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Association of polymorphism in cell death pathway gene FASLG with human male infertility

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ABSTRACT

Objective: To investigate –844C>T single nucleotide polymorphism (SNP) present in the promoter of cell death pathway gene FASLG with male infertile phenotype. **Methods:** Genotyping for SNP FASLG (rs763110) was done by polymerase chain reaction followed by analysis with specific endonuclease (PCR-RFLP). DNA sequencing was used to ascertain PCR-RFLP results. **Results:** FASLG –844C>T polymorphism, allele and genotype distribution did not differ significantly between patients and controls (*OR*: 1.03, 95% *CI*= 0.7638 to 1.3952, *P*=0.83). Thus SNP-844C>T of the FASLG gene is not associated with male infertility risk in the analyzed patients. **Conclusion:** Human male infertility is a complex disorder and thus other genetic or environmental factors may be contributing to the complex etiology, and further study in other region of Indian populations will verify whether it is associated with male infertility risk.

1. Introduction

Infertility affects 10%-12% couples worldwide and male factor accounts for nearly half of the infertility cases[1, 2]. The formation of mature spermatozoa is a complex phenomenon and requires homeostasis of many different types of cells within the testis[3]. It is regulated by number of genes involved in different pathways like cell proliferation, differentiation and apoptosis[4]. The process of spermatogenesis involves the renewal and differentiation of spermatogonial stem cells into rapidly proliferating spermatogonia, meiotic cells (spermatocytes) and haploid cells (round, elongating and elongated spermatids) before release of a spermatozoon[5]. But to maintain the number of

cells in the seminiferous tubules a dynamic balance between cell proliferation and apoptotic cell death is pre-requisite[6]. Apoptosis is involved in a range of physiological functions, such as regulating cell numbers and eliminating unwanted or potentially dangerous cells during the development of organisms, as well as in some pathological processes. Programmed germ cells death is a frequent event occurring during normal spermatogenesis[7]. The extrinsic pathway of apoptosis is key regulatory mechanism for apoptosis in testis. Fas and FasL express on germ cell and Sertoli cells respectively[8]. Expression of FasL is up-regulated in the testes of patients with Sertoli cell only (SCO) and maturation arrest (MA), which suggests that it may be associated with apoptotic elimination or altered maturation of Fas-expressing germ cells through the activation of caspase-3[9]. Fas/FasL expression in the human testis is developmentally regulated and it may be involved in quality control mechanism of the sperms[10]. The FASLG –844 C>T (rs763110) functional polymorphisms is located in the

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binding motif of transcription factors disrupt CAAT/enhancer-binding protein. Higher basal expression of FASLG is statistically significantly associated more with the FASLG –844C allele than with the FASLG –844T allele[11]. Based on the biological and pathologic consequence of FASLG, it is possible that functional genetic variations in the FASLG gene may contribute to the clinical outcomes of male infertility. Therefore, we evaluated the effect of the FASLG-844 C>T functional variant of FASLG in cases of idiopathic male infertility.

2. Material and methods

2.1. Subjects

Patients were recruited from the Out Patient Department of University hospital, Infertility Clinic, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The present case-control study consists of 204 idiopathic azoospermic infertile males (32.0 ± 4.8 years) and 217 fertile men of comparable age were taken as controls. Both cases and controls belong to same geographical location and have same ethnicity. Patients married for a minimum of two years, having unprotected intercourse were considered for the present study. Three semen analysis were carried out after three/ four days of sexual abstinence to ascertain their infertility status. The size, volume and consistency of testis, occurrence of varicocele, hydrocele or absence of secondary sexual characters were also recorded. Questionnaire was maintained for each patient to record details of their lifestyle, habits and family history. Informed consent was obtained from every participant of each group. Patients with obstructive azoospermia, hypogonadism, hypoandrogenism, chronic diseases, history of pelvic/spinal injuries, karyotype abnormalities and AZF microdeletions were excluded. The control group consists of healthy fertile males who have at least one child and no history of chronic illness. Approval of the University's ethical committee for research on Human material was obtained.

2.2. FASLG–844 C>T polymorphism genotyping

FASLG-844 C>T polymorphism was studied by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP). The C>T change at -844 position creates a restriction site for BsRD1 restriction endonuclease. Two previously described primer sequences were used for PCR: For (5'- CAGCTACTCGGAGGCCAAG -3')

and Rev (5'- GCTCTGAGGGGAGAGACCAT -3')[11]. The PCR conditions were 30 seconds at 94 °C, 55 seconds at 62 °C, and 55 seconds at 72 °C. The first cycle was preceded by 5 minute denaturation step and a single step of extension at 72 °C for 10 minutes followed the last cycle. The 401 bp PCR product was digested with 1 Unit of BsRD1 at 55 °C over night. Separation of the products was performed on a 2% agarose gel.

2.3. Sequencing

PCR product was sequenced with Taq-Dye deoxy-terminator-cycle sequencing kit (Applied Biosystem, USA) using an automated genetic analyzer 3130 sequencer to ascertain PCR-RFLP results. Reverse primer was used for FASLG -844 for cycle sequencing. The Sequence was aligned and analyzed using AB DNA Sequencing Analysis software (version 5.2).

2.4. Statistical analysis

Allele and genotype distribution among groups were evaluated using Chi-square test. The difference in frequency between the case and control groups was analyzed for statistical significance at the 95% confidence interval using χ^2 test. The allele frequency FASLG -844 C>T was in Hardy-Weinberg equilibrium. Odds ratios (ORs) were calculated and reported within the 95% confidence limits. All statistical tests were two-sided, and *P*-value < 0.05 was considered statistically significant.

3. Results

A total of 421 individuals (204 infertile patients and 217 fertile controls) were examined. PCR-RFLP analysis was performed to assess the genotypes of 844 C>T polymorphism in the promoter region of FASLG gene in cases and controls (Figure 1). The genotype and allele frequencies for 844 C>T polymorphism in the FASLG gene were compared between the two groups. For FASLG -844, T allele frequency in infertile patients was 35.3% and in controls was 29.5%. In the current study, there was no substantial difference in the distribution of both C and T allele between patients and controls (*OR*: 1.03, 95% *CI*= 0.7638 to 1.3952, *P*=0.83) (Table 1). However dominant model (TT *vs.* CT+CC) showed significant increased risk with male infertility.

Table 1

Distribution of genotypes and allele frequencies of C844T SNP of FASLG in the study population.

Polymorphism(Genotype)	Cases(n=204)	Controls(n=217)	Odds Ratio (95% CI)	P-value	YatesCorrected P value
Dominant alleles			1 (Reference)	-	-
TT	75 (36.8%)	101 (46.5%)	1.47(0.9898 to 2.190)	0.05	0.07
CT	114 (55.9%)	104 (47.9%)	1.68(0.7471 to 3.8010)	0.21	0.29
CC	15 (7.4 %)	12 (5.5 %)	1.49(1.014 to 2.1991)	0.04*	0.05*
CT+CC	129 (60.6%)	116 (53.5%)			
FASL-844 C/T alleles			1.03(0.7638 to 1.3952)	0.83	0.89
T	264 (64.7%)	306 (70.5%)			
C	114 (35.3%)	128 (29.5%)			

Note: OR= odds Ratio; CI= 95% confidence interval, *Significant.

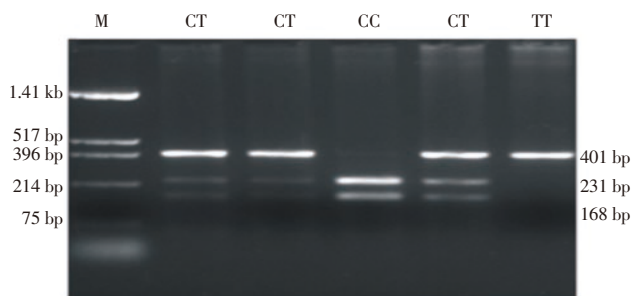


Figure 1. FASLG -844C>T mutation was detected by PCR-RFLP. 401bp PCR product was digested with BsRD1.

The allele C is cut by the enzyme and gives 231bp and 168bp, whereas the T allele remains uncut. Lane 1 shows the marker (puc/Hinf1), lane 2, 3, 5: CT heterozygous, lane 4: CC homozygous, lane 6: TT homozygous genotype.

4. Discussion

Spermatogenesis is a dynamic and synchronized process of maturation of spermatogonia into mature spermatozoa. The spermatogonia cells proliferate to give more number of cells, then differentiate into spermatocytes and finally mature sperm[12]. In the testis, Fas-induced apoptosis plays an important role during spermatogenesis. Fas (APO-1, CD95), a transmembrane receptor protein, transmit an apoptotic signal within cells when bound by Fas ligand[12]. Fas and FasL express on germ cells and Sertoli cells respectively. Studies have shown, when the testicular environment can no longer support spermatogenesis, the FasL system is activated leading to germ cell apoptosis, a process in which the Sertoli cells play a major role[7]. Testicular germ cells which are defective or in excessive number externalize Fas, when they come in contact of FasL on sertoli cells, undergo apoptosis and are resorbed. Thus, apoptosis plays an important role in (i) selecting germ cells and (ii) maintaining a proper amount of germ cells available for maturation by the Sertoli cells. Quality of semen is related with fertilization rate, implantation and development of offspring[13, 14]. Studies using the

generalized lymphoproliferative disease (gld) mice, which express a nonfunctional form of FasL further illustrate the importance of the Fas signaling pathway in the regulation of spontaneous germ cell apoptosis as well as increased germ cell death after toxicant-induced Sertoli cell injury[15,16]. Apoptosis is an essential process involved in range of physiological functions and some pathological processes. In the present study, functional polymorphism in cell death pathway gene FASLG was genotyped to investigate its role in human male infertility. In addition to this study Wang *et al*[17] have shown that FASLG–844C/T SNP may be a genetic predisposing factor of idiopathic azoospermia or severe oligozoospermia among Han Chinese men. In contrast, Guixiang *et al*[18] did not find any association of FASLG 844 C>T with altered sperm apoptosis and poor semen quality in Chinese population. The mutant C allele was found in a statistically identical prevalence among controls and patients. In the present study we have not found any association of FASLG 844>T SNP with male infertile phenotype in North Indian population. In the present study we have found association of FASLG -844 T SNP with male infertile phenotype in North Indian population using dominant genetic model (TT *vs.* CT+CC). In addition to this study Wang *et al* have shown that FASLG–844C/T SNP may be a genetic predisposing factor of idiopathic azoospermia or severe oligozoospermia among Han Chinese men. In contrast, Ji *et al* did not find any association of FASLG 844 C/T with altered sperm apoptosis and poor semen quality in Chinese population. The mutant C allele was found in a statistically identical prevalence among controls and patients. Human male infertility is a multifactorial trait thus both genetic as well as environmental factors contribute to its etiopathology. In summary, our study underscores the significance of genetic variant in the regulatory regions of FASLG in modulating risk of male infertility.

Conflict of interest statement

We declare that we have no conflict of interest.

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