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Spermatogenic Dysfunction in Azoospermic Japanese Men Caused by Y Chromosome Microdeletions

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SUMMARY

Genetic factors are responsible for approximately 15% of male infertility. The azoospermia factors AZFa, AZFb (P5/proximal P1), and AZFc (b2/b4) present on Yq are most important for spermatogenesis. Here, we evaluated frequencies of microdeletions of AZFa, AZFb, AZFc in azoospermia due to spermatogenic dysfunction in the Japanese population. The overall prevalence of Y chromosome microdeletions in infertile men was 8.1% (79/980). The prevalence of Y chromosome microdeletions in AZFa, AZFb, AZFabc, AZFbc (P5/distal P1 or P4/distal P1) and AZFc was 0.1% , 0.8% , 0.7% , 2.0% and 4.4% , respectively. Microdissection for testicular sperm extraction failed in all patients with microdeletion in AZFa, AZFb, AZFabc, and AZFbc, although sperm could be retrieved in 28/43 patients with AZFc deletions (62.2%). The presence of an AZFc deletion was associated with significantly better sperm retrieval than the 33.0% retrieval rate in idiopathic non-deleted azoospermic men undergoing microdissection for testicular sperm extraction at our institution during the study period. We conclude that Y chromosome microdeletion testing is essential for genetic and preoperative counseling in these patients.

Key Words : Male infertility, Azoospermia, Testicular sperm extraction, Y chromosome, Y microdeletions

INTRODUCTION

Infertility is a major reproductive health problem in Japan relating to the trend towards later marriage. Worldwide reports suggest that infertility affects 15% -20% of couples of reproductive age. Nearly 50% of these cases are attributable to the male partner[1]. The origin of male-factor infertility remains largely unexplained. Genetic factors cause approximately 10-15% of the male infertility[2]. Male fertility depends on successful spermatogenesis, in which a number of genes participates. Thus, it is evident that deletions or mutations in genes controlling spermatogenesis would result in infertility[3].

Cytogenetic studies in infertile men have revealed a gene that controls spermatogenesis ; this azoospermia factor (AZF) is located on the long arm of the Y chromosome (Yq11)[4]. Three spermatogenic loci in Yq11 have been classified into three regions, AZFa,

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AZFb and AZFc[5]. Each AZF region contains several genes that play a role in different stages of spermatogenesis. It is likely that further analysis of these individual genes in infertile males will result in more precise genotype-phenotype correlations. Interstitial or terminal deletions of all AZFa are rare and usually produce the severe phenotype of Sertoli-cell-only (SCO) syndrome[6]. Interstitial or terminal deletions of AZFb (P5/proximal P1) and AZFbc (P5/distal P1 or P4/distal P1) are uncommon and usually result in severe azoospermia[7]. Interstitial or terminal deletions of AZFc (b2/b4) only are relatively common and result in a variable infertility phenotype, ranging from azoospermia and SCO to severe or mild oligozoospermia[8]. The frequency of Y chromosome microdeletions in males with azoospermia or severe oligozoospermia is about 5% -15%[6].

In the present study we report our experience in the diagnosis and surgical management of azoospermia due to spermatogenic dysfunction in patients with Y chromosome microdeletions. Our series of microdissection-testicular sperm extractions (micro-TESE) in men with Y chromosome microdeletions is the largest in Japan.

MATERIALS and METHODS

Criteria for including patients

For the present study, we recruited 980 men with azoospermia due to spermatogenic dysfunction (ASD), aged 20-51 years. These subjects were ASD patients visiting the Dokkyo Medical University Koshigaya Hospital and affiliated institutions. All had primary infertility for more than a year. After a 2-7 day period of sexual abstinence, a semen sample was obtained from each patient. Complete semen analyses were repeated at least twice before a diagnosis of azoospermia was made. To determine the presence of sperm, the ejaculate was centrifuged and thoroughly examined visually.

Informed consent forms were obtained from each infertile man before inclusion in the study. The Institutional Review Board of the Dokkyo Medical University Koshigaya Hospital approved this study.

Lymphocyte chromosome spreads were prepared using routine methods and karyotypes were analyzed by G-banding. For each individual, a minimum of 30 metaphase spreads was analyzed. Patients with microdeletions associated with abnormal karyotypes (e.g. 46,XX male, 47,XXY and balanced rearrangements such as reciprocal translocations or inversions), and/or obstructive azoospermia were excluded from the present study.

PCR for sequence-tagged sites

Y chromosome microdeletion screening was performed by multiplex polymerase chain reaction (PCR) of DNA extracted from peripheral blood leukocytes. For each patient, genomic DNA was extracted from peripheral blood by the QIAamp DNA mini extraction kit (Qiagen, Venlo, The Netherlands). The Y Chromosome Deletion Detection System, Version 2.0 (Promega, Madison, WI) was used for to identify Y chromosome deletions. Nineteen sequence-tagged sites (STS) within the AZF region of Yq11 (SY81, SY86, SY84, SY182, SY121, SYPR3, SY124, SY127, SY128, SY130, SY134, SY145, SY152, SY242, SY208, SY254, SY255 and SY157) and the sex-determining region of the Y (SRY) gene, sY14, were targeted for PCR amplification. DNA from a fertile man served as a positive control and DNA from women was used as the negative control. To confirm multiplex PCR results that indicated a Y chromosome microdeletion, single primer PCR analyses were performed in duplicate for all deleted STSs and two flanking STSs. When Y chromosome microdeletion was found, patients were screened twice with multiplex PCR using a different blood sample. Given the lack of convincing data regarding the clinical relevance of partial deletions of the AZFc region, patients with such partial AZFc deletions were considered non-deleted in this analysis.

Micro-TESE

A single surgeon performed micro-TESE for all enrolled ASD patients. The outcomes in patients with Y chromosome microdeletions were compared with outcomes in patients without Y chromosome microdeletions. The significance of differences between sperm retrieval rates by micro-TESE in patients with or without Y chromosome microdeletions was estimated using Fisher's exact test.

Table 1 Prevalence of different Y microdeletions in patients with azoospermia due to dysfunctional spermatogenesis

	Number of patients	%
Total screened	980	
AZFa	1	0.1
AZFb	8	0.8
AZFa＋b＋c	7	0.7
AZFb＋c	20	2.0
AZFc	43	4.4
Any Y microdeletion	79	8.1

Table 2 Outcomes of micro-TESE in azoospermic men stratified by Y microdeletion status.

Etiology of azoospermia	Sperm retrieved	Sperm not retrieved	Sperm retrieval rate (%)	Pathology
AZFa	0	1	0	SCO：1
AZFb	0	8	0	MA：3；SCO：5
AZFa＋b＋c	0	7	0	SCO：7
AZFb＋c	0	20	0	SCO：20
AZFc	28	15	62.2*	MA：5；SCO：10**
Nondeletion	297	604	33.0*	

Note：TESE, testicular sperm extraction；AZF, azoospermic factor；MA, maturation arrest；SCO, Sertoli cell-only syndrome

* Sperm retrieval rate in men with AZFc deletions was significantly higher than in non-deleted ADS patients ($P<0.05$, Fisher's exact test).

** Diagnosis of patients with AZFc deletions from whom sperm could be retrieved was solely SCO

RESULTS

Of 980 ASD patients tested, we identified 79 with Y chromosome microdeletions (8.1%). The specific microdeletion status, and the number of men found to have each deletion are depicted in Table 1.

Outcomes of micro-TESE in these patients are presented in Table 2. All patients who were enrolled in this study underwent micro-TESE, including 901 ASD patients without Y chromosome microdeletions. Micro-TESE universally failed in men with AZFa, AZFb, AZFbc, and AZFabc deletions, but was successful in 28 of 43 patients with complete AZFc deletions (62.2%). Micro-TESE was more often successful in AZFc-deleted patients than ASD patients without Y chromosome microdeletions, for whom the surgical sperm retrieval rate was =33.0% ($P<0.05$). Both maturation arrest and SCO were present as pathologies in testicular specimens from patients with AZFb or AZFc deletions. On the other hand, in patients with AZFa, AZFabc and AZFbc deletions, the pathology observed was solely SCO, not maturation arrest. G-banding revealed that all patients with AZFabc deletion had 46, XYq- or 46, Xmar+.

DISCUSSION

In the present study we assessed the prevalence of Y chromosome microdeletions in Japanese ASD patients. To the best of our knowledge, the tested population reported in this study represents the largest cohort of ASD patients that has been tested for Y chromosome microdeletions in Japan. Amongst 980 ASD patients, we identified 79 men (8.1%) with microdeletions in the Y chromosome spanning the AZFa, AZFb and AZFc loci. The frequency of Y chromosome microdeletions in infertile men from Japan was thus similar to the frequency reported in other countries and regions[9].

Thirty-six patients (3.6%) had microdeletions including AZFa and/or AZFb in this study. We report only micro-TESE results, because we have not seen any men with deletions of the AZFa or AZFb regions that nonetheless had sperm on micro-TESE. Clinically, on counselling we now recommend primary use of donor sperm rather than TESE for men with deletions that involve complete loss of the AZFa or AZFb regions. For these unfortunate patients, Y chromosome microdeletion testing avoids undergoing unnecessary surgery and emphasizes the need for donor sperm or adoption.

We found AZFc microdeletions in 43 (4.4%) of the ASD patients tested. Many studies have reported that mature spermatozoa are present in about half of patients with AZFc deletions. Our data demonstrated that these patients had a significantly better microsurgical sperm retrieval rate (62.2%) relative to men with idiopathic ASD but without deletions (33.0%). The non-deleted ASD cohort excluded patients with identifiable conditions that have a higher rate of sperm retrieval with micro-TESE, including Klinefelter's syndrome[10]. Deletions in the AZFc region are invariably more frequently observed than deletions in the AZFa and AZFb loci in all published studies[11]. Azoospermic men have a higher incidence of microdeletions than oligozoospermic men and consequently the frequency of deletions reported from different laboratories may vary from 2 to 10% (or even higher) reflecting the composition of the study population[9].

In our experience, this information has been useful for preoperative counseling of couples who are considering micro-TESE. Y chromosome microdeletions are becoming common in Japan, justifying routine testing in couples considering assisted fertility interventions[12].

Among the available studies of STS in different loci, including those of the European Andrology Association (EAA), recommended markers had been used for detection of Y chromosome microdeletion in infertile men[9]. In azoospermic men, Y chromosome microdeletion testing not only provides essential information for genetic counseling, but it helps patients and their physicians make more informed decisions about surgical sperm retrieval.

On correlating the deletion pattern with testicular histology, the deletion of AZFa was found to be associated with complete absence of germ cells and the presence of Sertoli cells in the seminiferous tubules. The deletion of AZFb was associated with developmental arrest of germ cells at the pachytene stage. The deletion of AZFc was variably associated with SCO syndrome, developmental arrest of germ cells at the spermatid stage and maturation arrest. The genes at each locus act at a particular stage of germ cell differentiation, and deletion of a particular AZF locus results in a characteristic phenotype[13]. Results from our study of testicular biopsies are consistent with reports in the literature. Identification of gene products and their biological actions is necessary to understand the influence of AZF gene deletions on formation of mature spermatozoa. Further, copy-number variations in Y chromosomal AZF regions have been associated with the risk of spermatogenic failure[14]. Some studies have shown that the genetic basis of infertility should be addressed after a detailed consideration of different genetic factors, such as X chromosome mutations[15] and mitochondrial DNA mutations[16]. Recently, a genome-wide association study revealed that 41 single-nucleotide polymorphisms (SNPs) were significantly correlated with family size or birth rate[17]. In any case, further genome analysis for infertile patients should be considered as very important in the future.

CONCLUSIONS

The present report details one of the largest studies of ASD caused by Y chromosome microdeletions in Japan or elsewhere. As such, it is a significant contribution to the study of male infertility. Data presented here revealed a close relationship between microdeletions and spermatogenesis. Therefore, screening for microdeletions and chromosomal abnormalities should be performed and genetic counseling should be provided before micro-TESE in undertaken.

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