Assisted Reproductive Treatment Applications in Men With Normal Phenotype but 45,X/46,XY Mosaic Karyotype: Clinical and Genetic Perspectives

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SUMMARY

Objective: The 45,X/46,XY mosaic karyotype is expressed by a spectrum of genital phenotypes, ranging from normal males through to ambiguous genitalia and to normal females.

Case Reports: We present three cases of men with azoospermia or severe oligozoospermia, and a 45,X/46,XY mosaic karyotype and two with a Y-chromosome microdeletion. Phenotypically, they appeared as normal males, with normal penis, scrotum and secondary sex characteristics. Testicular sperm extraction and aspiration were applied to patients, and couples were prepared for assisted reproductive therapy. All men with azoospermia or severe oligozoospermia were evaluated for karyotype and Y-chromosome microdeletion even if they had normal phenotypes.

Conclusion: Possibilities for finding sperm and the biologic paternity in subjects with 45,X/46,XY karyotype should be considered. Furthermore, the increased risk for testicular neoplasia with mosaic karyotypes should be taken into consideration. [*Taiwan J Obstet Gynecol* 2010;49(2):199–202]

Key Words: assisted reproductive technologies, gonadal dysgenesis, Y microdeletion

Introduction

Mosaicism is defined as the presence in the same individual of two or more cell lines derived from a single stem cell line but with a different chromosomal constitution. The phenotype of 45,X/46,XY mosaicism is well recognized in females with gonadal dysgenesis, males with mixed gonadal dysgenesis, male pseudohermaphroditism, and in apparently normal males [1]. Isolated reports of phenotypically normal females and males with this disorder have been described, and most present with ambiguous genitalia and a short stature.

Mosaic 45,X/46,XY individuals are often infertile and karyotyping can uncover genetic abnormalities. Males with the mosaic karyotype can benefit from assisted reproductive therapies, but prenatal diagnosis plays an important role in preventing potential transmission of genetic abnormalities. Because of the increased incidence of testicular neoplasia (gonadoblastoma) in patients with X/XY mosaicism, it is important to document this chromosomal abnormality [2].

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Materials and Methods

Cytogenetic analysis

Metaphase cells were obtained from phytohemagglutinin-stimulated blood lymphocytes from all patients

using standard techniques. Slides were stained by a conventional Giemsa-trypsin banding method. Thirty metaphase spreads were routinely analyzed from each patient, detecting 10% mosaicism with 95% confidence [3]. If a second cell line was found, up to 200 metaphases were analyzed to establish the level of mosaicism.

Molecular cytogenetic analysis

We collected 2 mL blood samples from male patients and preserved the samples in EDTA-coated tubes to analyze microdeletions in the Y chromosome. DNA from the samples was isolated by a spin column technique. After DNA isolation, 1.5 μL of $MgCl_2$ (25mM), 0.5 μL of dNTPs (10mM), 0.2 μL of *Taq* polymerase, 1 μL (5μM) of each primer (forward and reverse), 3 μL of isolated DNA, 15.3 μL of distilled water and 2.3 μL of phosphate buffered saline were combined for polymerase chain reaction. Reactions were carried out on an automatic thermal cycler and consisted of an initial denaturation step for 6 minutes at 95°C, followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 58°C, and 45 seconds at 72°C. After the final cycle, an extension step for 10 minutes at 72°C was carried out. Approximately 12 μL of the polymerase chain reaction product was electrophoresed on a 2% agarose gel and stained with ethidium bromide to visualize. If a sequence tagged site from a particular individual failed to amplify, it was repeated three times before it was considered to be a deletion.

Case Reports

Case 1

A 28-year-old man was referred to our *in vitro* fertilization clinic with male factor infertility. Sperm analysis showed azoospermia. Phenotypically, he appeared as a normal male, with a normal penis, scrotum and secondary sex characteristics. Bilateral testicles were 3 × 3.5 cm in diameter. In his hormone profile, follicle-stimulating hormone (FSH) was 21 mIU/mL, luteinizing hormone (LH) was 16 mIU/mL, and total testosterone was 2.21 ng/mL. Cytogenetic and molecular cytogenetic analyses were performed from peripheral blood. We could not perform cytogenetic analysis in solid tissues because of ethical concerns. In total, 100 metaphase spreads were analyzed. Additionally, 250 cells in the interphase were evaluated using LSI SRY/CEP X fluorescence *in situ* hybridization probes (Abbott Molecular, Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instruction. The level of mosaicism was determined to be 45X(5%)/46XY(95%) by cytogenetic analysis, which was concordant with mixed gonadal dysgenesis. Moreover, Y-chromosome microdeletion analysis revealed deletions in the sY127 and sY134 regions. Subsequently, the patient underwent bilateral testicular biopsy. Pathologic examination revealed bilateral Sertoli cell only and spermatocytic arrest. Sperm could be obtained from testicular sperm extraction (TESE), and the young couple were enrolled in an assisted reproductive technology (ART) program. The possible consequences of ART and the need for prenatal genetic diagnosis (PGD) or chorionic villus sampling (CVS) were explained to the couple. Intracytoplasmic sperm injection was conducted with surgically removed spermatozoa, and we achieved three embryos. We planned to carry out CVS if pregnancy occurred, but unfortunately this did not happen.

Case 2

A 25-year-old man with normal physical characteristics and primary infertility was found to have severe oligozoospermia with only a few motile, viable spermatozoa in his sperm analysis. His testicular diameters were 3.2 × 2.5 cm; in his hormone profile, FSH was 26 mIU/mL, LH was 18 mIU/mL, and total testosterone was 2.8 ng/mL. Cytogenetic and fluorescence *in situ* hybridization analysis ascertained 45,X(20%)/46,XY (80%) mosaicism. The couple was enrolled in an ongoing ART program.

Case 3

A 32-year-old patient presenting with male factor infertility and normal physical appearance, but azoospermia in his sperm analysis was revealed to have 45,X(45%)/46,XY(55%) mosaicism in his karyotype analysis. The FSH value was 23 mIU/mL, LH was 17 mIU/mL, total testosterone was 2.77 ng/mL, and testicular diameters were 4.0 × 2.8 cm. We could not obtain any spermatozoa by TESE. We carried out a testicular biopsy for genetic and histologic evaluation. When we examined peripheral blood and testicular tissue samples for Y-chromosome microdeletions, the patient exhibited deletions in sY127 and sY134 of azoospermia factor (AZF)-b, and sY254 and sY255 of AZFc in blood samples (Figure). The patient also showed deletions in sY254 and sY255 of AZFc in testicular tissue samples.

Discussion

The three most common known genetic factors related to male infertility are cystic fibrosis gene mutations leading to congenital absence of the vas deferens, Y-chromosome microdeletions leading to spermatogenic impairment, and karyotype abnormalities [4].

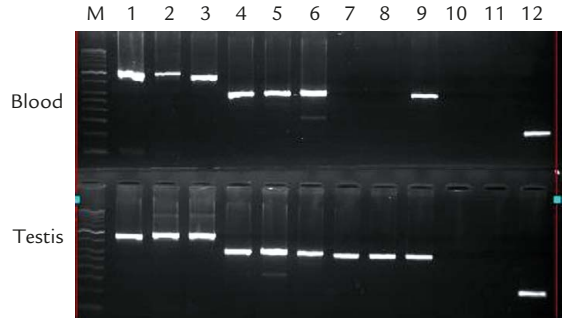

Figure. Gel agglutination image from the third patient's blood sample with azoospermia factor (AZF)-b (sY127, sY134) and AZFc (sY254, sY255) Y-chromosome microdeletions and the testicular sample with AZFc (sY254, sY255) microdeletions in the Y chromosome. Lane M, 50 bp marker; lane 1, SRY; lane 2, ZFY; lane 3, control for SRY and ZFY; lane 4, sY84; lane 5, sY86; lane 6, control for sY84 and sY86; lane 7, sY127; lane 8, sY134; lane 9, control for sY127 and sY134; lane 10, sY254; lane 11, sY255; lane 12, control for sY254 and sY255.

The frequency of genetic alterations is increased among men with severe spermatogenic impairment [5]. A 45X/46XY mosaicism is associated with a broad spectrum of phenotypes that include females with Turner syndrome, males with mixed gonadal dysgenesis, male pseudohermaphroditism, and apparently normal males [1]. A normal sized phallus, bilateral scrotal testes and a lack of evidence of müllerian structures in a pelvic ultrasound do not rule out the possibility of this chromosomal abnormality [6]. Men with pseudohermaphroditism may have penoscrotal, scrotal or perineal hypospadias as well as bilateral cryptorchidism. Coarctation of aorta, mild mental retardation, cystic hygroma and spina bifida may be additional clinical features.

The possibility of obtaining a pregnancy from mosaic and non-mosaic patients with Klinefelter syndrome has been previously presented [7,8]. These consequences lead us to a similar possibility for males with aneuploidy. Hence, in patients with 45X/46XY mosaicism, ART accompanied by PGD may result in a successful pregnancy.

Recent studies have shown that aneuploidy rates were higher in men who had unsuccessful intracytoplasmic sperm injection outcomes [9]. PGD for aneuploidy is proposed in reproductive medicine for improving the clinical outcome following ART. There is substantial evidence of increased implantation rates and a concomitant decrease in spontaneous abortions and aneuploidic pregnancies. This condition has severe clinical consequences, as approximately 33% of spontaneous abortions are aneuploid.

Foresta et al [10] evaluated 122 infertile, azoospermic men, and cytologic analysis identified Sertoli-cell-only syndrome, hypospermatogenesis, spermatogonial and/or spermatocytic arrest, spermatidic arrest, and normal germ line. Testicular volumes were found to be reduced in the Sertoli-cell-only, hypospermatogenesis and spermatogonial and/or spermatocytic arrest group [10]. However, our patients had normal testicular volumes, and the first patient had both Sertoli cell only and spermatocytic arrest as found in his testicular biopsy. In azoospermic subjects, testicular cytologic analysis permits the identification of different subtypes, and this classification may be very important in determining the appropriate therapy, particularly when choosing between surgical treatment and the use of assisted fertilization techniques by retrieval of epididymal or intratesticular spermatozoa and spermatids [10].

The Y chromosome is necessary for male germ cell development. Loss of Y-chromosome sequences in the euchromatic region of the long arm (Yq) is a major cause of male infertility. Between 10% and 20% of phenotypically normal men with idiopathic infertility and an apparently intact Y chromosome carry Yq microdeletions, resulting in the loss of genes necessary for fertility [11]. The first patient also revealed deletions in the sY127 and sY134 regions through Y-chromosome microdeletion analysis in addition to 45,X/46,XY mosaicism. Siffroi et al [12] showed that large and submicroscopic Yq deletions were associated with significantly increased percentages of 45,X cells in lymphocytes and of sperm cells nullisomic for genosomes, especially for the Y chromosome. Therefore, Yq microdeletion may be associated with Y-chromosome instability, leading to the formation of 45,X cell lines [12].

Case 3 contained AZFb and AZFc microdeletions in blood samples but exhibited only AZFc microdeletions in testicular tissue samples. In this case, we could not obtain any mature spermatozoa. There are few reports in the literature on this discordance in azoospermic patients with a 45,X/46,XY mosaic karyotype.

According to a previously published report, men with a 45,X/46,XY karyotype and malformations of the external genitalia are at increased risk of germ cell neoplasia of the gonads [2]. These tumors have been reported in subjects with 45,X/46,XY karyotype and gonadal dysgenesis, as it seems that Y-chromosome material participates in gonadoblastoma tumorigenesis. After ART procedures, gonadectomy can be proposed for patients who have genital abnormalities like hypospadias and cryptorchidism.

In conclusion, our cases demonstrate that all men with azoospermia or severe oligozoospermia should be evaluated for karyotype and Y-chromosome microdeletion analysis, even if they have normal phenotypes. The chance to obtain sperm in TESE materials and to experience biologic paternity for these patients should be kept in mind, but PGD or CVS for possible pregnancies

should be considered. Moreover, close follow-up for development of gonadal tumors should be mandatory in all patients with 45,X/46,XY karyotype and gonadal dysgenesis.

References

1. Telvi L, Lebbar A, Del Pino O, Barbet JP, Chaussain JL. 45,X/46,XY mosaicism: report of 27 cases. *Pediatrics* 1999; 104:304–8.
2. Müller J, Ritzén EM, Ivarsson SA, Rajpert-De Meyts E, Norjavaara E, Skakkebaek NE. Management of males with 45,X/46,XY gonadal dysgenesis. *Horm Res* 1999;52:11–4.
3. Hook, E. B. Exclusion of chromosomal mosaicism: Tables of 90%, 95%, and 99% confidence limits and comments on use. *Am J Hum Genet* 1977;29:94–7.
4. Kim ED, Bischoff FZ, Lipshultz LkI, Lamb DJ. Genetic concerns for the subfertile male in the era of ICSI. *Prenat Diagn* 1998;18:1349–65.
5. Niederberger C. Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. *J Urol* 2005;174:1046–7.
6. Aranoff GS, Morishima A. XO/XY mosaicism in delayed puberty. *J Adolesc Health Care* 1988;9:501–4.
7. Akashi T, Fuse H, Kojima Y, Hayashi M, Honda S. Birth after intracytoplasmic sperm injection of ejaculated spermatozoa from a man with mosaic Klinefelter's syndrome. *Asian J Androl* 2005;7:217–20.
8. Yarali H, Bozdag G. An ongoing pregnancy after frozen thawed embryo transfer in a patient with Klinefelter's syndrome. *Gynecol Obstet Invest* 2006;62:165–7.
9. Nicopoullos JD, Gilling-Smith C, Almeida PA, Homa S, Nice L, Tempest H, Ramsay JW. The role of sperm aneuploidy as a predictor of the success of intracytoplasmic sperm injection? *Hum Reprod* 2008;23:240–50.
10. Foresta C, Ferlin A, Bettella A, Rossato M, Varotto A. Diagnostic and clinical features in azoospermia. *Clin Endocrinol (Oxf)* 1995;43:537–43.
11. Papadimas J, Goulis DG, Giannouli C, Papanicolaou A, Tarlatzis B, Bontis JN. Ambiguous genitalia, 45,X/46,XY mosaic karyotype, and Y chromosome microdeletions in a 17-year-old man. *Fertil Steril* 2001;76:1261–3.
12. Siffroi JP, Le Bourhis C, Krausz C, et al. Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. *Hum Reprod* 2000;15:2559–62.