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Case of successful IVF treatment of an oligospermic male with 46,XX/46,XY chimerism

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Abstract

Introduction We present a case of an infertile male with 46,XX/46,XY chimerism fathering a child after ICSI procedure.

Methods Conventional cytogenetic analysis on chromosomes, derived from lymphocytes, using standard Q-banding procedures with a 450–550-band resolution and short-tandem-repeat analysis of 14 loci.

Results Analysis of 20 metaphases from lymphocytes indicated that the proband was a karyotypic mosaic with an almost equal distribution between male and female cell lines. In total, 12 of 20 (60%) metaphases exhibited a normal female karyotype 46,XX, while 8 of 20 (40%) metaphases demonstrated a normal male karyotype 46,XY. No structural chromosomal abnormalities were present. Out of 14 STR loci, two loci (D18S51 and D21S11) showed four different alleles in peripheral blood, buccal mucosal cells, conjunctival mucosal cells, and seminal fluid. In three loci (D2S1338, D7S820, and vWA), three alleles were detected with quantitative differences that indicated presence of four alleles. In DNA extracted from washed semen, four alleles were detected in one locus, and three alleles were detected in three loci. This pattern is consistent with tetragametic chimerism. There were no quantitative significant differences in peak heights between maternal and paternal alleles. STR-analysis on DNA from the son confirmed paternity.

Conclusion We report a unique case with 46,XX/46,XY chimerism confirmed to be tetragametic, demonstrated in several tissues, with male phenotype and no genital ambiguity with oligospermia fathering a healthy child after IVF with ICSI procedure.

Keywords Congenital chimera · Assisted reproductive technology · Infertility · Tetragametic chimerism · IVF

Introduction

Chimerism is the coexistence of more than one cell line in an individual due to the union of two originally separate "sibling" conceptions [1]. Human spontaneous congenital chimeras are thus products of a dizygote twinning event where the most frequent event is considered to be blood-exclusive chimerism, which is thought to arise from blood vessel anastomoses between chorionic placentas. Other hypothetical mechanism is the fertilization of two oocytes by two sperms and subsequent fusion of two zygotes into one single embryo or the dispermic type due to fertilization of two maternal nuclei by two sperm and a fusion of the two zygotes [2]. In all cases, the patient appears as tetragametic with two different haploid sets of maternal and paternal chromosomes.

Several chimeric cases have been reported since the first report in 1953 discovered during routine blood-typing testing [3]. Most chimeras are diagnosed at a young age due to ambiguous genitalia or abnormal blood grouping test [4, 5].

Chimeric patients with a 46,XX/46,XY karyotype are very rare. The phenotypic spectrum in these patients varies from normal male or female genitalia [6–9], to different degrees of ambiguous genitalia [4, 5, 10]. Most often, the patients are infertile, but there have been few reports on offspring in men [11] and women [7, 12] and also the successful in vitro fertilization and delivery of two healthy infants fathered by a 46,XX/46,XY true hermaphrodite [13, 14].

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We present a case of an infertile oligospermic male with apparent congenital tetragametic chimerism 46,XX/46,XY fathering a healthy boy after intra-cytoplastic sperm injection (ICSI) treatment.

Case report

A 25-year-old healthy male and his non-consanguineous spouse were referred to a public fertility clinic due to infertility. The couple had tried to obtain pregnancy for 1 year. The medical history of the patients was unremarkable with normal psychomotor development, no malformations, and no hereditary disease reported in the families. Clinical examination of genitals revealed a normal penis and both testes located in the scrotum, with a volume of 18 and 12 ml. Ultrasound examination of both testes was normal. MR scanning confirmed normal male genitals and no signs of ovarian tissue. Semen analysis was performed twice and revealed oligospermia with concentrations of 0.4 and 0.7 mill/ml (normal range > 15 mill/ml), respectively. Evaluation of reproductive hormone levels showed slightly elevated FSH level of 16.0 and 19.0 IE/L (normal range 1.2–15.8 IE/L) and LH level of 7.2 and 9.7 (range 1.7–8.6 IE/L), normal testosterone 16.2 nmol/L (range 11.0–34 nmol/L) and prolactin 295 MIE/L (90–580 MIE/L). A standard chromosome analysis of peripheral lymphocyte culture showed the karyotype mos 46,XX/46,XY. DNA analysis also revealed two cell lines in conjunctiva, buccal mucosa, and in seminal fluid. No Y chromosomal AZF deletion was detected according to EAA/EMQN guidelines [15].

After genetic counseling, the couple opted for infertility treatment. Only one IVF treatment with ICSI was performed, and a high-quality blastocyst was transferred resulting in pregnancy and the birth of a healthy son.

Materials and methods

Conventional cytogenetic analyses were performed on chromosomes derived from lymphocytes using standard procedures (Q-banding) with a 450–550 band resolution.

Short-tandem-repeat (STR) analysis: 14 STR loci were tested on DNA extracted from peripheral blood, buccal mucosal cells, conjunctival mucosal cells, seminal fluid, and washed semen from the proband, on DNA isolated from peripheral blood on his father and mother, and DNA extracted from buccal cells from the proband's son were all tested using AmpFLSTR™ Identifiler™ PCR Amplification KIT according to the instruction of the manufacturer (Thermo Scientific).

Results

Analysis of 20 metaphases from lymphocytes indicated that the proband was a karyotypic mosaic with an almost equal distribution between male and female cell lines. In total, 12 of 20 (60%) metaphases exhibited a normal female karyotype 46,XX, while 8 of 20 (40%) metaphases demonstrated a normal male karyotype 46,XY. No structural chromosomal abnormalities were present.

Table 1 The results of STR loci allele lengths in the proband, his child, and his parents. ni not informative

Loci (chromosome)	Proband Whole blood	Proband Buccal mucosa	Proband Conjunctival mucosa	Proband Seminal fluid	Proband Sperm	Child Buccal mucosa	Mother Whole blood	Father Whole blood
TPOX (2p)	233, 241	233, 241	233, 241	233, 241	233, 241	233	233	233, 241
D2S1338 (2q)	316, 324, 337	316, 324, 337	316, 324, 337	316, 324, 337	316, 324, 337	337, 349	316, 324	316, 337
D3S1358 (3p)	130, 134	130, 134	130, 134	130, 134	130, 134	130, 134	134	130, 138
CSF1PO (5q)	325, 329	325, 329	325, 329	325, 329	325, 329	329	325, 329	325, 329
D5S818 (5q)	153, 157	153, 157	153, 157	153, 157	153, 157	157, 161	153, 157	148, 157
D7S820 (7q)	262, 267, 285	262, 267, 285	262, 267, 285	262, 267, 285	262, 267	262, 278	262, 285	267, 270
D8S1179 (8)	133, 146	133, 146	133, 146	133, 146	133, 146	133	ni	133
TH01 (11p)	172, 187	172, 187	172, 187	172, 187	172, 187	172, 176	187	172
vWA (12p)	172, 176, 184	172, 176, 184	172, 176, 184	172, 176, 184	172, 176, 184	176, 184	176, 184	172, 176
D16S539 (16q)	282, 286	282, 286	282, 286	282, 286	282, 286	282, 286	282	282, 286
D18S51 (18q)	290, 294, 298, 306	290, 294, 298, 306	290, 294, 298, 306	290, 294, 298, 306	290, 294, 298	294, 298	290, 294	298, 306
D19S433 (19q)	118, 126	ni	118, 126	118, 126	118, 126	118	118, 125	126, 127
D21S11 (21q)	201, 206, 210, 216	201, 206, 210, 216	201, 206, 210, 216	201, 206, 210, 216	201, 206, 210, 216	210	201, 216	206, 210
AMEL (X,Y)	X,Y	X,Y	X,Y	X,Y	X, Y	X,Y	X	X,Y

Out of 14 STR loci, two loci (D18S51 and D21S11) showed four different alleles in peripheral blood, buccal mucosal cells, conjunctival mucosal cells, and seminal fluid. In three loci (D2S1338, D7S820, and vWA), three alleles were detected with quantitative differences that indicated presence of four alleles (Table 1). In DNA extracted from washed semen (sperm), four alleles were detected in one locus, and three alleles were detected in three loci. This pattern is consistent with tetragametic chimerism. There were no quantitative significant differences in peak heights between maternal and paternal alleles. STR-analysis on DNA from the son confirmed paternity.

Discussion

Peripheral blood karyotyping is recommended during the diagnostic workup of subjects with azoospermia and severe oligozoospermia. The finding of chimerism in the present case with a phenotypical normal male may be an incidental finding that cannot explain infertility. The results of the endocrinological investigations were normal, except for the slightly elevated LH level and the slightly smaller bitesticular volume, with a level of testosterone within the normal range, a condition which could be termed relative hypogonadism or compensated hypogonadism [16]. This may indicate that the patient will develop manifest hypogonadism in the future.

The mixture of male and female karyotypes can result in true hermaphroditism with ambiguous genitalia. The majority of reported 46,XX/46,XY chimeras are infertile, although fertile male and female has been described [2]. This indicates that both cell lines can determine the phenotype. It is not known how the distribution between male and female cells affects the development of the individual phenotype. According to the literature, normal male phenotype is especially rare, though it has been described [7, 8, 11]. In the present case, the amount and or distribution of cells with male karyotype 46,XY apparently resulted in a sufficient testosterone levels supporting the normal masculine development. If chimerism is present in all organs, it may be that the level of mosaicism in the gonads is predominantly male, explaining that the patient develops almost normal external genitalia and hypothalamic-pituitary-gonadal function. But at the same, the presence of a low-level 46,XX cell line in the gonads can explain the presence of compensated hypogonadism, which is otherwise not well explained.

The patient's mother reported that she had a bleeding episode during early pregnancy, which could indicate a pregnancy with vanishing twin and the possibility of exchange of blood between dizygote twins. However, we identified a pattern consistent of tetragametic chimerism in all examined mucosal samples as well as seminal fluid where no visible sign of blood and no quantitative differences between peak heights were present. It is difficult to verify that chimerism is exclusively confined to blood cells. The tetragametic pattern observed in DNA from mucosal cells could be caused by a mixture of male buccal epithelial cells with female leucocytes. The presence of a tetragametic pattern in seminal fluid, which mainly consists of leucocytes, could be consistent with chimerism confined to blood cells. A fibroblast cell culture from the patient may have shed more light on the origin of the chimerism; however, it was not ethically justified in the otherwise healthy patient to take biopsies from skin or other relevant tissues for cell culturing and analysis of fibroblasts. Chromosome analysis could have disclosed chimerism in fibroblasts, although a normal male/female karyotype would not have excluded chimerism outside the hematopoietic system in the patient.

In summary, we report a unique case with 46,XX/46,XY chimerism confirmed to be tetragametic, demonstrated in several tissues, with male phenotype and no genital ambiguity with oligospermia fathering a healthy child after IVF with ICSI procedure.

References

1. Gardner R, Sutherland G, Shaffer L. Chromosome abnormalities and genetic counseling. 4th ed. Oxford: Oxford University Press; 2011.
2. Tippett P. Blood group chimeras. A review. Vox Sang. 1983;44:333–59.
3. Dunsford I, Bowley CC, Hutchison AM, Thompson JS, Sanger R, Race RR. A human blood-group chimera. Br Med J. 1953;2:81.
4. Berger-Zaslav AL, Mehta L, Jacob J, Mercado T, Gadi I, Tepperberg JH, et al. Ovotesticular disorder of sexual development (true hermaphroditism). Urology. 2009;73:293–6.
5. Shin SY, Yoo HW, Lee BH, Kim KS, Seo EJ. Identification of the mechanism underlying a human chimera by SNP array analysis. Am J Med Genet A. 2012;158A:2119–23.
6. Hong X, Ying Y, Xu X, Liu Y, Chen Z, Lan X, et al. A dispermic chimera was identified in a healthy man with mixed field agglutination reaction in ABO blood grouping and mosaic 46, XY/46, XX karyotype. Transfus Apher Sci. 2013;48:223–8.
7. Bromilow IM, Duguid J. The Liverpool chimaera. Vox Sang. 1989;57:147–9.
8. Schoenle E, Schmid W, Schinzel A, Mahler M, Ritter M, Schenker T, et al. 46,XX/46,XY chimerism in a phenotypically normal man. Hum Genet. 1983;64:86–9.
9. Sudik R, Jakubiczka S, Nawroth F, Gilberg E, Wieacker PF. Chimerism in a fertile woman with 46,XY karyotype and female phenotype. Hum Reprod. 2001;16:56–8.
10. Krob G, Braun A, Kuhnle U. True hermaphroditism: geographical distribution, clinical findings, chromosomes and gonadal histology. Eur J Pediatr. 1994;153:2–10.
11. Watkins WM, Yates AD, Greenwell P, Bird GW, Gibson M, Roy TC, et al. A human dispermic chimaera first suspected from analyses of the blood group gene-specified glycosyltransferases. J Immunogenet. 1981;8:113–28.

12. Verp MS, Harrison HH, Ober C, Oliveri D, Amarose AP, Lindgren V, et al. Chimerism as the etiology of a 46,XX/46,XY fertile true hermaphrodite. Fertil Steril. 1992;57:346–9.
13. Sugawara N, Tokunaga Y, Maeda M, Komaba R, Araki Y. A successful pregnancy outcome using frozen testicular sperm from a chimeric infertile male with a 46, XX/46, XY karyotype: case report. Hum Reprod. 2005;20:147–8.
14. Sugawara N, Kimura Y, Araki Y. Successful second delivery outcome using refrozen thawed testicular sperm from an infertile male true hermaphrodite with a 46, XX/46, XY karyotype: case report. Hum Cell. 2012;25:96–9.
15. Simoni M, Bakker E, Eurlings MC, Matthijs G, Moro E, Muller CR, et al. Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. Int J Androl. 1999;22:292–9.
16. Aksglaede L, Andersson AM, Jorgensen N, Jensen TK, Carlsen E, McLachlan RI, et al. Primary testicular failure in Klinefelter's syndrome: the use of bivariate luteinizing hormone-testosterone reference charts. Clin Endocrinol. 2007;66:276–81.