

Comparative analysis of Y chromosomal microdeletions in Korean infertile men of 47,XXY and 46,XY karyotypes

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In the azoospermic patients, there are many of undiagnosed factors related to genetic bases. Among them, Klinefelter's syndrome (47,XXY; KS) and Y-chromosomal microdeletion with normal karyotype (46,XY; YMNK) are the most frequent causes of male infertility. This research focused on the comparative analysis of YMNK (n = 66) and KS (n = 30) patients suffered from male infertility in Korean population. We used the polymerase chain reaction (PCR) approach including 19 pairs of sequence-tagged site (STS) primers for detecting the Y-chromosomal microdeletion on AZFa, b, c regions, indicating that Y chromosomal microdeletions were almost evenly occurred in AZF all regions in Korean population. Comparative analysis indicated that 34.9% YMNK and 73.4% KS patients harbored the microdeleted Y-chromosome. It seems to be high instability of Y-chromosome in KS patients than that of YMNK infertility patients. Taken together, genome instability containing microdeletion could bring male infertility with the disturbance of normal spermatogenesis.

Key words – Klinefelter's syndrome, Y-chromosomal microdeletion, male infertility, AZF

Introduction

In the modern society, tendency of late marriage, environmental problem, malnutrition, endocrinological disorders, and genetic factors caused the high level of infertility rate [14]. Among them, genetic factors such as microdeletion and chromosomal abnormalities have been estimated to account for at least 30% of male infertility [3]. Klinefelter's syndrome is the most frequent genetic cause of infertile men with chromosomal abnormality. This syndrome had been the first reported by Klinefelter and co-workers with the symptom of gynecomastia, aspermatogenesis without Leydigism, and higher concentration of follicle-stimulating hormone (FSH) [19]. Their prevalence was reported by up to 3.1% in the infertile male population [28,33]. Most of Klinefelter's syndrome patients have a nonmosaic 47,XXY karyotype caused by paternal and maternal non-disjunction at different meiosis stages [24]. Normal spermatogenesis was observed very rarely in Klinefelter's syndrome patients, that they were usually

considered sterile. However, the aid of advanced assisted reproductive techniques (ART), such as intracytoplasmic sperm injection (ICSI), microepididymal sperm aspiration (MESA), and testicular sperm extraction (TESE), enabled the nonmosaic Klinefelter's syndrome patients could be a father [9]. Most infant born by ART with sperm from Klinefelter's syndrome men has a normal 46,XY karyotype. Nevertheless, we could not fully understand the fundamental genetic causes of that syndrome related to male infertility [12,15,25].

The Yq11 region defined as "azoospermia factor (AZF) region" was focused on their importance to complete the normal spermatogenesis by the investigation of microscopically detectable deletions in infertile men [35]. This region had been further divided into three non-overlapping regions of AZFa, AZFb and AZFc [12,37]. Among these regions, the AZFc region was noticed by the frequent microdeletions, comprising about 80% of all detected microdeletions [22,38]. Recently, various genes which were essential for spermatogenesis on AZF regions were identified [4,5,10,23,27].

Although, the exact relationship of Y-chromosomal microdeletion and spermatogenesis was poorly understood, detecting method of microdeletion with STS primer was

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used widely in the various clinical center for male infertility patients. As the elucidation of relationship between etiology and prognosis is very important for the treating of male infertility patients, more screening with different population and heterogeneous group of patients will be needed. Moreover, some cases of ART treatment reported that infertile men assisted by ART technique could be a father, and their sons also were identified by the possession of Y-chromosomal microdeletion. That means their sons also were exposed by the risk of male infertility [6,16,21]. For the screening of Y-chromosomal microdeletion, various STS primers were used in heterogenous group of infertility patients. Their frequency of microdeletion was varied from 1.0% to 55.5% according to different population and symptoms [1,8,17,18,31,32,34,36].

In this investigation, we examined the Y-chromosomal microdeletion with the 19 STS markers in 66 infertile men with normal karyotype and 30 infertile men diagnosed by Klinefelter's syndrome in Korean population. The comparative study of microdeletion analysis between 46,XY and 46,XXY infertile men could give good insights to infertility research and clinical treatment.

Materials and Methods

Patients

We analyzed 96 male infertility patients who diagnosed as male infertility in Pusan National University Hospital. Among them, 66 men had a normal Karyotype (46,XY) and 30 men diagnosed by Klinefelter's syndrome had abnormal Karyotype (46,XXY).

DNA extraction and STS markers for microdeletion analysis

Blood samples from the patients were collected and stored in EDTA vacuum tubes (Greiner) and genomic DNA was isolated from peripheral leucocytes by a standard protocol of AccuPrep Genomic DNA Extraction kit (Bioneer). Totally, 19 STS markers (sY14, sY84, sY87, sY90, sY117, sY127, sY132, sY134, sY136, sY142, sY153, sY152, sY220, sY155, sY149, sY254, sY157, sY255, sY283) were used for detecting the Y-chromosomal microdeletion (Table 1) [1,13,29].

PCR amplification for microdeletion analysis

For the microdeletion analysis, Polymerase Chain Reaction

Table 1. STS primers used in this study

Primers	Sequence (5'-3')	Size (bp)
sY14	S-GAATATTCCCCTCTCCGGA AS-GCTGGTGCTCCATTCTTGAG	500
sY84	S-AGAAGGGTCTGAAAGCAGGT AS-GCCTACTACCTGGAGGCTTC	326
sY87	S-TCTGTTGCTTGAAGAGGG AS-ACTGCAGGAAGAATCAGCTG	252
sY90	S-CAGTGCCCCATAACACTTC AS-ATGGTAATACAGCAGCTCCG	176
sY117	S-GTTGGTTCCATGCTCCATAC AS-CAGGGAGAGACCTTTTACC	262
sY127	S-GGCTCACAAACGAAAAGAAA AS-CTGCAGGCAGTAATAAGGGA	274
sY132	S-GAGAGTCATAATGCCGACGT AS-TGGTCTCAGGAAGTTTTGC	159
sY134	S-GTCTGCCTCACCATAAACG AS-ACCACTGCCAAAACCTTTCAA	301
sY136	S-CACATGAAGCACTGGAACCTG AS-GTTGTCTGGAAATCCCTGTG	235
sY142	S-AGCTTCTATTGAGGGCTTC AS-CTCTCTGCAATCCCTGACAT	196
sY149	S-TGTACACTGCCCTAATCCT AS-TGGTCATGACAAAAGACGAA	132
sY152	S-AAGACAGTCTGCCATGTTTCA AS-ACAGGAGGGTACTTAGCAGT	125
sY153	S-GCATCCTCATTTTATGTCCA AS-CAACCCAAAAGCACTGAGTA	139
sY155	S-ATTTTGCCTTGCATTGCTAG AS-TTTTTAAGCCTGTGACCTGG	349
sY157	S-CTTAGGAAAAGTGAAGCCG AS-CCTGCTGTGAGCAAGATACA	285
sY220	S-ATGGGTGAGAAGCCTGATTGT AS-TGGGAAAAGCCTGAACTGCC	109
sY254	S-GGGTGTACCAGAAGGCAAA AS-GAACCGTATCTACCAAAGCAGC	350
sY255	S-GTTACAGGATTCGGCGTGAT AS-CTCGTCATGTGCAGCCAC	126
sY283	S-GAGTGATACACTCGGACTTGTGTA AS-GTTATTTGAAAAGTACACGGG	500

(PCR) was performed using TaKaRa Ex Taq polymerase (Takara). The PCR condition was performed as follows. After the initial denaturation step at 94°C for 3 min, DNA was amplified for 30 cycles at 94°C for 1 min, annealing temperature for 1 min and 72°C for 1.5 min. The PCR machine is MJ-100 of MJ research. Annealing temperature was different by various STS primers. All DNA samples were tested by the STS marker of sY14 for detection of sex determining region Y (SRY) gene.

Results

In order to evaluate the deletion frequency of Korean infertile men on Y chromosome, PCR analysis was performed using 19 STS marker sets. Totally, 96 men suffering from male infertility were examined for deletion screening. All tested patients were divided into two groups. The First group had Y-chromosomal microdeletion with normal kar-

ypotype (46,XY; YMNK) and the second group had Klinefelter's syndrome (47,XXY; KS). Among the second group, two patients (no. 5, 8) were diagnosed as mosaic Klinefelter's syndrome and others were nonmosaic Klinefelter's syndrome.

In the first group, 23 patients appeared to have at least one microdeleted region on their Y chromosome (Fig. 1).

The result indicated that 34.9% of tested Korean infertile men, possessed normal karyotype (46,XY), were screened to have microdeletion in their Y chromosome. Patients who had a deleted Y chromosome in AZFa region were 8 men, 5 men were in AZFb and 7 men were in AZFc region, indicating that 12.1 % of patients were deleted in AZFa, 7.5% in AZFb, 10.6% in AZFc region. Among the

No	AZFa			AZFb							AZFc									
	SRY	sY14	sY84	sY87	sY90	sY117	sY127	sY132	sY134	sY136	sY142	sY153	sY152	sY220	sY155	sY149	sY254	sY157	sY255	sY283
1*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
36	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
37*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
38	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
41*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
43*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
46*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
47*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
48*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
49	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
51	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
52	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
53	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
54	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
55*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
56*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
58*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
59*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
60	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
61	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
62*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
63*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
64	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
65	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
66*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Fig. 1. Schematic depiction of the Y chromosomal microdeletion in 66 infertile men (46, XY). "+" and "-" indicated that the presence and absence of STS markers, respectively. "*" indicated that the men who had no deletion.

first group, 4 men (no. 36, 51, 60, 64) showed deletion event more than one locus. Surprisingly, 2 men (no. 51, 60) possessed totally deleted AZFc region on their Y chromosome. As a matter of course, analysis of sY14 marker for SRY gene showed positive signals in their patients.

The second group was composed of Klinefelter's syndrome patients diagnosed by male infertility. Total 30 men were tested for Y chromosomal microdeletion analyses. All tested genomic DNA samples were amplified with sY14 marker for SRY gene as positive control. Only 8 men showed no deletion of Y chromosome, and 21 men showed microdeleted Y chromosome, indicating that more than 73% of tested Klinefelter's syndrome patients showed Y chromosomal abnormality (Fig. 2). AZFa region deletion was shown in 10 patients (33.3%), AZFb region deletion were in 17 patients (56.6%), AZFc region deletion were in 12 patients (40.0%). In the second group, 14 men (no. 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22) were observed to contain various deletion loci in their Y chromosome.

Discussion

The human Y chromosome has been known to be a very unique and important chromosome to complete the sper-

matogenesis after the first report of infertile men who had a long microdeletion in their Y chromosome [35]. Many research papers are reported for the importance of Y chromosomal microdeletion and many clinical centers used microdeletion screening methods for infertility patients. But, we could not explain the exact impact of Y-chromosomal microdeletion in male infertility patients. Only their correlation was recognized by major of scientists who researched the andrology [20]. In the last decade, various groups of population (Chinese, Japanese, Irish, Turkish, Slovenian, Finnish, New Zealand, Swedish, Croatia, India infertile men) were tested for microdeletion analyses on their Y chromosome [1,2,7,3,18,26,27,29,30,32]. To know the difference of their characteristics between the populations is important for the adequate infertility treatments of their own population. We examined the Korean population (no=96) to investigate the Y-chromosomal microdeletion rate. We conducted the comparative analyses with same STS marker for the evaluation of the difference between 46,XY and 46,XXY (Klinefelter's syndrome) infertility patients with same STS marker sets. Korean population analyses showed consistent microdeletion rate (34.9%) with those of previous analyses of other population (1% to 55.5%). However, the distribution of microdeletion on the

No	SRY	AZFa			AZFb						AZFc								
	sY14	sY84	sY87	sY90	sY117	sY127	sY132	sY134	sY136	sY142	sY153	sY152	sY220	sY155	sY149	sY254	sY157	sY255	sY283
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Fig. 2. Schematic depiction of the Y chromosomal microdeletion in 30 infertile men (Klinefelter's syndrome, 46, XXY). "+" and "-" indicated that the presence and absence of STS markers, respectively. "*" indicated that the men who had no deletion.

Y chromosome was different from previous studies. Usually the results of previous studies indicated that AZFc region was most commonly deleted compared to other regions [14,27,32]. However, in case of Korean population, AZFa, b regions also seems to be important for spermatogenesis.

In the case of Klinefelter's syndrome patients, very surprising results were found. More than 73% patients showed microdeletion in their Y chromosome. What make their Y chromosomal microdeletions often? At the first report of Klinefelter's syndrome, many research papers reported the detailed symptoms of that syndrome, but nobody could explain the original causes of the Klinefelter's syndrome. Although, many symptoms of Klinefelter's syndrome could be improved by the early awareness and treatments such as testosterone replacement [39], there was no positive improvement on their fertility. From our analyses of Y-chromosomal microdeletion, we hypothesized that Klinefelter's syndrome might be derived by the genomic instability such as microdeletion or chromosomal rearrangement. Various clinic centers developed the ART methods for Klinefelter's syndrome patients, and many cases have reported that most babies born by the ART methods with sperm from Klinefelter's syndrome patients have a normal karyotype. Nevertheless, such kind of ART methods must be performed very carefully [12,15,25]. Because their babies' genome also could be microdeleted by the genome instability. Although our data sets were not enough to reflect the whole population of Klinefelter's syndrome patients, the results of no deleted Y chromosomal proportion (26.6%) symbolized genomic features of Y chromosome in 46,XXY population.

Obviously, genome instability of Y chromosome could be different from the normal 46,XY infertile men and Klinefelter's syndrome patients. Many other comparative data related to Klinefelter's syndrome and normal 46,XY infertile men are needed to reveal the mystery of male infertility in further studies. Taken together, our results indicated that Y chromosomal microdeletions were almost evenly occurred in AZF a, b, c regions in Korean population. Comparative analysis between YMNK and KS patients indicated that high frequent microdeletions on Y chromosome are occurred in KS patients.

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초록 : 47,XXY와 46,XY 핵형을 가진 한국인 불임남성의 Y 염색체의 미세결실에 대한 비교 분석

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무정자증 환자의 경우, 정확히 알려지지 않은 유전적인 요인들이 남성불임과 연관되어 있다. 그들 중 클라인펠트 증후군(KS)과 정상 핵형의 남성에게서 발견되는 Y염색체상의 미세결실 증상(YMNK)은 남성 불임의 가장 빈번한 원인이라고 할 수 있다. 본 연구는 한국인 집단에서 남성불임으로 고통 받고 있는 YMNK (66 개체)와 KS (30 개체) 환자들을 비교 분석 하였다. Y염색체 상의 AZFa,b,c 영역의 미세결실을 분석하기 위해 19개의 STS 프라이머를 이용해 PCR분석을 하였다. 실험 결과 YMNK의 34.9%와 KS의 73.4%가 미세결실을 포함하고 있었다. 이 점으로 미루어보아 YMNK환자보다 KS환자의 경우가 Y염색체의 불안정성이 더욱 높은 것으로 사료된다. 결론적으로 미세결실을 포함하는 제놈의 불안정성은 정상적인 정자형성 과정을 방해하여 남성불임을 초래할 수 있을 것이다.