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Determination of the parent of origin in nine cases of prenatally detected chromosome aberrations found after intracytoplasmic sperm injection

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Prenatal cytogenetic analysis of 71 fetuses conceived by intracytoplasmic sperm injection (ICSI) resulted in the detection of nine (12.7%) chromosome aberrations including two cases of 47,XXY, four cases involving a 45,X cell line and three autosomal trisomies. Molecular analysis of the parental origin of the deleted or supernumerary chromosome was performed by using polymorphic microsatellite markers. Six cases involving a sex chromosome abnormality were found to be of paternal origin while the two trisomic cases that could be analysed were of maternal origin. Two cases involved the same infertile couple who had two consecutive ICSI pregnancies terminated because of a chromosome abnormality. The replaced embryos in both cases originated from a single batch of ICSI fertilized oocytes of which part was used to initiate the first pregnancy and part was cryopreserved and used to initiate the second pregnancy.

Key words: chromosome abnormality/intracytoplasmic sperm injection/IVF/parent of origin/prenatal

Introduction

The finding of a number of sex chromosomal aberrations in intracytoplasmic sperm injection (ICSI) pregnancies (In't Veld *et al.*, 1995a, Liebaers *et al.*, 1995) has initiated a discussion about a possible causal relationship between such (sex) chromosome abnormalities and an enhanced level of (sex) chromosome aneuploidy reported in sperm cells of infertile males (Moosani *et al.*, 1995). In this respect it is important to establish the parent of origin of the additional or missing chromosome in cytogenetically abnormal ICSI pregnancies as it may help to identify the cause of the chromosome aberration concerned and establish whether such chromosome aberrations are indeed of a predominantly paternal origin. In the present study we have performed molecular studies using polymorphic microsatellite markers to identify the parent of origin of nine chromosome aberrations found in a series of prenatally investigated ICSI pregnancies.

Materials and methods

Patients

Case no. 1

Oligoasthenoteratozoospermia (OAT) with 12×10^6 sperm cells/ml, a progressive motility of 31% and 0% normal morphology were observed in a 36 year old male. Because of advanced maternal age of 38 years in his partner, transabdominal chorionic villus sampling (TA-CVS) was performed at 13 weeks gestation. A 46,X,del(Y)(q11)/46,X,idic(Y)(q11)/45,X karyotype was found and confirmed in amniotic fluid cells (In't Veld *et al.*, 1995b). Both parents were karyotyped and displayed normal chromosome complements in peripheral blood lymphocytes. The pregnancy was terminated at the parents' request, and the cytogenetic abnormalities were confirmed in cultured skin fibroblasts.

Case no.2

Extreme OAT with <100 000 sperm cells/ml, a progressive motility of 1% and 0% normal morphology were observed in a 38 year old male. Maternal age was 35 years. Two fertilized oocytes were replaced, resulting in a singleton pregnancy. TA-CVS was performed at 11 weeks gestation and chromosome analysis on the semi-direct villus preparations yielded a 45,X karyotype. Ultrasound examination at 13 weeks indicated nuchal oedema and hydrops fetalis. On the basis of these results the parents requested termination of pregnancy. Both parents displayed a normal karyotype in peripheral blood lymphocytes. Cultured fetal skin fibroblasts confirmed a 45,X karyotype in all cells examined.

Case no. 3

OAT with 12×10^6 sperm cells/ml, a progressive motility of 37% and 5% normal morphology were observed in a 38 year old male. Maternal age was 37 years. Amniocentesis was performed at 16 weeks gestation and resulted in a 47,XXY karyotype. Both parents displayed a normal karyotype in 100 peripheral lymphocytes examined. The parents opted for continuation of the pregnancy and a 3050 g boy without phenotypic abnormalities was born at week 40.

Case no. 4

Extreme OAT with 49 spermatozoa/ml, 2% progressive motility and 0% normal morphology were observed in a 34 year old male. Maternal age was 35 years. Two fertilized oocytes were transferred. Amniocentesis was performed at 16 weeks gestation and resulted in a 47,XXY karyotype. The parents opted for continuation of the pregnancy and a 3930 g boy without phenotypic abnormalities was born at week 40. Both parents displayed a normal karyotype in 100 peripheral lymphocytes examined.

Case no. 5

OAT with 10.8×10^6 spermatozoa/ml and a progressive motility of 7% were observed in a 38 year old male. Maternal age was 37 years.

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Embryo transfer resulted in a twin pregnancy. TA-CVS at 12 weeks gestation resulted in a mosaic 45,X/46,XY karyotype in fetus 1 and 46,XY in fetus 2. The karyotypes were confirmed by interphase fluorescent in-situ hybridization (FISH) on uncultured amniotic fluid cells at week 14 of gestation. The parents opted for a selective feticide. No fetal tissues were available for confirmatory chromosome studies. Both parents presented with a normal karyotype in peripheral blood lymphocytes.

Case no. 6
OAT with 4×10^6 sperm cells/ml, 0% progressive motility and 0% normal morphology were observed in a 33 year old male. Maternal age was 32 years. Amniocentesis was performed at 21 weeks gestation because of an ultrasound abnormality (double bubble; duodenal obstruction). A 47,XY,+21 karyotype was observed and the pregnancy was terminated at the parents' request. Subsequent chromosome analysis of cultured fetal skin fibroblasts confirmed the trisomy 21 karyotype.

Case no. 7
Parents were identical to those in case no. 1; three fertilized oocytes cryopreserved after the first ICSI procedure were implanted approximately one year later. Intrauterine fetal death was observed at week 15 and TA-CVS resulted in a 47,XY,+18 karyotype. No fetal tissues were available for confirmatory chromosome studies.

Case no. 8
Oligoasthenozoospermia (OA) with 1.2×10^6 sperm cells/ml, a normal morphology and a progressive motility of 17% were observed in a 33 year old male. Maternal age was 37 years. TA-CVS was performed at 16 weeks gestation because of ultrasound abnormalities (hygroma colli, hydrothorax, hydrops fetalis) and resulted in a 45,X karyotype. The pregnancy resulted in an intrauterine fetal death at 17 weeks gestation. The cytogenetic result was confirmed in cultured fetal skin fibroblasts.

Case no. 9
Microsurgical epididymal sperm aspiration (MESA) in a 37 year old azoospermic male with congenital bilateral absence of the vas deferens (CBAVD) yielded 0.1×10^6 sperm cells. The patient displayed heterozygosity for the deltaF508 cystic fibrosis mutation. Because of a maternal age of 39 years, amniocentesis was performed at 16 weeks gestation resulting in a 47,XX,+18 karyotype. The pregnancy was terminated at the parents request, and the trisomy 18 confirmed in cultured fetal skin fibroblasts.

DNA extraction

DNA was extracted from peripheral blood of both parents in all cases and from cultured amniotic fluid cells (case nos 3,4,6) or cultured fetal skin fibroblasts (case nos 2,8,9) according to standard techniques. DNA was extracted from chorionic villus chromosome slides (case nos 5,7) by scraping the cells into 10 mM NaCl/10 mM EDTA and centrifugation for 15 s at 10 000 *g*. The cell pellet was then resuspended in 25 µl 50 mM NaOH and boiled for 20 min. After neutralization with 1 M Tris–HCl pH 7.5 the suspension was spun down to remove cell debris.

Parent of origin studies

Molecular analysis of parental origin of the additional or missing chromosome was performed using polymerase chain reaction (PCR) amplification of polymorphic microsatellite repeats and a detection system which was either based on the use of radioactive nucleotides or on fluorophore labelled primers. In case of a radioactive PCR reaction, 60 ng of genomic DNA and 0.5–2.0 µl of the DNA solution obtained from the chromosome slides were amplified in a total volume of 15 µl containing 60 ng of each primer, 0.1 µl 100 mM dNTP-mix with a lower dCTP concentration, 0.45 µl 50 mM $MgCl_2$, 1.5 µl 10×PCR buffer (Gibco BRL, Gaithersburg, USA), 0.3 µl 25 mM spermidine, 0.75 µl 1% W1 (BRL), 0.1 µl α^{32}P-dCTP, and 0.1 µl Taq-polymerase (5 U/µl) (Gibco, BRL). Samples were processed through 25 cycles for fresh and 40 cycles for slide-extracted DNA samples, consisting of 1 min at 94°C for denaturation, 1 min at 55°C for annealing and 1.5 min at 72°C for extension. The amplifications were performed in a Perkin Elmer Cetus DNA thermal cycler 9600. Alleles were separated by electrophoresis on a 6% denaturing polyacrylamide gel. Gels were fixed with 10% acetic acid/10% methanol, dried and exposed to X-ray film overnight at room temperature.

Part of the genotyping was performed with fluorescently labelled primers by semi-automated methods (Reed *et al.*, 1994) using an ABI prism 377 DNA sequencer.

Loci that were examined for each of the chromosomes involved are listed in Tables I and II. Markers that were tested but did not show an informative pattern included the chromosome 18 specific markers D18S541, D18S851, D18S976 and the chromosome 21 specific markers D21S258, D21S13E, D21S111, D21S265, glur5, D21S219, D21S167, D21S156, IFNAR, PFKL, D21S270, D21S268.

Results

Prenatal chromosome studies were performed in 71 fetuses resulting from 57 singleton and seven twin pregnancies, all established by ICSI, and referred for advanced maternal age (MA>35 years) ($n = 25$), ultrasound abnormalities ($n = 2$) or a previous child with congenital abnormalities ($n = 2$); in all other pregnancies the sole reason for referral was the ICSI procedure itself. Mean maternal age in all 64 pregnancies was 33.3 years.

Nine cytogenetic abnormalities were identified including two cases with Klinefelter syndrome (47,XXY), two cases of 45,X, one case of a mosaic 45,X/46,XY, one case with a mosaic 46,X,del(Y)(q11)/46,X,idic(Y)(q11)/45,X, two cases of trisomy 18 and one case of trisomy 21 (Table III).

The parent of origin was determined via the analysis of polymorphic microsatellite repeats, except in case 1, in which a structurally abnormal Y chromosome was present, which was considered to be of paternal origin by definition, and case 7, in which insufficient fetal DNA could be obtained.

A series of markers on the X chromosome was tested in cases 2–5 and 8. Polymorphic microsatellite markers were chosen that were distributed over the entire X chromosome and multiple informative markers were analysed for each individual case. In the three 45,X cases (cases 2, 5 and 8) the parent of origin of the remaining sex chromosome was established by comparison of the allelic data of father, mother and fetus (Table I). Analysis of the allelic data for marker DXS538 in case 2 showed three different alleles: the father is carrier of allele 1, the mother of alleles 2 and 3, and the 45,X fetus of allele 2. These findings demonstrate that in the fetus the marker DXS538 is maternally derived as only the mother carries allele 2. Similar analyses can be made for the other cases and markers involved. It can be concluded from these analyses that the remaining X chromosome in the three 45,X cases is of maternal origin. In the two 47,XXY cases (cases 3 and 4) a similar analysis can be made. When marker DXS454 in case 3 is taken as an example, it can be seen (Table I) that

Table I. Parent of origin analysis of X chromosome abnormalities

Marker	Location	Case no.				
		2	3	4	5	8
DXS996	p22.32	NT	2/1.3/2.3	NT	NT	2/1.3/3
DXS999	p22.2	NT	2/1.2/2.2	NT	NT	2/1.2/1
DXS451	p22.13	NT	2/1.3/2.3	NT	NT	2/1.1/1
DXS538	p21.1–p11.21	1/2.3/2	1/1.2/1.1	2/1.3/1.2	3/1.2/2	2/1.2/2
DXS993	p11.4	NT	2/1.2/1.2	NT	NT	2/1.3/3
DXS1068	p11.4	NT	1/1.1/1.1	2/1.1/1.2	1/1.1/1	3/1.2/2
DXS426	p11.3–p11.23	3/1.2/2	1/1.2/1.1	NT	NT	NT
DXS991	p11.21	1/1.2/1	1/1.1/1.1	2/1.3/2.3	2/1.3/1	NT
DXS453	q12	1/2.3/2	2/1.3/1.2	NT	NT	NT
DXS990	q21.33–q22	2/1.2/1	2/1.3/1.2	1/2.2/1.2	1/1.2/1	2/1.2/1
DXS454	q21.33	1/1.1/1	3/1.2/1.3	NT	1/2.3/2	NT
DXS1001	q24	NT	NT	3/1.2/2.3	NT	NT
DXS984	q27.1	NT	1/2.2/1.2	NT	NT	1/1.2/1
DXS998	q27.3	NT	1/2.2/1.2	NT	NT	1/2.3/2

Allelic data are given in the sequence father, mother, fetus; informative alleles are underlined; NT = not tested.

Table II. Parent of origin analysis of trisomy 18 and trisomy 21

Marker	Location	Case no.	
		6	9
D18S542	–	NT	3.4/1.2/1.2.3
D21S65	q21–q22.1	3.3/1.2/1.2.3	NT
D21S171	q22.3	1.4/2.3/2.3.4	NT

Allelic data are given in the sequence father, mother, fetus; NT = not tested.

Table III. Cytogenetic abnormalities in ICSI pregnancies

Case no.	Karyotype	Parent of origin
1	46,X, del(Y)(q11)/46,X,idic(Y)(q11)/45,X	pat[b]
2	45,X	pat[b]
3	47,XXY	pat[a]
4	47,XXY	pat[a]
5	45,X/46,XY	pat[b]
6	47,XX,+21	mat[a]
7	47,XY,+18	NT
8	45,X	pat[b]
9	47,XX,+18	mat[a]

Parent of origin of the additional[a] or deleted[b] chromosome; NT = not tested.

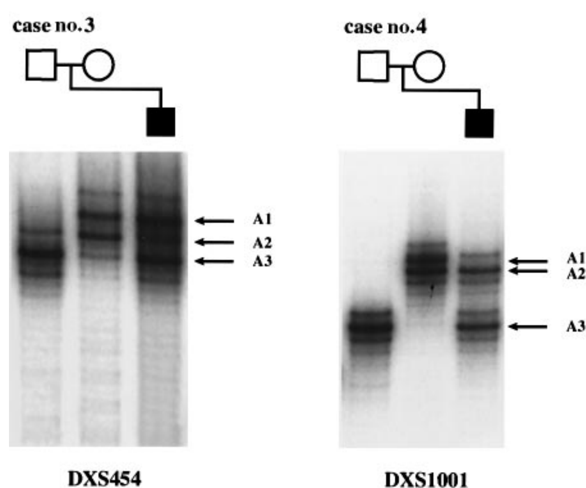

Figure 1. Autoradiograms of polymorphic repeat markers (DXS454 and DXS1001) on chromosome X demonstrating the paternal origin of the additional X chromosome in two cases of 47,XXY. In both cases one maternal (A1 in case 3 and A2 in case 4) and one paternal allele (A3) are present in the fetus. Allelic data are given in the sequence father, mother, fetus, from left to right.

the father carries allele 3, the mother alleles 1 and 2, and the fetus alleles 1 and 3. The fetus therefore contains both a maternal and paternal allele (Figure 1). It can be concluded from the marker data that the additional X chromosome in both cases of 47,XXY is of paternal origin. In both the trisomy 21 case (case 6) and the single trisomy 18 case that could be investigated (case 9), two maternal and one paternal autosome can be detected (Table II). It can thus be concluded that the supernumerary chromosome is of maternal origin.

Discussion

Chromosome abnormalities prenatally detected in ICSI pregnancies are frequently found to involve the sex chromosomes. Apart from the six cases described in the present report, five cases were found in a series of 585 prenatal karyotypes performed in the context of a large prospective study (two cases of 47,XXY, one case of 47,XXX, one case of 47,XYY and one case of a mosaic 46,XX/47,XXX) (Bonduelle *et al.*, 1994, 1995, 1996; Liebaers *et al.*, 1995). On the basis of these two series of results, which differ markedly in size and in composition of the patient group involved, a discussion has evolved about a possible increased incidence of (sex) chromosome abnormalities in ICSI pregnancies. In an initial report (In't Veld *et al.*, 1995a), a cluster of five sex chromosomal abnormalities was described on a total of 15 prenatal diagnoses performed in a group of women referred for maternal age. It was postulated that the male fertility problems that led to ICSI might in some cases be accompanied by an increased incidence of sex chromosomal abnormalities in sperm cells. This view is based on the relatively frequent occurrence of sex chromosomal abnormalities in infertile males. Chromosome abnormalties are reported to be present in peripheral blood lymphocytes in

16.6% of azoospermic males and in 6.6% of oligozoospermic males (reviewed in De Braekeleer and Dao, 1991). The 47,XXY karyotype is frequent in both groups. Meiotic division in (mosaic) carriers of such a chromosome defect may lead to hyperhaploid XY-containing spermatozoa (Cozzi *et al.*, 1994; Chevret *et al.*, 1996). In chromosomally normal infertile males an increased incidence of XY disomic spermatozoa was noted (Moosani *et al.*, 1995), possibly resulting from gonadal mosaicism not detected in peripheral blood (Persson *et al.*, 1996).

The present report extends our initial 15 prenatal diagnostic samples to 71 cases in which a total of nine chromosome abnormalities were found, of which six were of a sex chromosomal nature. If a (sex) chromosome aberration in an ICSI-derived pregnancy is due to aneuploidy in spermatozoa, the supernumerary or missing chromosome should be of paternal origin. We therefore performed a parent of origin analysis and found that all six sex chromosome abnormalities were indeed paternally derived. In the two cases of XXY the abnormality was most likely due to an error in male meiosis and related to chromosomally abnormal spermatozoa. In the four cases involving a 45,X cell line the mechanism is less clear: two cases presenting a non-mosaic 45,X karyotype most likely resulted from a paternal meiotic error, although mosaicism and post-zygotic chromosome loss can never be entirely excluded. Mosaicism is found in most patients with Turner's syndrome (Chu *et al.*, 1996) suggesting that most non-mosaic 45,X cases do not come to term. In this respect it is interesting to note that the two non-mosaic 45,X cases were characterized by severe ultrasound abnormalities early in gestation with consequent intrauterine fetal death in one case. In the two cases of mosaic 45,X a post-zygotic mitotic error is the likely causal mechanism; in case 1 this error may be due to the instability of the idic (Y) chromosome resulting in either the partial or complete deletion of the chromosome. The idic (Y) itself probably originates from an error in the paternal meiosis, or is inherited from the father in whom a gonadal mosaicism, possibly related to the oligoasthenoteratozoospermia, could not be excluded.

Although in some cases the paternal origin of the sex chromosome abnormalities is suggestive of a sperm-related origin of the defect, it should be stressed that these results are not necessarily related to the fertility problems in the male, as in half of all cases of 47,XXY encountered in the general population, the additional chromosome is of paternal origin (reviewed in Abruzzo and Hassold, 1995). Similarly, the deleted chromosome is paternal in 80% of cases with a 45,X karyotype (Hassold *et al.*, 1988). More data are therefore needed on the parent of origin of sex chromosome abnormalities in ICSI pregnancies before conclusions can be drawn as to a possible bias towards a paternal origin of the abnormality in offspring of infertile males in comparison to the general population. The finding of two maternally-derived autosomal trisomies conforms to the observation that 93–96% of all trisomies 18 and 21 are of maternal origin (Abruzzo and Hassold, 1995) and does not support a sperm-based origin of these defects. These autosomal abnormalities are more likely to be related to the maternal age of the mothers involved (32, 39 and 39 years). The second case of trisomy 18 is of particular interest as it involves the couple's second consecutive abnormal ICSI pregnancy. Both pregnancies were derived from a single batch of ICSI fertilized oocytes. In the first pregnancy a complex mosaic involving a 45,X cell line and a derivative Y chromosome was found; in the second pregnancy, in which frozen embryos were used, a trisomy 18 was detected. It should thus be concluded that the initial batch of ICSI fertilized oocytes contained at least two chromosomally abnormal embryos both of which progressed to the second trimester of pregnancy. The first case involved a paternally-derived defect, while the second case could not be analysed. Although such a repeated abnormal cytogenetic result might be coincidental it may also point to an increased risk of chromosome anomalies in ICSI fertilized oocytes or to a defect in post-implantation selection mechanisms.

The 12.7% chromosome abnormalities in the present series is an order of magnitude higher than the ~1% reported in the Brussels study (Bonduelle *et al.*, 1996). However, comparison of these incidences is hazardous as the latter is a prospective study involving consecutive ICSI pregnancies from a single centre while the present series consists of a selected population referred for prenatal diagnosis and involves five different ICSI centres. It is evident that a reliable estimate of the frequency of chromosome abnormalities can only be derived from large prospective studies. Since major differences in methodology exist between centres (Feichtinger *et al.*, 1995), and patient selection criteria are not standardized, such studies should encompass multiple centres. In this respect the patient selection criteria are of particular importance as the various sub-types of male infertility are characterized by different types and frequencies of genetic abnormalities. In particular the sperm count taken as a guideline for referral for ICSI may be of importance. As some genetic risks like chromosome aberrations in infertile males are linked to the degree of oligozoospermia (Chandley, 1984), it cannot be excluded that differences in patient selection criteria may lead to differences between centres in the frequency of chromosome aberrations found in ICSI offspring.

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