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Y chromosome microdeletions in idiopathic azoospermia and non-mosaic type of klinefelter syndrome

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Abbreviations: AZF, azoospermia factor; STS, sequence tagged site; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; *SRY*, sex determining region Y; *CDY*, chromodomain Y; *DBY*, dead box Y; *DFFRY*, drosophilia fat facets related Y

Abstract

The objective of this study was to elucidate the cause of the spermatogenic defect in idiopathic azoospermia and non-mosaic type of Klinefelter syndrome. Genomic DNAs from 9 cases of Korean idiopathic azoospermia and 6 of Korean non-mosaic type of Klinefelter syndrome were used for the detection of Y chromosome microdeletions by polymerase chain reaction using 60 primers. Microdeletions of the Y chromosome were found in 1 of 9 (11.1%) patients with idiopathic azoospermia, whereas none was deleted in non-mosaic type of Klinefelter syndrome. This result suggests that Y chromosome microdeletions could be one of the etiologic factors in idiopathic azoospermia.

Keywords: Y chromosome, microdeletion, idiopathic azoospermia, Klinefelter syndrome

Introduction

Approximately 15% of all couples attempting pregnancy experience failure of conception (Mosher WD, 1987). Male factor, either the sole cause or a contributing cause, has been identified in 50% of infertility cases (Bhasin *et al*., 1994; De Krester, 1997). Varicocele, infection, hypogonadism, cryptorchidism, structural abnormalities in reproductive organs, psychological problems, genetic defects and idiopathic infertility are encountered as causes of male infertility (Kim *et al*., 1997). Up to 66% of all infertile men have idiopathic azoospermia or severe oligospermia, depending on the definition of idiopathic that is no known causes (Hendry *et al*., 1973; Nieschlag, 1997). Since conventional cytogenetic technology cannot reveal any molecular defects of spermatogenesis in idiopathic azoospermia, microdeletions were used for molecular defects of Y chromosome.

The role of Y chromosome controlling spermatogenesis was first recognized in 1976 when partial deletions in the long arm of Y chromosome were discovered (Tiepolo and Zuffardi, 1976). After dividing Y chromosome into 7 regions (Vergnaud *et al*., 1986), the 3-nonoverlapping regions of AZFs (AZFa, AZFb, AZFc) have been mapped within intervals 5 and 6 of the Y chromosome which lie within Yq11.21-Yq11.23. Although the large majority of microdeletions were located in the distal part (AZFc) with variable size of deletions (Reijo *et al*., 1995; Najmabadi *et al*., 1996; Qureshi *et al*., 1996; Stuppia *et al*., 1996; Vogt *et al*., 1996; Foresta *et al*., 1997; Pryor *et al*., 1997), men with deletions involving the more proximal parts (AZFa, AZFb) generally represent as azoospermic, and severe oligospermia may also be encountered in AZFb deletion.

Klinefelter syndrome is the most common chromosomal abnormality associated with male infertility and azoospermia. Its mechanism leading to spermatogenic defect is unknown.

The purpose of this study is to investigate the Y chromosome microdeletions in idiopathic azoospermia and non-mosaic type of Klinefelter syndrome using polymerase chain reaction.

Materials and Methods

Patients

Nine cases of idiopathic azoospermia and six cases of non-mosaic type of Klinefelter syndrome from the Infertility Clinic of Korea University Anam Hospital were recruited for this study. Men of azoospermia secondary to infection, obstruction, and other causes of testicular injury are excluded. Azoospermia was confirmed with semen analysis according to the World Health Organization Guidelines (World Health Organization, 1993). As a control group 8 proven fathers were tested. This study was approved by Institutional Review Board and written informed consent was obtained from all patients.

Genomic DNA preparation

DNA extraction was performed by conventional method as follows. Briefly, 10 ml of peripheral blood was collect-

ed in EDTA bottles. RBC lysis buffer (12 mM Tris, pH 7.5; 5 mM $MgCl_2$) was added to lymphocytes obtained from centrifugation. The resulting lymphocytes were supplemented with 20% sodium dodecyl sulfate, and proteinase K and were incubated overnight at 55°C, following this 6M NaCl was added. To precipitate DNA, 2 volumes of 99.5% ethanol was added and then the mixture was washed in 70% ethanol, and dissolved in distilled water. The concentration of DNA was measured by spectrophotometer at 260 nm.

PCR

A set of 60 Y specific STS (sequence tagged site) (accessed from Gene Bank, USA) was tested in each patient. The *SRY, CDY, DBY* and *DFFRY* gene and sY45, sY66, sY67, sY68, sY78, sY81, sY82, sY83, sY86, sY87, sY90, sY95, sY102, sY106, sY117, sY119, sY121, sY124, sY130, sY133, sY134, sY136, sY139, sY142, sY143, sY146, sY149, sY152, sY153, sY155, sY157, sY158, sY160, sY200, sY202, sY205, sY210, sY211, sY254, sY274, sY276, sY277, sY283, sY591, sY592, sY594, sY595, sY600, sY601, sY602, sY603, sY610, sY620, sY624, sY634, sY638 were used. Among them, sY205, sY254, sY277, sY283, sY624 were encoded in DAZ gene.

The polymerase chain reaction was performed with 100 ng of genomic DNA in a final volume of 50 μl, including a reaction buffer, diethylnitrophenyl thiophosphate mix, each primer, and Taq polymerase. Thermocycling (Hybrid, UK) consisted of an initial denaturation of 5 min at 94°C and 35 cycles of 1 min at 94°C for denaturation, 1 min at 56°C for annealing, and 1 min at 72°C for extension, and then final extension of 10 min at 72°C. In the sY121, sY160, sY211, sY254, sY283, sY592, sY610, sY638, the annealing temperature was 58°C. The products were separated on 2% agarose gel by electrophoresis. And stained with ethidium bromide, and visualized using UV transillumination. A deletion was confirmed when two independent consecutive PCR amplifications yielded negative results.

Results

PCR screening of 60 loci in the Y chromosome was performed in a group of patients with azoospermia who had idiopathic etiology (N=9) or Klinefelter syndrome (N=6), and 8 healthy fertile men as a control.

Deletion of Y chromosome was found in 1 case of the 9 (11.1%) cases with idiopathic azoospermia. The deleted sites were sY136 and sY277, and the 2 deleted sites were seen in the same patient (Figure 1 and Figure 2).

PCR was repeated in cases of deleted STSs, and the same results were obtained. There were no problems during amplification, since the positivity with sY136 and sY277 was observed in all other cases. The deleted sites were confined to the region of AZFb (sY136) and AZFc (sY277), which were limited to interval 6.

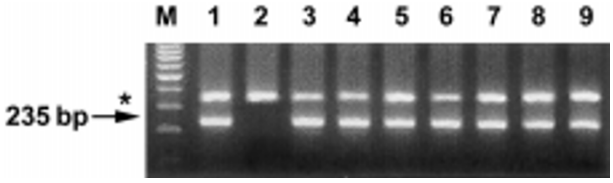

Figure 1. Deletion of sY136 in 1 of 9 patients with idiopathic azoospermia. Lane 2 shows deletion of sY136 corresponding to 235 bp. Primer sequence of SY136: Forward-CACATGAAGCACTGGAACTG, Reverse-GTTGTCTGGAAATCCCTGTG. M: marker. * : sY274 corresponding to 361 bp.

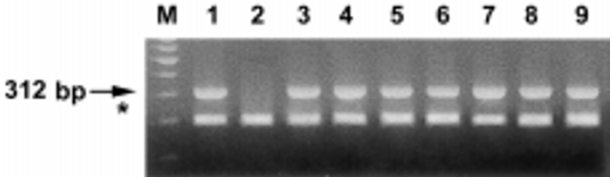

Figure 2. Deletion of sY277 in 1 of 9 patients with idiopathic azoospermia. Lane 2 shows deletion of sY277 corresponding to 312 bp. Primer sequence of SY277: Forward-GGGTTTTGCCTGCATACGTAATTA, Reverse-CCTAAAAGCAATTCTAAACCTCCAG. M: marker. * : sY276 corresponding to 216 bp

None of the 6 patients with azoospermic Klinefelter syndrome showed any deletion of the 60 loci tested, and no deletions were observed in any of the fertile men studied.

Discussion

Main function of Y chromosome carrying about 1% of the human genome is the propagation of species through sex determination and control of spermatogenesis.

Defects of genes/loci on the Y chromosome can influence the regulation of spermatogenesis (Bardoni *et al.*, 1991; Ma *et al.*, 1992; Vogt *et al.*, 1992; Ma *et al.*, 1993; Chandley *et al.*, 1994; Kobayashi *et al.*, 1994; Reijo *et al.*, 1995; Kent-First *et al.*, 1996; Najmabadi *et al.*, 1996; Qureshi *et al.*, 1996; Reijo *et al.*, 1996; Stuppia *et al.*, 1996; Vogt *et al.*, 1996; Foresta *et al.*, 1997; Mulhall *et al.*, 1997; Pryor *et al.*, 1997; Kim *et al.*, 1999). Structure and function of the genes on the Y chromosome to be elucidated is in progress, however the published data lead to the idea that microdeletions of the Y chromosome could play a role in the step of spermatogenesis, partly.

Considering the microdeletions of Y chromosome, some questions concerning real frequency, indication of molecular diagnosis, and significance of the number and sites of primers investigated are raised.

The reported incidence of Y deletions in men with azoospermia or severe oligospermia varies among studies, ranging from 1% (van der Ven *et al.*, 1997) to 55% (Foresta *et al.*, 1998); although most studies reported the incidence below 15%.

Microdeletions are found more frequently in azoospermic men than in oligozoospermic men (Simoni *et al*., 1998). The prevalence of microdeletions in azoospermic men was between the ranges of 6.7% (Kleiman *et al*., 1999) to 37.5% (Foresta *et al*., 1997). On the other hand, that in oligospermic men was between 1.5% (Oliva *et al*., 1998) to 22.7% (Foresta *et al*., 1997).

In our study, the frequency of microdeletion in idiopathic Korean azoospermia is 11.1% and it falls within the range reported previously.

Indication of molecular testing is not precisely defined at present. From the reports experienced to date in several centers, there has been an agreement with the fact that Y chromosome analysis should be performed in all patients with a sperm concentration $< 5 \times 10^6$/ml. Molecular analysis would not help anatomical problems in case of varicocele and/or cryptorchidism, because molecular pathology in Y chromosome can also be found with these conditions (Pryor *et al*., 1997; Simoni *et al*., 1997). In addition, analysis of microdeletions should be recommended in all patients who are considering the use of IVF (*in vitro* fertilization) and ICSI (intracytoplasmic sperm injection).

To date, the question of which and how many loci should be analysed cannot be defined. The published data state that the frequency of microdeletions found is not dependent on the number of primers used. A higher number of STSs may lower inaccuracy, but very large numbers of primers to detect the microdeletions of Y chromosome might be polymorphic variants and/or of clinical irrelevance.

This study used 60 primers to detect microdeletions but the deletion frequency was not increased compared to those of other reports. Microdeletions in infertile men, but not in their fertile fathers, suggested that the event occurred *de novo* in infertile patients (Ma *et al*., 1992; Reijo *et al*., 1995; Reijo *et al*., 1996) and it provided that the deletions were the cause of the spermatogenic failure observed in these men. Another possible explanation is gonadal mosaicism, the mosaic father might transmit the microdeletions to their sons. Of those who were not deleted in any region in germ-line DNA, their DNA from the testis tissue also might to be normal. To detect gonadal mosaicism, single cell analysis of spermatogenic cells may be required in patients considering the use of IVF & ICSI.

The incidence of sex chromosome and autosomal anomalies revealed 4.2% and 1.5% of the azoospermic and oligospermic infertile men, respectively (Johnson, 1998). The predominate percentage of sex chromosome anomalies among infertile men was attributed to the Klinefelter syndrome (47,XXY), which was the most common karyotypic anomaly to be found among infertile men. Azoospermia is almost the rule in men with Klinefelter syndrome who have the 47,XXY karyotype, although some studies have reported the presence of motile sperm in the ejaculate and testicular sperm in XY/XXY mosaicism (Cozzi *et al*., 1994). To avoid the bias, we selected the group of Klinefelter syndrome that is non-mosaic type and azoospermia.

This study demonstrated that deletion was observed with a frequency of 11.1% using 60 STS primers in 9 patients with idiopathic azoospermia but not in 6 patients with Klinefelter's syndrome without spermatogenesis. This suggests that AZF deletion of Y chromosome may contribute to the cause of idiopathic azoospermia, but different mechanisms of azoospermia in Klinefelter syndrome may exist. We suggested that over dosage of X chromosome interferes with the function of Y chromosome in non-mosaic type of Klinefelter syndrome.

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