Detailed Analysis of Isodicentric Y in a Case with Azoospermia and 45,X/46,X,Idic(Y) Mosaicism

Jaehyeon Lee1, Jong Kwan Park2,3, Dal Sik Kim1,3, Hye Soo Lee1,3, Sam Im Choi1,3, and Yong Gon Cho1,3

1Department of Laboratory Medicine, Chonbuk National University Medical School, Jeonju, 2Department of Urology, Chonbuk National University Medical School, Jeonju, and 3Research Institute of Clinical Medicine of Chonbuk National University-Biomedical Research Institute of Chonbuk National University Hospital, Korea

Abstract. Isodicentric chromosomes are the most commonly reported aberrations of the human Y chromosome. We characterized the isodicentric Y chromosome from an azoospermic male by fluorescence in situ hybridization (FISH) and PCR. The patient was a 33-year-old man who visited our hospital for evaluation of infertility. He expressed male external genitalia and showed normal testosterone levels. However, severe varicocele on both sides and hydrocele of the right side were found upon ultrasonography of the scrotum, and no sperm were found on semen analysis. The patient showed 46,X,idic(Y)(q11.22)[58]/45,X[12] karyotype. FISH demonstrated the presence of two centromeres and two SRY regions (74.0% of cells counted). PCR showed the breakpoint between SY161 and SY121 in Yq11.221-q11.222.

Introduction

Isodicentric Y chromosomes are usually unstable during cell division and remain so until one of their centromeres is inactivated [1,2]. Most reported cases are chromosomal mosaics, generally including a 45,X cell line (95% of cases) [3]. Phenotypes vary from male to abnormal female or individual with ambiguous genitalia, depending on the location of the breakpoints as well as on the proportion of each cell line [4].

We characterized the isodicentric Y chromosome from an azoospermic male by FISH and PCR, which demonstrated the breakpoint between SY161 and SY121 in Yq11.221-q11.222 region.

Cases Presentation. A 33-year-old male had visited the outpatient urology department for evaluation of his infertility. The initial complete blood count was within normal limits, including white blood cells at 4.5x10^9/L, hemoglobin of 15.4 g/dL, and platelets at 184x10^9/L. Some laboratory findings were abnormal, including fasting serum, AST 62 IU/L, ALT 96 IU/L, total cholesterol 240 mg/dL, triglyceride 378 mg/dL, FSH 22.5 mIU/mL, and LH 14.6 mIU/mL. However, the patient’s serum testosterone level was within normal limits at 4.7 ng/mL. He had a small volume (6mL) of testes and decreased semen volume without sperm in the semen analysis. Scrotal sonography showed a hydrocele in the right scrotal sac and severe varicocele in both testes.

Cytogenetic study was performed with informed consent on peripheral blood lymphocytes and demonstrated chromosomal mosaics in his karyotype of 46,X,idic(Y) (q11.22)[58]/45,X[12]. FISH studies were performed on blood samples using CEP X SpectrumOrange (Xp11.1-q11.1)/Y SpectrumGreen (Yq12) direct labeled fluorescent DNA probe kit, the LSI SRY (Yp11.3) probe, and CEP X (DXZ1, Xp11.1-q11.1) SpectrumGreen/Y (DYZ3, Yp11.1-q11.1) SpectrumOrange Probe kit (Abbott Molecular Inc., Des Plaines, IL, USA) according to manufacturer’s instructions. FISH characterization of the rearranged chromosome Y demonstrated the presence of a double hybridization signal (74.0% of cells counted) using the Y alpha-satellite (DYZ3) probe and the presence of two SRY regions, confirming the suspicion of a dicentric Yp chromosome (Figure 1). Patient DNA was analyzed by PCR using 12 characterized Y-specific primer pair to find the deleted regions on the Y chromosome. Table 1 shows the Y-specific primers and product for PCR amplification. The molecular study performed by 12 Y sequence-tagged sites (STS) showed the presence of SY14, SY34, SY69, SY78, SY161 loci, but starting with SY121 in the AZFb region, the remaining loci tested were deleted. This result allowed us to establish the chromosomal breakpoint in the Yq11.221-q11.222 region corresponding to the 5I-5N deletion map interval (Figure 2)[5]. No data were available from testicular biopsy.
Y-chromosome infertility is characterized by azoospermia (absence of sperm), severe oligozoospermia (<1x10^6 sperm/mL semen), moderate oligozoospermia (1-5x10^6 sperm/ml semen), or mild oligozoospermia (5-20x10^6 sperm/mL semen) and is usually caused by deletions of genetic material in a region of the Y chromosome called azoospermia factor (AZF) A, B, or C [6]. Males with Y chromosome infertility usually have no obvious symptoms, although physical examination may reveal small testes.

Approximately 2-8% of men with unexplained infertility associated with azoospermia or oligozoospermia have chromosome abnormalities, mostly involving the sex chromosomes [7]. Isodicentric Y chromosomes are a common structural rearrangement of the Y chromosome in azoospermic males, resulting in both deletion of part of the Y long arm and duplication of the Y short arm and proximal Y long arm, and are mostly found in a mosaic form [8-11]. Routine cytogenetic studies and FISH analyses performed on peripheral blood lymphocytes and using probes specific for Y-linked genes can detect the rearranged Y chromosome, including long arm deletions. The detection of Y chromosome rearrangements is important because some are often associated with a 45,X cell line, and deleted regions in AZF region are known to be associated with infertility [12-15]. Hsu et al. suggested that the percentage of 45,X cells in the urogenital ridge play a significant role in sex determination and differentiation of the gonads, as well as the structure of the dicentric Y chromosome [8].

The patient in the study was considered to have Y-chromosome infertility and had no symptoms except a small volume (6mL) of testes. The patient had chromosomal mosaics, showing a higher percentage of cells with rearranged Y having two SRY gene than 45,X cells in the peripheral blood. He was male phenotypically, having the external genitalia of a normal male and didn’t have any reported Turner features. We have no data available for testicular biopsy or buccal smear in the patient. It has been described that the phenotypic sex depends on the percentage of cells with the SRY gene in the gonads, and a cytogenetic study performed on the blood cells only was not informative [16]. However, we could explain the association of male phenotype in this case with the presence of more copies of the SRY gene in peripheral blood cells, suggesting a possible dosage effect. The breakpoint between STS SY161 and SY121 in region 5 of the deletion map does not seem to interfere with the stability of the rearranged Y, leading to a higher percentage of 45,X cells and a female phenotype, as previously reported [17].

The idic(Y) of the patient has a breakpoint between SY161 and SY121, resulting in deletions of AZFb and AZFc all. The association of those deletions with severe spermatogenic impairment was revealed by molecular studies [18,19]. It has been shown by
assembly of a complete 4.3 Mb map of AZFb that the AZFb region has large repeated sequences and testis-specific genes involved in spermatogenesis, so deletion in this region should result in spermatogenic impairment [20]. A recently published article describing a 14-patient case series delineates the association between idic(Y) and infertility, which may overlap this case’s finding [21]. However, ours is a well-studied case of a Korean patient that uses the different molecular markers for idic(Y) than the recent study [21]. Our STS markers were useful for characterizing the chromosomal breakpoints and the deletion interval. FISH analysis is also useful for defining the Y rearrangement and extending the number of cells investigated, allowing a more precise evaluation of the mosaicism.

References