (Primer3.ut.ee) (Table II).

To analyze the aforementioned DNA sequences, PCR-Sequencing technique was performed with an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 45 sec, annealing at 60°C for 45 sec and extension at 72°C for 45 sec. The PCR products were confirmed by running on 1.5% agarose gel, and were applied for sequencing by an ABI 3730XL automated DNA sequencer (Macrogen, Seoul, Korea).

Statistical analysis

Fisher exact test was used to investigate the relationship between three sample groups (control, azoospermic and asthenospermic) and allele frequency. All statistical analysis were performed using SPSS (Statistical Package for the Social Sciences version 17.0, SPSS Inc., Chicago, IL, USA) software. A $p < 0.05$ was considered as statistically significant.

Results

Sequence analysis data revealed the nucleotide transition G5508A in *SEPT12* gene of the patients with respect to normal controls (Fig.1). The patients were divided into the two groups of azoospermia and asthenospermia. As indicated in Table I, among the azoospermic patients (50 individuals) 36 (72.0%) samples were normal homozygous (GG), 11 (22.0%) of them had the heterozygous (GA) mutation and 3 (6.0%) individuals were observed with both alleles mutated (AA). In asthenospermia patients, number of individuals harboring normal homozygous (GG), heterozygous (GA) and completely mutated (AA) alleles were, 11 (64.7%), 5 (29.4%) and 1 (5.9%), respectively. Statistical analysis of the data showed that the G5508A variation of *SEPT12* gene revealed

significantly different genotype distributions between the normal control group and the patients groups ($p=0.005$). Also, the frequency of the normal G allele was significantly higher in the control group compared with the patients groups ($p=0.001$). However, there was no significant change between the two

groups of azoospermic and asthenospermic for this allele ($p=0.05$). On the other side, the frequency of the A allele in normal group was significantly lower compared with the both patients groups ($p=0.001$). Again, no significant change was observed between the two patients groups ($p=0.05$).

Table I. Distribution of the allelic alteration of G5508A between infertile patients and normal controls

	Normal	Azoospermia	Asthenospermia	
	n (%)	n (%)	n (%)	p-value
Genotype-wise comparison, n (%)				
GG	89 (89.0)a	36 (72.0)b	11 (64.7)b	0.005
GA	11 (11.0)a	11 (22.0)ab	5 (29.4)b	
AA	0 (0.0)a	3 (6.0)b	1 (5.9)b	
Allele-wise comparison, n (%)				
G	189 (94.5)a	83 (83.0)b	27(79.4)b	0.001
A	11 (5.5)a	17 (17.0)b	7(20.6)b	

The same letter in each rows show not significant difference between groups ($p\text{-value} > 0.05$) according to Fisher exact test.

Table II. Primer pairs used in this study

Coding region	Primers (5'→3')	Product size (bp)
G1-G3	F: GTTGATCTGGTCCCGAAG R: TAAACGCCCCACCCTAACTG	332
G4	F: TGATGTCTCTCGTCAAAGCAC R: CCCTGCTGCTGCTGTTTAT	341

bp: base pair

F: forward

R: reverse.

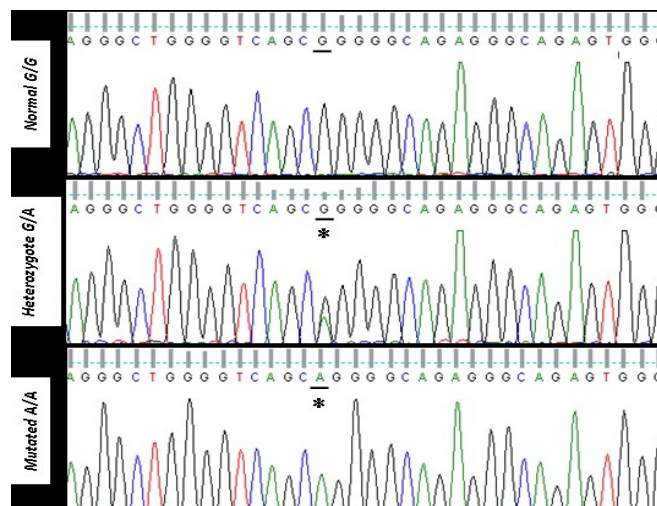


Figure 1. Electropherogram showing the heterozygote and homozygote DNA sequences of the *SEPT12* gene variants G→A compared with the normal-person sequence. The stars indicate location of the variations.

Discussion

There are several reports introducing novel genes involved in spermatogenesis, in the way that their down-regulation in the testes tissues of infertile men has been identified by high throughput expression analyses (9). *SEPT12* is one of these genes which its lower

expression in the testicular biopsies of infertile men is significantly related with azoospermia. In this study, we hypothesized genetic variations of *SEPT12* gene may be associated with male infertility caused by spermatogenesis failure.

The present association study revealed significantly different allele frequency of

G5508A in *SEPT12* gene, among patients with azoospermia and asthenospermia with respect to control men with normal spermograms. Although the number of analyzed patients is not enough to have a final decision, our preliminary finding observed in Iranian patients suggests that this G>A alteration may play a causative role in disruption of spermatogenesis. According to the recent reports showing that some *SEPT12* SNPs may predispose men to spermatogenic failure (16-18), the current data propose that the novel G5508A polymorphism can be considered as a biomarker for idiopathic male infertility.

Today, in vitro fertilization has been established as an efficient technique to resolve infertility in couples with female factors, but it cannot be useful for severe male factors with lacks in spermatogenesis. Although testicular sperm extraction-intracytoplasmic sperm injection is now successfully used for these cases, however, it cannot benefit patients with complete failure in spermatogenesis. So, genetic diagnosis of severe male infertility is a critical subject for assisted reproductive technology.

Conclusion

In conclusion, we suggest that the novel G5508A polymorphism in *SEPT12* gene may be associated with idiopathic male infertility in Iranian men. However, as this genetic alteration is in the intronic region, the molecular mechanism of its effectiveness is really unknown. Further study is needed to test this synonymous coding polymorphism for potential alteration of splicing between exonic elements.

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Conflict of interest

We have no conflict of interest.

References

1. De Kretser DM, Baker HW. Infertility in men: recent advances and continuing controversies. *J Clin Endocrinol Metab* 1999; 84: 3443-3450.
2. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-

- Cervical Mucus Interaction. Cambridge University Press, Cambridge; 1999.
3. Sadeghi-Nejad H, Farrokhi F. Genetics of azoospermia: current knowledge, clinical implications, and future directions. Part I. *Urol J* 2006; 3: 193-203.
4. Aryanpur M, Tarahomi M, Sharifi H, Heydari Gh, Hessami Z, Akhoundi M, et al. Comparison of spermatozoa quality in male smokers and nonsmokers of Iranian infertile couples. *Int J Fertil Steril* 2011; 5: 152-157.
5. Schultz N, Hamra FK, Garbers DL. A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. *Proc Natl Acad Sci USA* 2003; 100: 12201-12206.
6. Schlecht U, Demougin P, Koch R, Hermida L, Wiederkehr C, Descombes P, et al. Expression profiling of mammalian male meiosis and gametogenesis identifies novel candidate genes for roles in the regulation of fertility. *Mol Biol Cell* 2004; 15: 1031-1043.
7. Wang H, Zhou Z, Xu M, Li J, Xiao J, Xu ZY, et al. A spermatogenesis-related gene expression profile in human spermatozoa and its potential clinical applications. *J Mol Med* 2004; 82: 317-324.
8. Lin YH, Lin YM, Teng YN, Hsieh TY, Lin YS, Kuo PL. Identification of ten novel genes involved in human spermatogenesis by microarray analysis of testicular tissue. *Fertil Steril* 2006; 86: 1650-1658.
9. Gava MM, Chagas Ede O, Bianco B, Christofolini DM, Pompeo AC, Glina S, et al. Methylenetetrahydrofolate reductase polymorphisms are related to male infertility in Brazilian men. *Genet Test Mol Biomarkers* 2011; 15: 153-157.
10. Li X, Pan J, Liu Q, Xiong E, Chen Z, Zhou Z, et al. Glutathione S-transferases gene polymorphisms and risk of male idiopathic infertility: a systematic review and meta-analysis. *Mol Biol Rep* 2013; 40: 2431-2438.
11. Nuti F, Krausz C. Gene polymorphisms/mutations relevant to abnormal spermatogenesis. *Reprod Biomed Online* 2008; 16: 504-513.
12. Hall PA, Finger FP. Septins and human disease. In: Hall PA, Russell SHE, Pringle JR. The Septins. John Wiley & Sons, Hoboken. New Jersey, USA, 2008: 295-317.
13. Shafipour M, Sabbaghian M, Shahhoseini M, Sadighi MA. Comparative expression analysis of Septin 14 in testes of infertile men with normal spermatogenesis and spermatogenic failure. *Iran J Reprod Med* 2014; 12: 205-208.
14. Weirich CS, Erzberger JP, Barral Y. The septin family of GTPases: architecture and dynamics. *Nat Rev Mol Cell Biol* 2008; 9: 478-489.
15. 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: 794-807.
16. 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; 174:1857-1868.
17. Miyakawa H, Miyamoto T, Koh E, Tsujimura A, Miyagawa Y, Saijo Y, et al. Single-nucleotide polymorphisms in the SEPTIN12 gene may be a genetic risk factor for Japanese patients with Sertoli cell-only syndrome. *J Androl* 2012; 33: 483-487.
18. Miyamoto T, Tsujimura A, Miyagawa Y, Koh E, Namiki M, Horikawa M, et al. Single nucleotide polymorphisms in the SEPTIN12 gene may be associated with azoospermia by meiotic arrest in Japanese men. *J Assist Reprod Genet* 2012; 29: 47-51.