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原著論文

Genetic Variation in the Testis-Specific *GSG3/CAPZA3* Gene Encoding for the Actin Regulatory Protein in Infertile Males

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アクチン調節蛋白質をコードするヒト *Gsg3/CAPZA3* 遺伝子の遺伝子多型

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要 旨

アクチンキャッピング蛋白質 GSG3/CAPZA3 は、受精可能な精子の形態形成に重要な役割を担っている。*GSG3/CAPZA3* 遺伝子は、イントロンレス遺伝子として哺乳類に保存され、マウスにおいてミッセンス変異が精子の減少と形態異常を誘導し、雄性不妊症の原因となることが知られている。日本人において男性不妊症と *GSG3/CAPZA3* 遺伝子多型の関係を調べるため、261人の男性不妊症患者と139人の妊孕性が確認された男性ボランティアの遺伝子多型を調べた。その結果、妊孕性が確認された男性ボランティアの一人にアミノ酸置換を伴う一塩基多型がヘテロ接合型として検出された。このことから、*GSG3/CAPZA3* 遺伝子には、他の精子細胞特異的イントロンレス遺伝子に比べて遺伝子多型が極めてわずかしか存在しないことが示唆された。

キーワード

精子、男性不妊症、ゲノム、SNPs、精子形成、精巣

Abstract

The actin capping protein GSG3/CAPZA3 plays a physiologically important role in maintaining sperm architecture for fertilization. The *GSG3/CAPZA3* gene is conserved in mammals and lacks introns. A missense mutation in the *CAPZA3* gene in mice causes male infertility by reducing the concentration and changing the shape of sperm. To investigate possible associations between *GSG3/CAPZA3* gene variations and impaired spermatogenesis in Japanese males, we screened for mutations in *GSG3/CAPZA3* using DNA from 261 sterile male patients and 139 male volunteers who were fertile. A single nucleotide polymorphism (SNP) was found in one sample in the heterozygous state in the fertile male volunteers. The results indicate that compared with other germ-cell-specific intronless genes the change was restricted in GSG3/CAPZA3 protein.

Key words

fertilization, genome, male infertility, SNPs, sperm, spermatogenesis

Introduction

Germ cell-specific gene 3 (*Gsg3*) was cloned in a germ cell-specific subtracted library that was prepared by subtracting W/W^v mutant testes mRNAs from adult testes cDNAs.[1] Computer-assisted analyses of the mouse GSG3 protein indicated that it belongs to a family of actin capping proteins, which play a role in actin fiber network regulation. Genomic analyses have revealed that mouse *GSG3* is an intron-less gene located on chromosome 6 that is conserved in mammals.[2,3] The putative transcriptional promoter region of *GSG3* contains cyclic AMP-response element motifs.[2,4] In rats, testis-specific actin capping protein (TSACP) is expressed post-meiotically in round spermatids, and its localization coincides with the position of the developing acrosome.[5] The bovine capping protein (CPα3) and two other actin-regulatory proteins exhibit dynamic distribution that is altered in both the head and tail of sperm during epididymal maturation and the acrosomal reaction.[6] In humans, the capping protein GSG3/CAPZA3 is mainly localized in the neck region of ejaculated sperm, with moderate and faint signals in the tail and post-acrosomal region, respectively.[4] In mice, the GSG3/CAPZA protein is specifically expressed in testes, and its distribution changes from the flagellum to the head during epididymal maturation.[7] Moreover, the actin polymerization inhibitor latrunculin A was unable to alter CAPZA3 movement in sperm.[8] Recently, males homozygous for the repro32 ENU-induced mutation produced by the Reproductive Genomics program at The Jackson Laboratory were shown to be infertile.[9] The mutant mice have low epididymal sperm concentrations and produce sperm with abnormally shaped heads and poor motility. These results suggest that GSG3/CAPZA3 has an important role in reorganization of the actin cytoskeleton during shaping of the acrosome and capacitation of sperm.

To examine whether *GSG3/CAPZA3* is the hereditary cause of male infertility, nucleotide polymorphisms in the *GSG3/CAPZA3* coding region were assessed by direct sequencing of polymerase chain reaction (PCR)-amplified DNA from male patients.

2. Materials and Methods

2.1. *Participants*

Japanese subjects with nonobstructive infertility ($n=261$) were divided into subgroups according to the degree of defective spermatogenesis: 180 patients (69%) had nonobstructive azoospermia, whereas 81 patients (31%) had severe ologospermia ($<5\times10^6$ cells/mL). All patients displayed idiopathic infertility and had no history of prior medical conditions, including, but not limited to, cryptorchidism, recurrent infections, trauma, orchitis, or varicocele. All subjects were diagnosed with primary idiopathic infertility based on cytogenetic analyses. The control group consisted of fertile males who had fathered children born at a maternity clinic ($n=139$).

All donors were informed of the purpose of the study and gave permission for their blood to be used for genomic DNA analyses. This study was conducted with approval from the institutional review board and an independent ethics committee at Osaka University.

2.2. *Identification of SNPs in GSG3/CAPZA3 by direct sequencing of PCR-Amplified DNA*

DNA samples from fertile (n=139) and infertile patients (n=261) were extracted from blood leukocytes. Genomic DNA was isolated from the blood samples using standard protease treatment and phenol extraction procedures.

The PCR primers hCAPA (5′-CAGGA GGCTCAGACCTTGCCAGAC-3′) and hCA PB (5′-GCTAAGTGAGAGACATATCTCTAC -3′) were designed to amplify *GSG3/CAPZA3*.[4] PCR reactions were performed in the manufacturer's recommended reaction buffer (50 μl) containing 0.1 μg of human genomic DNA, 0.2 μM of each primer, 2.5 μM each of dGTP, dATP, dCTP, and dTTP, and LA Taq polymerase (Takara, Shiga, Japan). Cycling conditions included 96℃ for 3 min, followed by 35 cycles of denaturation at 96℃ for 45 s, annealing at 65℃ for 45 s, and extension at 72℃ for 90 s.[4]

PCR-amplified fragments were purified using AMPure® (Agencourt Bioscience Corporation, Beverly, MA, USA) and sequenced using the same PCR primers and the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The reaction products were purified using BigDye® XTerminator and analyzed using an ABI–PRISM 3730xl Genetic Analyzer (Applied Biosystems). Subjects with sequence ambiguities were excluded. Screening of variations in the sequences was performed using SeqScape® software (Applied Biosystems).

2.3. *Statistical Analyses*

X^2-tests were used to compare the genotype distribution between infertile subjects and fertile controls. P-values <0.05 were considered statistically significant.

3. Results and Discussion

We cloned and characterized germ cell-specific genes from mice using germ cell-specific antibodies and cDNA subtraction methods.[10] Because nearly all mouse male germ cell-specific genes have human orthologs, we isolated and characterized the human genes using mouse cDNA probes with a human testis cDNA library and computer analyses. In Japan, as in many European countries, more than 10% of married couples are affected by infertility.[11] To understand infertility, gene mutations in human male infertility patients have been discovered. We assessed the prevalence of SNPs in germ cell-specific genes by direct sequencing of PCR-amplified DNA from male patients who were undergoing fertility evaluations.[12] From these results, we found that some SNPs are clearly related to male infertility.[13–22] Additionally, many SNPs that are not related to male infertility exist in germ cell-specific genes.

In this study, we analyzed nucleotide polymorphisms in *GSG3/CAPZA3*. One primer set was used to amplify a DNA fragment of the *GSG3/CAPZA3* transcriptional region on chromosome 12p12.3. One SNP (c532G>A) introduced an amino acid substitution (V178I) and was found to be heterozygous in the control group (Figures 1 and 2). The minor homozygous genotype (A/A) was not found in 400 Japanese males (Table 1). The presence of SNPs did not result in significant differences between the fertility and infertility groups.

The GSG3/CAPZA3 protein has a role in

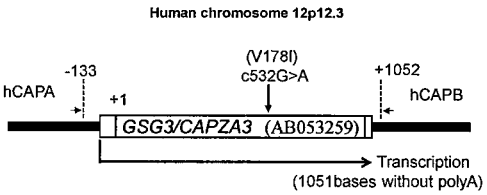

Figure 1. Schematic presentation of the *GSG3/CAPZA3* gene.

The *GSG3/CAPZA* intron-less gene is localized at 12p12.3. The nucleic acid sequence position relative to the first nucleotide of the start codon (upper). Horizontal arrows show the primers used for PCR and sequencing. The gray box indicates the open reading frame. The vertical arrow points to the position of the single nucleotide polymorphism. The DNA sequence between nucleic acid position −118 and 1,037 was analyzed.

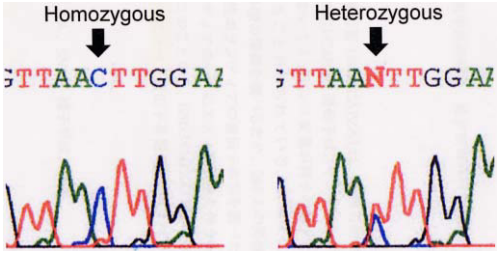

Figure 2. Detection of the c532G/A SNP in *GSG3/CAPZA3*.

Complementary strands of amplified DNA fragments were sequenced using the hCAPB primer. The homozygous C/C (left) and heterozygous C/T (right) signals were distinctly identified upon screening.

spermiogenesis and sperm capacitation. The *GSG3/CAPZA3* gene is a testis-specific retrotransposed gene that lacks introns (Figure 1). GSG3/CAPZA3 functions in various mammals. Homozygous deletion of repro32 ENU-induced mutations in *GSG3/CAPZA3* resulted in infertility due to sperm defects but was not embryonic lethal. Previously, we analyzed SNPs in the germ cell-specific genes *SCOT-t*[14] and *PGAM4*,[21] which play important roles in the energy metabolism of sperm.[21, 23] *SCOT-t* and *PGAM4* are also retrotransposed, intron-less genes. They also included SNPs associated with male infertility and SNPs not associated with infertility. Moreover, these SNPs also introduced amino acid substitutions. Some SNPs were also found in the germ cell-specific, intronless *HANP1*[16] and *CETN1* genes.[22] HANP1 is a haploid germ cell-specific histone-like protein, while the CETN1 protein is centrin-specifically expressed in spermatids. SCOT-t (520 amino acids), HANP1 (255 amino acids), PGAM4 (254 amino acids) and CETN1 (172 amino acids) include three, five, two and four SNP-introduced amino acid substitutions, respectively. We observed a total of 78 amino acid substitutions per 200 persons, 398 amino acid substitutions per 530 persons, 42 amino acid substitutions per 630 persons and 119 amino acid substitutions per 378 persons in SCOT-t, HANP1, PGAM4 and CETN1, respectively. *PGAM4* existed under unique conditions because *PGAM4* was haploidy localized on the X chromosome in one male.

Table 1. Single ncleotide polymorphism in *GSG3/CAPZA3* in infertile or rocen fertile male

	Position		Genotype	Number of SNP	
	Nucleotide	Amino Acid		Infertile	Proven fertile
c.532G/A	532	178	G/G	261	138
			G/A	0	1
Total				261	139

On the other hand, GSG3/CAPZA3 (299 amino acids) included one SNP-introduced amino acid substitution among 400 persons. *GSG3/CAPZA3* is a genetically stable gene compared with *SCOT-t, HANP1,* and *CETN1.*

Although the reason for the significant differences ($P < 0.05$) in the number of SNPs in *GSG3/CAPZA3* is unknown at present, it may depend on the chromosome in which the *GSG3/CAPZA3* gene exists. The conformational domain that induces delicate and active changes may not exist in the GSG3/CAPZA3 protein, and amino acid changes in the protein may be restricted. Our current results show that the retrotransposed *GSG3/CAPZA3* gene was genetically more stable than other retrotransposed germ-cell-specific genes.

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