

Association of single nucleotide polymorphism c.673C>A/p.Gln225Lys in *SEPT12* gene with spermatogenesis failure in male idiopathic infertility in Northeast China

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Abstract

Male infertility is a complex multifactorial disease affecting approximately 10% of couples who want to have children. Some cases of infertility can be explained by genetic factors. Septins are members of the GTPase superfamily, which are involved in diverse biological processes including morphogenesis, compartmentalization, cytokinesis, and apoptosis. The septin 12 gene, *SEPT12*, is expressed exclusively in post-meiotic male germ cells and is considered as a critical gene for spermatogenesis. In this study, we evaluated 200 patients with non-obstructive azoospermia and detected mutations of 25 spermatogenesis-associated genes by targeted exome sequencing. We report a missense *SEPT12* variant, c.673C>A/p.Gln225Lys, in an infertile man with non-obstructive azoospermia. The variation was located inside the GTPase domain and had a SIFT score of 0.02 (<0.50) and was considered to be 'probably damaging' by PolyPhen. This case may provide clues to help establish the relationship between *SEPT12* gene alterations and some cases of idiopathic male infertility. The role of this variant should thus be investigated further.

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Keywords

SEPT12, single nucleotide polymorphism, spermatogenesis failure, male infertility, azoospermia, septin

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Introduction

Male infertility is a complex multifactorial disease that affects approximately 10% of couples wanting to have children, with consequently substantial clinical and social impacts.¹ However, partly due to the nature of the condition, the causes of male infertility remain largely unexplained.² Approximately 10% to 15% of infertility cases are known to be due to genetic causes, including chromosomal abnormalities and single-gene mutations.^{3,4} A large proportion of infertile men do not receive a clear diagnosis. However, aberrations in many additional genes are likely to underlie a significant proportion of cases of male infertility/subfertility, given that spermatogenesis requires the coordinated action of thousands of genes,² alterations in the structure or expression of any of which might result in male infertility.⁴⁻¹⁰ Genetic polymorphisms may also increase the susceptibility to some forms of male infertility.^{11,12}

Few causal mutations have been associated with spermatogenesis defects in recent decades; however, the use of parallel sequencing technologies has allowed the identification of increasing numbers of genes essential for spermatogenesis and mutations involved in spermatogenesis failure.¹³

We aimed to monitor genetic variations in some genes associated with spermatogenic defects in infertile Chinese men with non-obstructive azoospermia. We also present a patient in whom next generation sequencing suggested the existence of a single nucleotide polymorphism (SNP) variation in the septin 12 gene (*SEPT12*).

Septins are a family of polymerizing GTP-binding proteins.¹ *SEPT12* encodes a testis-specific septin and is considered as a critical gene for spermatogenesis.¹⁴ Septins are members of the GTPase superfamily, which are involved in diverse biological processes including morphogenesis, compartmentalization, cytokinesis, and apoptosis.¹⁵ In association with other septins, septin 12 is an essential annulus component in mature mammalian sperm.¹⁶ High expression levels of *SEPT12* mRNA have been observed exclusively in the testis of sexually mature male humans and mice, but not in men who were sterile as a result of the inability to produce mature spermatozoa.⁷ *SEPT12* knock out in mouse embryonic stem cells resulted in chimeric mice with severe spermatogenic defects. *SEPT12* has thus been suggested to play a crucial role in the process of spermatogenesis.¹⁴

This evidence suggests that *SEPT12* may be a potential sterility gene in humans, and genetic mutations or polymorphisms of *SEPT12* may participate in male infertility. In this study and case report, we aimed to provide clues to establish the existence of any relationship between *SEPT12* gene alterations and idiopathic male infertility.

Methods and case report

This study was reviewed and approved by the Medical Ethics Committee of First Hospital of Jilin University (No. 2014-321) and performed according to the tenets of the Declaration of Helsinki. All the patients were Chinese and provided written informed consent. Written informed

consent for specific patient information and images to be published was provided by the Medical Ethics Committee of First Hospital of Jilin University. We evaluated a cohort of 200 patients who consulted for sterility and displayed non-obstructive azoospermia. They underwent comprehensive examination, including a detailed medical history, physical examination, hormone profiles, chromosome analysis, and molecular testing for Y-chromosome microdeletions. Patients without Y-chromosome microdeletions were included in the study and were subjected to analysis of spermatogenesis-associated genes.

Mutation screening of spermatogenesis-associated genes was performed by targeted exome sequencing. Genomic DNA was isolated from blood lymphocytes and subjected to exome capture using an in-house targeted gene panel (Peking Medriv Academy of Genetics and Reproduction, Peking, China), followed by next generation sequencing on the Illumina MiSeq sequencing platform (HiSeq2000, Illumina, San Diego, CA, USA), which includes 25 spermatogenesis-associated genes. Capture probes were also prepared based on the 25 spermatogenesis-associated genes. Sequence reads were mapped to the human reference genome assembly (NCBI build 37/hg19) using the Burrow-Wheeler Aligner (BWA version 0.7.12). Duplicated reads from the library and polymerase chain reaction (PCR) preparation were removed using Picard tools. After alignment to the human reference genome (GRCh37/hg19), the most likely non-deleterious variants were filtered as described previously.¹⁷ Variants with minor allele frequencies >1% in the dbSNP, 1000 Genomes Project, Exome Aggregation Consortium, and Exome Variant Server databases were excluded because they were unlikely to be deleterious. Intronic variants and synonymous variants located within intronic regions, regulatory regions, and non-regulatory intergenic regions were also

excluded. Nonsynonymous variants and splice site variants were retained.

Possible pathogenic effects of the candidate variants were evaluated using PolyPhen2 (<https://genetics.bwh.harvard.edu/pph2/>, version 2.2), with probabilistic scores set at <0.15, 0.15 to 0.85, and >0.85 for benign, possibly damaging, and probably damaging, respectively,¹⁸ and SIFT (<https://sift.jcvi.org>, version 1.03) with a cut-off score of ≤0.05 for deleterious variants.¹⁹

A missense variant was confirmed by conventional PCR and Sanger sequencing (ABI 3730XL, Applied Biosystems, Carlsbad, CA, USA) carried out by BGI Genomics, Beijing Genomics Institute, Shenzhen, China. The primers for the detected missense variant c.673C>A/p.Gln225Lys in *SEPT12* (GenBank accession number NM_144605.4) were 5'-CCTT GAGCTTGTGGATGAGTAGA-3' (forward) and 5'-GGAAATGGTGTCAGGT GGTGC-3' (reverse).

Case report

A 29-year-old Chinese man was shown to have the *SEPT12* c.673C>A/p.Gln225Lys variant. He and his partner had been attempting to conceive for 1 year. Azoospermia was confirmed. The patient reported no genital traumas and there was no sign of varicocele. His testicular volumes were 8 mL left and 10 mL right. He had a high serum concentration of follicle-stimulating hormone (23.88 IU/L) and slightly elevated luteinizing hormone (10.9 IU/L), and a normal level of free testosterone (10.79 pmol/L). Chromosome analysis showed a normal 46, XY karyotype and no Y-chromosome microdeletions. The definitive histological results showed Sertoli cell-only syndrome (SCOS).

Results

Targeted exome sequencing for mutations in 25 spermatogenesis-associated genes in

200 individuals (average age 30.25 ± 5.24 years, range 19 to 47 years) with non-obstructive azoospermia detected a *SEPT12* variant in an infertile man. The variant was heterozygous, and involved a single nucleotide change located within exon 7. The SNP was not novel (rs771771089), according to the Single Nucleotide Polymorphism database (dbSNP), but it had not previously been reported in association with spermatogenesis failure. This SNP was nonsynonymous (c.673C>A/p.Gln225Lys) and had a SIFT score of 0.02 (<0.50), and was considered to be 'probably damaging' by PolyPhen. PCR and Sanger sequencing confirmed the heterozygous missense variant in the patient (Figure 1a).

Discussion

Previous studies have indicated that spermatogenesis failure in patients with azoospermia may result from chromosomal abnormalities, Y-chromosome microdeletions, or single gene variants.^{20,21} However, the underlying genes and molecular mechanisms remain poorly known.

The septins are a family of polymerizing GTP-binding proteins, originally discovered in a group of cell cycle mutants of budding yeast with defects in cytokinesis.²² The functions of septins include cytoskeletal remodeling, mitosis, vesicle trafficking, and membrane compartmentalization. Septins have also been implicated in the pathogenesis of many diseases, including hereditary neuralgic amyotrophy, leukemia, breast cancer, ovarian tumors, Alzheimer's disease, and male infertility.²³

The roles of septins in spermatogenesis have begun to be revealed. Among the 14 septin family members, septins 4, 7, and 12 are major constituents of the annulus,²⁴ a submembrane ring structure demarcating the principle and mid-piece of the sperm tail.¹⁵ Septin 4 plays important roles in maintaining the proper mitochondrial

architecture and establishing the annulus during spermatogenesis.²³ Male *Sept4* knockout mice were sterile due to immotile sperm with a defective annulus, and the immotile sperm showed dis-localization of septins 1, 6, and 7 from the annulus.²⁵ Annulus (septins 1/4/6/7) complexes tend to be absent in mature spermatozoa from men with asthenozoospermia.²² Investigation of expression patterns suggested that septin 7 may be involved in the regulation of subcellular-compartment formation during spermiogenesis, and that the absence of a septin 7 signal correlated with multiple sperm defects.²⁶

In this study, a *SEPT12* genetic variant (c.673C>A/p.Gln225Lys) was detected in an infertile man with non-obstructive azoospermia. This point mutation was detected in exon 7 and located in the GTPase domain, resulting in the replacement of glutamine with lysine. The mutant protein (septin 12^{Q225K}) may lose its GTP-hydrolysis ability, thus adversely affecting septin filament formation compared with wild-type septin 12.¹⁵ The GTPase domain is critical for the polymerization of septin subunits into filaments, which are required for formation of the sperm annulus/septin ring, which is in turn involved in the normal structure and function of sperm and in male fertility.²⁷

Some *SEPT12* gene variants have been associated with male infertility (Figure 1b). Several recent studies showed that some *SEPT12* SNPs may predispose men to azoospermia.^{28,29} Miyamoto et al. carried out mutational analysis of *SEPT12* in 30 men with azoospermia due to meiotic arrest and 140 fertile male controls, and found that the frequencies of the c.204G>C allele and CC genotype were significantly higher in the cases compared with the controls. This *SEPT12* variant previously demonstrated a relationship with increased susceptibility to azoospermia caused by meiotic arrest.²⁸ Miyakawa et al. investigated 100 Japanese

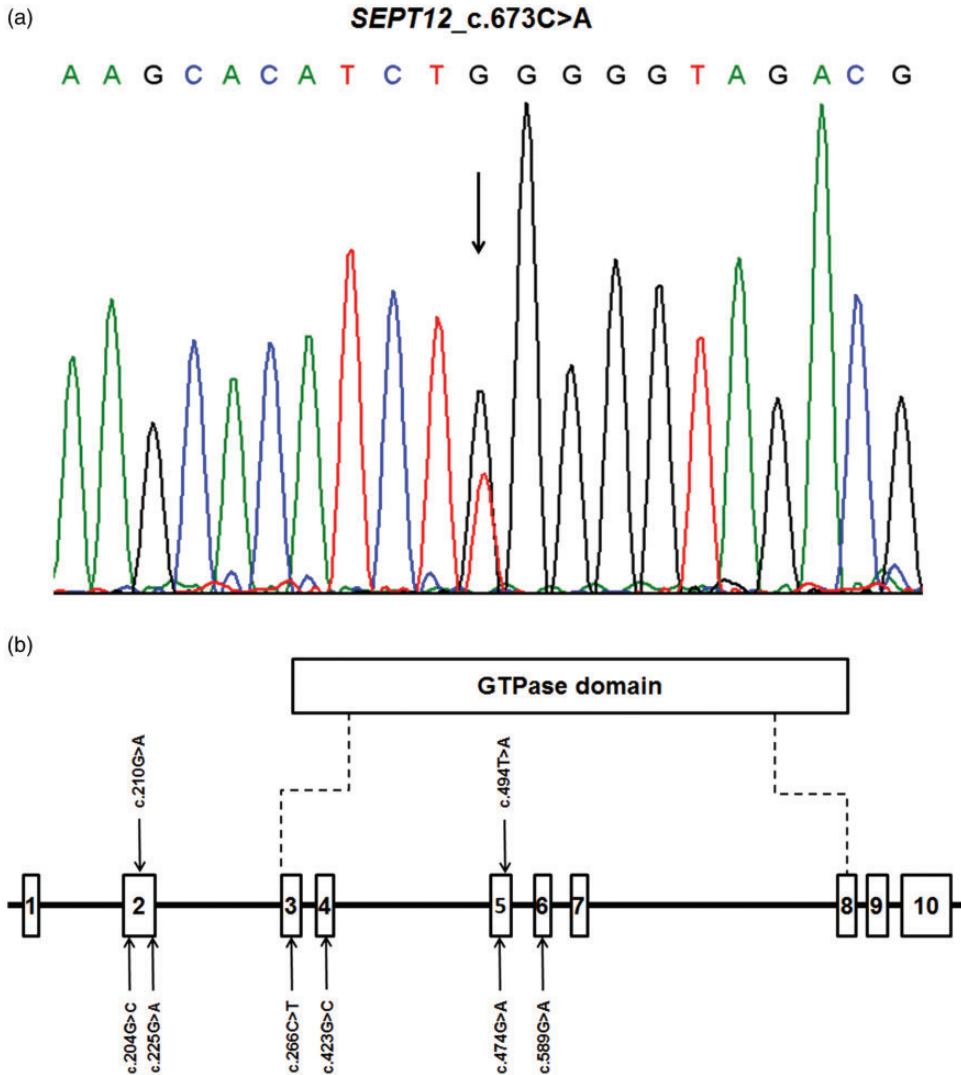


Figure 1. (a) *SEPT12* c.673C>A/p.Gln225Lys heterozygous variant in an infertile patient was confirmed by Sanger sequencing. Arrow indicates location of the variation. (b) Previously reported genetic variants of *SEPT12* significantly associated with male infertility.^{15,20–22} Open bars indicate exons. Exon 3 to exon 8 encodes the GTPase domain of *SEPT12*.

patients with azoospermia caused by SCOS by direct sequencing of the coding regions of the *SEPT12* gene. They detected eight coding SNPs, some of which had notably higher genotype and allele frequencies in SCOS patients compared with the control group.²⁹ These findings suggested that

SEPT12 and its mutations might be associated with human spermatogenesis. In the current study, we reported a *SEPT12* variant, c.673C>A/p.Gln225Lys, in a man with non-obstructive azoospermia caused by SCOS. Overall, these results suggest that the c.673C>A/p.Gln225Lys variant was

likely to be related to spermatogenesis failure in this patient.

Other studies have also reported an association between *SEPT12* mutations and oligoasthenoteratozoospermia.^{15,30} Lin et al. found that most individuals homozygous for the *SEPT12* c.474G>A allele had teratozoospermia (14%), and their sperm showed bent tails and de-condensed nuclei, with significant DNA damage.³⁰ Kuo et al. reported two missense mutations in *SEPT12* in infertile men, c.266C>T/p.Thr89Met and c.589G>A/p.Asp197Asn, both of which were located inside the GTPase domain with potential effects on protein structure.¹⁵ Patients carrying the two variants presented with oligoasthenozoospermia and asthenoteratozoospermia, respectively. These results suggested that *SEPT12* loss-of-function mutations can disrupt the structural integrity of sperm by perturbing septin filament formation.¹⁵ The variant c.673C>A/p.Gln225Lys detected in the current study was a missense mutation, also located inside the GTPase domain and therefore also with the potential to alter the protein structure. We therefore hypothesized that it may be associated with spermatogenesis failure. However, further functional assays are needed to verify this hypothesis and to provide new insights into the cause of spermatogenesis failure in humans.

In conclusion, the c.673C>A/p.Gln225Lys genetic variant detected in the *SEPT12* gene in a patient with non-obstructive azoospermia suggests that this SNP may play a causative role in spermatogenesis failure. This SNP is not novel, but it has not previously been reported in association with spermatogenesis failure. Although this study was not conclusive, it provides the basis for further studies aimed at clarifying the relationship between *SEPT12* gene alterations and idiopathic male infertility, with the potential for this variant to be considered as a biomarker for idiopathic male infertility in the future.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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