

原 著 論 文

Discovery of a *TEKTIN-t/TEKT2* Gene Variant Encoding Sperm Flagellar Protein in Japanese Males

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鞭毛機能に必須な *TEKTIN-t/TEKT2* 遺伝子の日本人男性における  
遺伝子多型の解析

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要 旨

テクチンは、ダイニンとともに精子の鞭毛や繊毛の形成に関与したタンパク質である。テクチンには、ヒトにおいて少なくとも6種類の遺伝子の存在が報告されている。テクチン遺伝子のうち、*Tektin-t/Tekt2*、*Tekt3*、もしくは*Tekt4*が欠失することによって、精子の鞭毛が機能不全を起こし、なかでも*Tektin-t/Tekt2*遺伝子の欠失は、雄性不妊症になることがマウスで示されている。雄性不妊症の原因にテクチン遺伝子の機能不全が関与することが予想される。私たちは、ヒト *TEKTIN-t/TEKT2* 遺伝子の遺伝子多型と男性不妊症との関係について調べるため、282人の原因不明の男性不妊症患者と89人の妊孕性が確認された男性ボランティアの遺伝子の解析を行った。その結果、日本人男性不妊症患者に有意に検出される *TEKTIN-t/TEKT2* 遺伝子の変化は見られなかった。これらの結果は、*TEKTIN-t/TEKT2* は、日本人男性不妊症の原因遺伝子となる割合は非常に低いことを示すとともに、今後の大規模な男性不妊症の原因となる遺伝子の解析に役に立つものと考えられる。

キーワード

不妊症、ゲノム、一塩基多型、精子形成、精巣

Abstract

*TEKTIN* proteins contribute to the formation of cilia and flagella together with dynein. At least six types of *TEKTIN* genes have been reported in humans. The disruption of *Tektin-t/Tekt2*, *Tekt3*, or *Tekt4* in mice causes sperm flagellar dysfunction, and *Tektin-t/Tekt2* null male mice are infertile. To investigate the possible association between variations in *TEKTIN-t/TEKT2* and impaired spermatogenesis in Japanese males, we screened for mutations in *TEKTIN-t/TEKT2* using DNA from 282 sterile male patients and 89 proven-fertile male volunteers. Six polymorphisms were found in the open reading frame of *TEKTIN-t/TEKT2*, but no significant differences in genotype frequency were identified in the infertile subjects ( $P>0.05$ ). We also did not detect a previously reported *TEKTIN-t/TEKT2* gene variant in our subjects. These data may be applied to future large-scale genetic analyses of the association between genetic background and male infertility.

Key words

Infertility, genome, SNPs, spermatogenesis, testis

## 1. Introduction

During spermiogenesis, drastic morphological changes occur that transform spermatids to sperm. The completed sperm cells consist of a nucleus and a flagellum,<sup>1)</sup> but they possess almost no cytoplasm. Previously, we isolated cDNA clones that were specifically expressed in mouse haploid germ cells from a subtracted cDNA library.<sup>2)</sup> Mouse *Tektin-t/Tekt2* was identified as a gene that is specifically expressed in haploid germ cells, and TEKTIN-t/TEKT2 protein is localized in flagella. Tektin A, B, and C proteins were first isolated from sea urchin flagella.<sup>3)</sup> Structural analyses of sea urchin sperm indicate that Tektin proteins play important roles in determining the conformation of flagella by acting as protofilament components.<sup>4)</sup> To understand the physiological role of TEKTIN-t/TEKT2, we studied *Tektin-t/Tekt2* mutant mice generated by Lexicon Pharmaceuticals Inc. (The Woodlands, TX) from ES cells that corresponded to OST12401 (OmniBank sequence tag) and were targeted by gene trapping. *Tektin-t*-deficient mice are viable, and the females are fertile but the males are infertile. Defects in sperm and tracheal cilium motility result from defective dynein function.<sup>5)</sup> Subsequent studies of *TEKTINs* showed that the human genome includes at least six *TEKTIN* genes,<sup>6)</sup> and *Tekt4* and 3 mutant male mice exhibit asthenozoospermia (i.e., impaired sperm motility).<sup>7,8)</sup> Analyses of *Tektin* mutant mice showed that a deficiency in one *Tektin* gene can lead to the production of dysfunctional flagella. Moreover, the dysfunction noted in *Tektin*-disrupted mice has been observed in both the flagella of sperm cells and cilia of other body cells. These results indicate that

the formation of functional flagella on human sperm requires TEKTINs and other proteins. Recently, a heterozygous mutation (A229V) was reported in one of 90 non-syndromic asthenozoospermia patients in Italy.<sup>9)</sup>

To examine whether *TEKTIN-t/TEKT2* is a hereditary cause of male infertility in Japan, the existence of nucleotide polymorphisms in the coding region of *TEKTIN-t/TEKT2* was assessed by the direct sequencing of PCR-amplified DNA from male patients. We did not detect a previously reported genetic mutation, but we identified a novel genetic polymorphism. Based on these results, further investigation using a larger population of infertile cases is warranted.

## 2. Materials and Methods

### 2.1 Participants

Infertile Japanese subjects (N=282) were divided into subgroups according to the degree of defective spermatogenesis: 192 (68%) patients had non-obstructive azoospermia, while 90 (32%) had severe oligospermia ( $<5 \times 10^6$  cells/ml). All of the patients displayed idiopathic infertility based on a cytogenetic analysis, and they possessed no history of prior medical conditions, including but not limited to cryptorchidism, recurrent infections, trauma, orchitis, and varicocele. The control group consisted of fertile males who had fathered children born at a maternity clinic (N=89). 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 carried out with the approval of the institutional review board and independent ethics committee of Osaka University (Osaka, Japan).

## 2.2 Identification of single nucleotide polymorphisms (SNPs) in *TEKTIN-t/TEKT2* by the direct sequencing of PCR-amplified DNA

DNA was extracted from leukocytes of proven-fertile (N=89) and infertile patients (N=282). Genomic DNA was isolated from the blood samples using standard protease treatment and phenol extraction procedures. PCR was carried out using the manufacturer's

recommended reaction buffer (50 $\mu$ l) containing 0.1 $\mu$ g of human genomic DNA; 0.2 $\mu$ M each primer; 2.5 $\mu$ M each of dGTP, dATP, dCTP, and dTTP; and Ex Taq Polymerase (Takara Bio Inc., Otsu, Japan).

The sequences containing the encoded region in exons 1–9 of *TEKT2* were amplified by PCR using specific primers (Table 1, and Figure 1) under the conditions described in

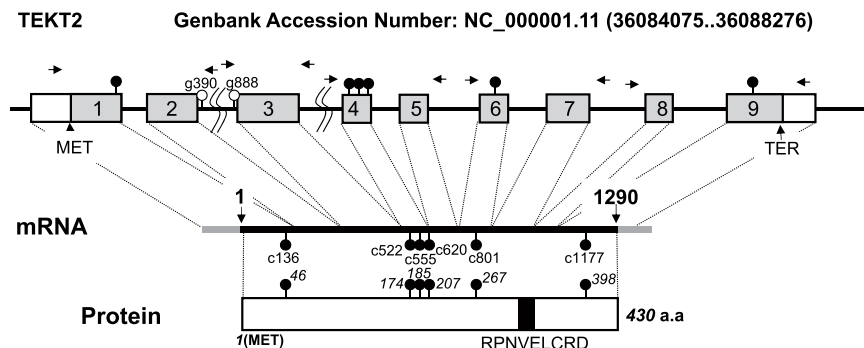


Figure 1. Schematic view of the *TEKTIN-t/TEKT2* gene, mRNA transcript, and encoded protein (430 amino acids). Exons 1–9 are depicted as thick boxes; introns are shown as lines. The numbers in the thick boxes indicate the positions of the exons. Six single nucleotide polymorphisms (SNPs) in the open reading frame are indicated by black circles. The two white circles indicate SNPs in introns. Each SNP is named based on its position relative to the first nucleotide of the start codon. Italicized numbers (lower) indicate the positions of the amino acids in relation to the first methionine (MET). The consensus amino acid sequence of *TEKTIN-t/TEKT2* is indicated by thick boxes.

Table 1 Primers used for SNPs analyses by direct sequencing

Gene	Name of primer	Sequence	
<i>TEKTIN-t/TEKT2</i>	EXON 1, 2	TEK12F TEK12R	AGGAGGTCTCCGAAAGGTCTCC GAAGGAGGGGAGGTTTCGGCAGC
	EXON 3	TEK3F TEK3R	CTCAAGTGTTCCATCCAACCTCGGC TTTCTGTACAGGCACCAGAGGGC
EXON 4, 5	TEK45F TEK45R	TTAGTACAGTGAGGCCTGCCTCTG ACTGGGACCATCCATTAGCTGTGG	
	EXON 6, 7	TEK67F TEK67R	GGCAGCCCTGAGTGTAGACCCTCC CCCTTGCTCCATCAGGAATATGAG
EXON 8, 9	TEK89F TEK89R	ACAGTGTGGCTGACTTGGAACCTCC TTCCATTCAATTTCTCTCCTCCCTC	

Table 2. The sequences containing exons 1–2, 3, 4–5, 6–7, and 8–9 were amplified using the following primer pairs: TEK12F and TEK12R, TEK3F and TEK3R, TEK45F and TEK45R, TEK67F and TEK67R, and TEK89F and TEK89R, respectively.

The amplified fragments were purified using a SUPREC PCR Spin Column (Takara Bio Inc.), and then sequenced using the same PCR primers with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The reaction products were analyzed using an ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems).

### 2.3 Statistical analysis

The  $\chi^2$  test was used to compare the genotype distribution between the infertile subjects and proven-fertile controls. A *P*-value of <0.05 was considered to be statistically significant.

## 3. Results and Discussion

Infertility affects approximately 15% of couples, and in about half of those cases the problem resides with the male. At least 40% of cases of human infertility have no obvious underlying cause.<sup>10</sup> Since haploid germ cell-specific genes do not affect the development of

somatic cells, defects in haploid germ cell-specific genes may be considered a cause of idiopathic male infertility. To date, polymorphisms in haploid germ cell-specific genes that are associated with male infertility have been identified using genomic DNA from Japanese males.<sup>11–23</sup>

In this study, an analysis of genetic variants of *TEKTIN-t/TEKT2*, which is specifically expressed in haploid germ cells, was carried out using DNA from Japanese males because the disruption of *TEKTIN-t/TEKT2* causes impaired sperm flagellar function and male infertility in mice. A base exchange that introduced a four amino acid substitution and two silent mutations was found in the open reading frame in a total of 371 Japanese males (Table 3, and Figure 1). The base exchange (c136c>t) was identified in a homozygous or heterozygous state. Minor homozygous genotypes were not found for the other base exchanges (c522c>t, c555c>t, c620g>a, c801g>t, and c1177a>g). c522 and c555 were silent mutations. Additionally, two SNPs (g390c/t and g888c/t) in an intron were detected during a DNA sequence analysis of the exons. A homozygous SNP (g390c>t) appeared in the intron only in the infertile population. However, no significant differ-

Table 2 Condition of PCR for SNPs analyses

Gene	Primers	Annealing temp. and time	Product size
<i>TEKTIN-T/TEKT2</i>			
EXON 1, 2	TEK12F, TEK12R	63°C, 30 sec	520 bp
EXON 3	TEK3F, TEK3R	68°C, 45 sec	428 bp
EXON 4, 5	TEK45F, TEK45R	63°C, 30 sec	444 bp
EXON 6, 7	TEK67F, TEK67R	63°C, 30 sec	498 bp
EXON 8, 9	TEK89F, TEK89R	62°C, 30 sec	572 bp

Denaturing temperature for all the reactions was 98°C for 10 sec.

Cycle number and extension time all the reactions were 35 cycles and 72°C, 30 sec.

ences in the genotype frequencies of those SNPs were observed to be specific to the infertile subjects ( $P > 0.05$ ). All of the SNPs were found in the dbSNP of the National Center for Biotechnology Information (NCBI, Bethesda, MD). Although many SNPs in the *TEKT2* genomic sequence were registered in the dbSNP, we did not find them except for eight SNPs in Japanese males. These results may be due to ethnic differences between the samples included in our study and those in the database.

Researchers in Italy identified a heterozygous mutation (A229V) in 90 non-syndromic asthenozoospermia patients.<sup>9)</sup> In humans and mice, at least six *tektin* genes have been reported, and mice carrying a mutation in *Tektin-t/Tekt2*, *Tekt3*, or *Tekt4* were found to have dysfunctional flagella on their sperm.

TEKTINs and other protein complexes might affect the conformation of flagella in collaboration with other proteins<sup>24)</sup>; a dominant heterozygous mutation in *Tektin-t/Tekt2* could cause sperm flagellar dysfunction by disrupting that collaboration. The mutation A229V was not found in our analysis. The patients included in our analysis had azoospermia or severe oligospermia, but none of them had asthenozoospermia. This could explain any differences in the results between the Italian study<sup>9)</sup> and ours.

Even if a dominant-negative mutation exists, haploid germ cell-specific genes may be inherited through females. However, such a mutation in *Tektin-t/Tekt2* was not found. Impaired flagellar function may stem from the inheritance of a gene substitution that affects tracheal cilium motility in males and

**Table 3** Prevalence of single nucleotide polymorphisms in *TEKTIN-t/TEKT2* in infertile or proven fertile populations

	Position		Genotype	Number (%) of SNP		Reference (NCBI dbSNP rs#)
	Nucleotide	Amino acid		Infertile	Proven fertile	
TEKTIN-t/ TEKT2	c136	R	c/c	197 (69.9)	56 (62.9)	rs12043423
		R/C	c/t	76 (26.9)	31 (34.8)	
		C	t/t	9 (3.2)	2 (2.2)	
g390			c/c	257 (91.1)	84 (94.4)	rs117718807
			c/t	24 (8.5)	5 (5.6)	
			t/t	1 (0.4)	0 (0)	
g888			c/c	272 (96.5)	87 (97.8)	rs3767702
			c/t	10 (3.5)	2 (2.2)	
			t/t	0 (0)	0 (0)	
c522	174	S	c/c	281 (99.6)	89 (100)	rs142894717
			c/t	1 (0.4)	0 (0)	
			t/t	0 (0)	0 (0)	
c555	185	I	c/c	282 (100)	88 (98.9)	rs200428414
			c/t	0 (0)	1 (1.1)	
			t/t	0 (0)	0 (0)	
c620	207	R	g/g	277 (98.2)	87 (97.8)	rs116893490
		R/H	g/a	5 (1.8)	2 (2.2)	
		H	a/a	0 (0)	0 (0)	
c801	267	K	g/g	278 (98.6)	88 (98.9)	rs142743253
		K/N	g/t	4 (1.4)	1 (1.1)	
		N	t/t	0 (0)	0 (0)	
c1177	398	T	a/a	278 (98.6)	87 (97.8)	rs200994339
		T/A	a/g	4 (1.4)	2 (2.2)	
		A	g/g	0 (0)	0 (0)	
Total				282	89	

The translation start site was + 1 on *TEKTIN-t/TEKT2* gene and cDNA.

females rather than a defect in the sperm-specific *Scot-t* and *PGAM4* genes<sup>11, 21)</sup> because *Tektin-t* null mice exhibit defects in both sperm and tracheal cilium motility.

DNA from 282 infertile male patients and 89 male volunteers proven to be fertile was screened for mutations in *TEKTIN-t/TEKT2*. Six polymorphisms were found in the open reading frame of *TEKTIN-t/TEKT2*. No significant differences in genotype frequency were identified in the infertile subjects ( $P > 0.05$ ). This analysis will contribute greatly to future large-scale studies of the genetic background of infertility in Japanese males.

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