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研究ノート

SNPs within the Intron-less *TAF7* Gene Encoding a General Transcription Factor in Japanese Males

Hiromitsu TANAKA[1)]*, Tatsuaki YOSHIMOTO[1)], Nobuhiro NAKAMURA[1)], Tsutomu TAKEDA[1)], Morimasa WADA[1)], Yasushi MIYAGAWA[2)] and Akira TSUJIMURA[3)]

([1)]Faculty of Pharmaceutical Sciences, Nagasaki International University, [2)]Department of Urology, Graduate School of Medicine, Osaka University, [3)]Department of Urology, Juntendo University Hospital, *Corresponding author.)

基本転写因子をコードする *TAF7* イントロンレス遺伝子の日本人男性における遺伝子多型解析

田中宏光[1)]*, 吉本辰暁[1)], 中村信博[1)]
武田司[1)], 和田守正[1)], 宮川康[2)]
辻村晃[3)]

([1)]長崎国際大学 薬学部 薬学科、[2)]大阪大学大学院 医学系研究科 器官制御外科学（泌尿器科学）、[3)]順天堂大学 医学部 泌尿器科、*連絡対応著者）

要 旨

TAF7 は転写開始と伸長に必須な転写開始前複合体に含まれる。精巣において、TAF7 は細胞の増殖を伴う精原細胞と第一精母細胞の核に局在し、また、TAF7 の精巣特異的アイソザイムである TAF7L は、精子細胞分化過程で核に局在する。このことから、雄性生殖細胞では、細胞増殖を伴う精原細胞では TAF7 が、その後の精子分化過程では TAF7L が転写調節に機能していることが考えられる。今回私たちは、細胞増殖をともなう精原細胞で発現する TAF7 イントロンレス遺伝子について、男性不妊症と TAF7 の遺伝子多型の関係を調べるため、282人の日本人男性不妊症患者と96人の妊孕性が確認された日本人男性ボランティアの遺伝子多型を調べた。その結果、男性不妊症性患者において、5′ 非翻訳領域に CTC の３塩基欠失と、アミノ酸置換を伴う一塩基多型がそれぞれヘテロ接合型として各々一人づつ検出された。The National Center for Biotechnology Information には TAF7 に関して多くの一塩基多型が登録されているが、日本人男性に関してそれらの遺伝子多型はほとんどが検出されなかった。新たに同定されたこれらの遺伝子多型は、体質と遺伝子多型の大規模な解析に役立つものと考えられる。

キーワード

不妊症、精巣、生殖細胞、精子形成、精原細胞、RNA ポリメラーゼ

Abstract

TAF7 is a part of the protein complex that is indispensable for the start of transcription. In the testis, TAF7 localizes on nuclei in spermatogonia and primary spermatocytes during proliferation. To examine whether genetic polymorphisms of the intron-less *TAF7* gene are associated with male infertility, we screened *TAF7* for genetic polymorphisms using DNA from 282 sterile and 96 fertile male volunteers. We identified 11 genetic polymorphisms in the *TAF7* region. Although many single nucleotide polymorphisms（SNPs）have been reported in the SNP database of the National Center for Biotechnology Information, we found a novel CTC deletion and one SNP with an amino acid substitution in the *TAF7* genomic region in infertile patients. These genetic

polymorphisms might be causes of male sterility. These results will be useful for analyzing the association of traits and genetic polymorphisms in further large-scale genetic analyses.

Key words
Infertility, Testis, Germ Cell, Spermatogenesis, Spermatogonia, RNA polymerase

Introduction

The assembly of the transcriptional complex is necessary to start transcription by RNA polymerase Ⅱ (pol Ⅱ). In this process, the first step involves the binding of transcription factor ⅡD (TFⅡD) to a promoter DNA sequence. TFⅡD is multi-protein complex that includes TATA-binding protein (TBP) and TBP associated factors (TAF), which include at least 12 proteins. There are many TAF subtypes. TAF7 plays important roles in the start of transcription in proliferating cells, but not in differentiated cells.[1] In testicular cells, TAF7 localizes on the nuclei in spermatogonia and primary spermatocytes. TAF7L, a germ cell-specific isozyme of TAF7, is expressed in the cytoplasm in primary spermatocytes and is localized on nuclei in pachytene spermatocytes and round spermatids.[2] These results indicate that TAF7 plays important roles in spermatogonia during cell proliferation and TAF7L is activated in spermatids during differentiation and transcription in the testis.[3), 4)] *TAF7* is an intron-less gene on chromosome 5. *TAF7L* mRNA is processed from 12–14 exons with alternative RNA splicing on the X chromosome. To understand infertility, we assessed the prevalence of SNPs in genes expressed in germ cells. To examine whether *TAF7* is a hereditary cause of male infertility, nucleotide polymorphisms in *TAF7* were assessed by direct sequencing of polymerase chain reaction (PCR)-amplified DNA from male patients.

MATERIALS AND METHODS

Participants

Japanese infertile subjects (n=282) were divided into subgroups according to the degree of defective spermatogenesis: 192 (68%) patients had non-obstructive azoospermia and 90 (32%) had severe oligospermia ($<5\times10^6$ cells/mL). All patients had idiopathic infertility based on a cytogenetic analysis and had no history of other medical conditions, including, but not limited to, cryptorchidism, recurrent infections, trauma, orchitis, or varicocele. The control group consisted of fertile males who had fathered children born at a maternity clinic (n=96). All donors were informed of the purpose of the study and gave permission for their blood to be subjected to genomic DNA analysis. This study was approved by the institutional review board and independent ethics committee of Osaka University.

Identification of Nucleotide Polymorphisms in *TAF7*

Genomic DNAs were isolated from a blood sample by protease treatment and phenol extraction. Sequences corresponding to the region encoded by the exon of *TAF7* were amplified by PCR using the primers TAFFr1 (A) (5′-GCACTTCCGTTTTTGCTGGGTAGG-3′) and TAFRv1(D) (5′-ATTGCTTAGTC-ACTAAACTCCACC-3′) (Fig. 1). PCR was performed using Prime-STAR or *Ex Taq* Hot Start (Takara, Shiga) and consisted of 40

cycles at 96℃ for 45 s, 65℃ for 45 s, and 72℃ for 90 s. PCR-amplified fragments were purified using SUPREC PCR spin columns (Takara). The DNA fragments were sequenced independently from both ends using the same PCR and internal primers: TAF7Fr 2(B) (5-GCTAGCACTGATCCTAAAGCAAGC-3′) and TAFRv2(C) (5′-CCACCGAGTACTAACAGCTTCAGC-3′), with thermal cycle sequencing kits (Applied Biosystems; Foster City, CA, USA) (Figure 1). The reaction products were analyzed using an ABI-PRISM 310 Genetic Analyzer (Applied Biosystems).

Statistical Analysis

Differences between the experimental and control conditions were compared using Fisher's exact test ($P<0.05$).

Results and Discussion

In Japan, more than 20% of married couples are affected by infertility, with the cause involving the male in two thirds.[5] We assessed the prevalence of SNPs in genes expressed in germ cells by direct sequencing of PCR-amplified DNA from males who were undergoing fertility evaluations.[6] Here, genetic polymorphisms in the *TAF7* gene expressed in spermatogonia were analyzed. The entire coding sequence of *TAF7* (NCBI accession number: Chromosome 5, NC_000005.10 (141318490... 141320784, complement)) is intron-less (Figure 1). Eleven polymorphisms were found in *TAF7* (Table 1). Nucleic acid base exchange introducing one amino acid substitution and two silent mutations were found in the *TAF7* open reading frame. Five genetic polymorphisms and three SNPs were detected in the 5′-untranslated region (5′–UTR) and 3′–UTR, respectively. We found one novel SNP causing an amino acid substitution and one deletion of a CTC nucleic sequence only in infertile males. Although many SNPs have been registered in the NCBI dbSNP database, none of two in the *TAF7* transcriptional region were found in the 378 Japanese males studied. This result may be due to race differences. Three SNPs (c555C>G, c1212G>A, and c1447T>C) were found in major and minor homozygous or heterozygous states. None of the minor homozygous states was detected in the proven fertile subjects. The CTC deletion and SNP (c560A>G) that introduced an amino acid substitution were found in infertile males. Although no significant differences in genotype frequency were identified in the infertile subjects ($P>0.05$),

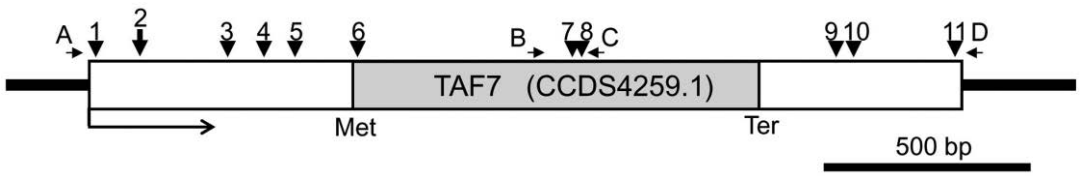

Figure 1. Schematic view of the *TAF7* gene region
The *TAF7* intron-less gene is localized at 5q31 (NC_000005.10). The box indicates the transcribed zone in the *TAF7* genome region and the open reading frame is shaded. The horizontal arrow below the box indicates the direction of transcription. The small horizontal arrows above the box indicate the locations of the PCR and DNA sequencing primers (A, TAF7Fr; B, TAF7Fr2; C, TAF7Rv2; D, TAF7Rv1). The vertical arrow indicates the location of the CTC deletion and the arrowheads the other SNPs. CCDS4295.1 is the Accession Number in NCBI.

the genetic polymorphisms observed only in infertile patients might be related to the cause of male infertility. This is the first analysis of *TAF7* genetic polymorphisms in males with non-obstructive azoospermia. These results will contribute greatly to future large-scale studies of the genetic background of infertility in Japanese males.

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Table 1 The prevalence of genetic polymorphisms in *TAF7* in infertile and proven fertile populations

	Position			Genotype	Number (%) of SNP		Reference
	Nucleotide	Amino acid			Infertile	Proven fertile	(NCBI dbSNP rs#)
TAF	−698			c/c	272/279 (97.5)	96 (100)	
				c/t	7/279 (2.5)	0 (0)	rs140942166
				t/t	0/279 (0)	0 (0)	
	−526−524			ctc	281 (99.6)	96 (100)	
				CTC/deletion	1 (0.4)	0 (0)	
				deletion	0 (0)	0 (0)	
	−302			c/c	278 (98.6)	93 (96.9)	
				c/t	4 (1.4)	3 (3.1)	rs146447648
				t/t	0 (0)	0 (0)	
	−229			a/a	279 (98.9)	95 (99.0)	
				a/c	3 (1.1)	1 (1.0)	rs11547633
				c/c	0 (0)	0 (0)	
	−148			a/a	281 (99.6)	95 (99.0)	
				a/g	1 (0.4)	1 (1.0)	rs149745811
				g/g	0 (0)	0 (0)	
	12	4	S	c/c	277 (98.2)	96 (100)	
				c/t	5 (1.8)	0 (0)	rs745733156
				t/t	0 (0)	0 (0)	
	555	185	A	c/c	243 (86.2)	91 (94.8)	
				c/g	38 (13.5)	5 (5.2)	rs2230133
				g/g	1 (0.4)	0 (0)	
	560	187	D	a/a	281 (99.6)	96 (100)	
			D/G	a/g	1 (0.4)	0 (0)	
			G	g/g	0 (0)	0 (0)	
	1166			t/t	277 (98.2)	95 (99.0)	
				t/c	5 (1.8)	1 (1.0)	rs140606827
				c/c	0 (0.0)	0 (0)	
	1212			a/a	219 (77.7)	75 (78.1)	
				a/g	58 (20.6)	21 (21.9)	rs10310
				g/g	5 (1.8)	0 (0)	
	1447			t/t	181 (64.2)	69 (71.9)	
				t/c	93 (33.0)	27 (28.1)	rs7730
				c/c	8 (2.8)	0 (0)	
Total					282	96	

Each genetic polymorphisms is named based on its position relative to the first nucleotide of the start codon.

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