Delineating the association between isodicentric chromosome Y and infertility: a retrospective study

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Objective: To report on 14 infertile patients who had a de novo form of the same isodicentric (idic)(Yq) karyotype with variable degrees of mosaicism.
Design: Retrospective study and review of the literature.
Setting: Medical genetics laboratory in a research institute for reproductive biomedicine.
Patient(s): Fourteen infertile patients, including 13 male patients and 1 female patient who had infertility with the same idic(Y) karyotype.
Intervention(s): Conventional cytogenetic methods, fluorescence in situ hybridization (FISH) on seminal germ cells and blood, and polymerase chain reaction (PCR)-based molecular approaches.
Main Outcome Measure(s): Karyotype, FISH, and PCR results.
Result(s): Cytogenetic results revealed abnormal Y chromosome: 45,X/46,X,idic(Y)(q11.22). The FISH technique on blood lymphocytes confirmed a rearranged Y chromosome, with two centromeres and two *SRY* signals, and marker chromosome with various levels of mosaicism. Moreover, aneuploidy of sex chromosomes was also detected in haploid seminal germ cells. Multiplex PCR analysis of blood samples demonstrated microdeletion in AZFb and AZFc loci.
Conclusion(s): Because of the resemblance between inversion of chromosome Y and idics(Y), use of confirmatory techniques (e.g., FISH or PCR-based methods) could help prevent medical errors in healthcare systems and precisely delineate chromosomal aberrations in infertile patients when clinical data fail to clarify the cause of infertility. (Fertil Steril® 2014;101:1091–6. ©2014 by American Society for Reproductive Medicine.)
Key Words: Mosaicism, isodicentric Yq chromosome, multiplex PCR, fluorescence in situ hybridization (FISH), azoospermia, semen germ cells

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Isodicentrics (idics) are among the most cytogenetically complex aberrations of the Y chromosome, originating during spermatogenesis via aberrant homologous crossing-over between opposite arms of a palindrome (1), and are associated with male nonobstructive infertility (2, 3). It is asserted that because of mitotic instability of idics, they nearly always occur as a mosaic with a 45,X cell line with a wide spectrum of manifestations, ranging from females with Turner symptoms to males with spermatogenic failure (2–5). This variation depends on the numbers of 45,X cells in the urogenital ridge and also the percentage of *SRY*-positive cells in the gonads.

One useful method for detecting chromosomal abnormalities could be the fluorescence in situ hybridization (FISH) technique, which can be performed on different kinds of cells (6). Unfortunately, azoospermic samples do not have any sperm to be scored following this approach. On the other hand, round cells commonly can be found in the semen of both fertile and infertile men. These consist of

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germ cells as well as somatic cells, including white blood cells and genital epithelial cells (7). Haploid germ cells can be used as an alternative source for assessment by FISH. Additionally, it has been proposed that recognition of seminal germ cells from oligo- or azoospermic patients can provide a source of material for systematic investigation (8). The biological importance of seminal immature germ cells is still undefined. However, clinically the presence of these sorts of cells is of potential significance as a predictive parameter for testicular sperm extraction, and the probands could be consulted for testicular sperm extraction–intracytoplasmic sperm injection (9). Nevertheless, the significance of the presence of immature germ cells in semen is still a subject under debate (10).

This retrospective study was inspired by previous publications that imply the correlation between idic(Y) rearrangement, mosaicism, and percentage of 45,X cell line and tissue specificity with the resulting clinical outcomes in patients with infertility (4, 11, 12).

Here the clinical (e.g., pathologic and hormonal), cytogenetic, FISH-, and polymerase chain reaction (PCR)-based molecular results of 14 carriers of idic(Yq) who were referred to our center because of infertility are presented.

MATERIALS AND METHODS

According to the guidelines of the Ethics Committee of Royan Reproductive Biomedicine Research Center, the activity did not require institutional review board approval, because our research project involved use of existing information collected from human participants (e.g., secondary datasets, existing biological samples), but there were not any identifiers linking individuals to the data/samples. Meanwhile, written consent had been obtained from all patients for the use of materials.

Fourteen cases diagnosed with the same rearranged idic(Y) (pter→q11.22::q11.22→pter) chromosome (age range, 29–41 years) were included in this study. One was an infertile woman with primary amenorrhea and Turner syndrome–like phenotype; the rest were azoospermic men with different testes histopathology manifestations.

All patients underwent routine hormone and semen analysis. Serum LH, FSH, and T were analyzed by fully automated equipment operating on electrochemiluminescence (ECL) technology (Hitachi) (Table 1). All but three patients had mosaic pattern karyotype, although further investigation using FISH on blood samples revealed another cryptic mosaic case (Table 2).

Although some literature emphasizes the priority of gonadal tissue for investigation of rearranged Y chromosome(s) in patients with spermatogenic failure (4), because of religious and ethical challenges we could not obtain Ethics Committee approval for testicular biopsy of azoospermic patients for cytogenetic studies. Thus all of our examinations were carried out on peripheral blood lymphocytes and seminal germ cells of patients. Since our research activity involves the use of existing information collected from secondary datasets, existing biological samples, and diagnostic specimens, so, according to the policy of US Government Printing office, 7 C.F.R. § 1c.101, it may categorized in the group that exempted from providing institutional review board (IRB) approval.

TABLE 1

Patient FSH, LH and T hormonal profiles.

Case no.	FSH	LH	T
1	24	19	1.9
2	2.1	2.2	6.8
3	17.27	5.23	3.53
4	14	8	3.8
5	1.8	3.9	7.45
6	11.8	6.5	2.83
7	1.77	2.8	7.31
8	11.6	5.9	2.2
9	1.2	0.8	7.6
10	9.1	4	4.2
11	32	4.6	3.1
12	12.5	8.4	5.5
13	1.6	2.1	8.1
14	38	3	NA

Note: Normal ranges were 1–10 mIU/mL for LH, 1–14 mIU/mL for FSH, and 2.8–8 ng/mL for T.

Kalantari. idic(Y) aberration effects on fertility. Fertil Steril 2014.

Cytogenetic Test

A high-resolution chromosome analysis using a GTG-banding technique was carried out on peripheral blood lymphocytes according to standard cytogenetic protocols (13). At least 15 metaphase cells were routinely analyzed for each sample. To confirm the results from the GTG-banding technique, additional C-banding was performed.

Fluorescence In Situ Hybridization

Two different samples were used to determine the sex chromosome constitution of the cells: blood lymphocytes and haploid seminal germ cell (because no distinction between somatic and diploid germ cells was attempted, this work concentrates on the study of haploid germ cells). The slides were prepared according to standard cytogenetic procedures, and two distinct FISH probe sets were used. For a group of slides, triple-color FISH probes including control probe for CEP 18 and CEP Y (DYZ3) and CEP X probes (CEP 18 SpectrumAqua/CEP X [DXZ1] SpectrumGreen/CEP Y [DYZ3] SpectrumOrange Probe Direct Labeled Fluorescent DNA Probe Kit; Vysis, Abbott Molecular) were applied. For others, different probes were used: TelVysion Xq/Yq SpectrumOrange, TelVysion Xp/Yp SpectrumGreen, CEP X (DXZ1) SpectrumGreen/CEP Y (DYZ3) SpectrumOrange Probe Direct Labeled Fluorescent DNA Probe Kit (Vysis, Abbott Molecular).

The procedure of slide preparation (e.g., probe application, co-denaturation, hybridization, and posthybridization washes) was performed according to the manufacturer's instructions. Appropriate viewing, analysis, and imaging of FISH results was accomplished by use of a well-tuned fluorescent microscope (Olympus BX51) equipped with necessary and optimum filter sets (Spectrum Orange/Spectrum Green/6-diamino-2-phenylindole single band-pass filter sets; Abbott Molecular) and image acquisition and processing software (Cytovision V4.0; Applied Imaging, Genetix). Fifteen to twenty interphasic germ cells nuclei (1N) and at least 20 metaphases for blood samples were scored by FISH, and subsequent results are briefly demonstrated in Table 2.