Sperm analyses, genetic counselling and therapy in an infertile carrier of a supernumerary marker chromosome 15

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Abstract

Purpose: A supernumerary marker chromosome (SMC) was analysed after lymphocyte culture of a patient with oligoasthenoteratozoospermia (OAT) before ICSI treatment.

Material and methods: By additional molecular cytogenetic investigations the marker could be identified as a heterochromatic derivate of chromosome 15 [karyotype: 47,XY,+der(15)].

Results: Sperm analyses by interphase FISH showed a normal monosomy 15 in 82% and an additional marker in 17% of the cells. In spite of these findings a pregnancy could not be induced. The brother of the patient showed the same chromosome abnormality and an OAT-syndrome as well.

Conclusions: ICSI-treatment lead to a normal pregnancy and to the birth of a healthy boy. The genetic risk factors of both marker carriers are analysed in detail.

Key words: supernumerary marker chromosome (SMC), derivate of chromosome 15, fluorescence in situ hybridization (FISH), infertile males, oligozoospermia, ICSI-treatment.

Introduction

Chromosome aberrations play an important role in the aetiology of male infertility. Among infertile males, the increase is about 10-fold compared to the general population [1,2]. These abnormalities include numerical changes of the sex chromosomes but frequently balanced sex chromosome and autosome rearrangements also, such as the translocation 13/14 or supernumerary heterochromatic marker chromosomes [3].

Here, we report the case of a patient and his family with a supernumerary marker chromosome (SMC) which was identified as a derivate of chromosome 15. Investigations of somatic cells could be combined with sperm analyses thus allowing a better risk estimation in genetic counseling of the couple.

Material and methods

Clinical and andrological findings

An infertile couple who had wished for children for 10 years, attended the Section of Endocrinology and Reproduction at the Department of Obstetrics and Gynecology of the University of Bonn for further diagnosis and therapy.

Both partners were healthy. Exposition to mutagenic agents could be excluded. The husband was 41 years and his wife 38 years old at the time of examination. The wife had undergone an operative laparascopy 8 years ago, where peritubal adhesions on the left side were removed and minimal endometriosis was diagnosed. Hydropertubation showed that both Fallopian tubes were patent. Apart from a slight luteal insufficiency, all other gynaecological and endocrinological parameters were normal in the woman. The husband was repeatedly diagnosed in the past with mild to moderate oligoasthenoteratozoospermia (OAT). In the first semen analysis at the andrological laboratory of our institution, the sperm count was 12x10^6/ml, sperm motility was 40% and less than 5% of the spermatozoa showed a normal morphology. The diagnosis was confirmed in a second investigation 9 months later (*Tab. 1*).

Cytogenetics

Conventional chromosome analysis in lymphocytes Chromosome analyses of the couple were performed on metaphase chromosomes from cultured peripheral blood lymphocytes using standard banding techniques (QFQ-, GTG-, RBA-, CBG-banding, NOR and DA/DAPI staining). The woman

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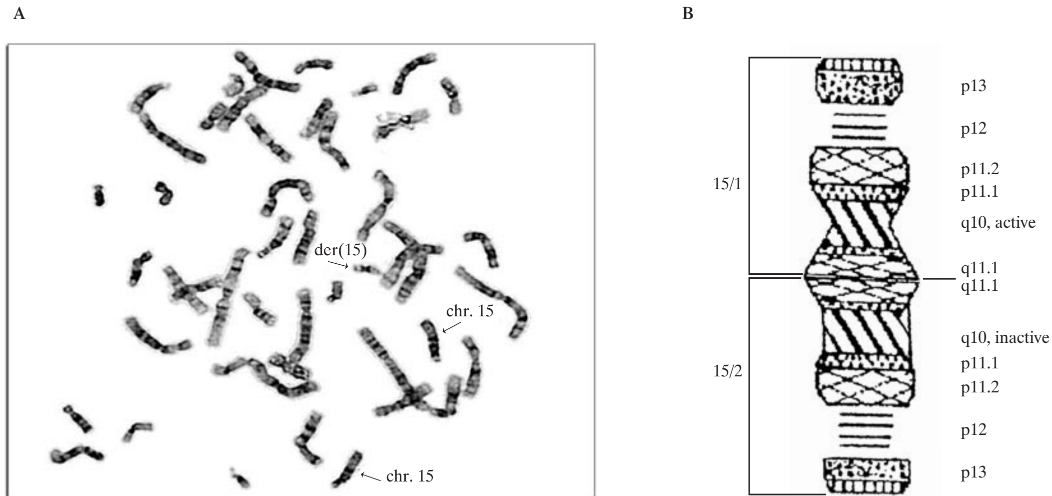
Table 1. **Semen parameters in the patient with oligoasthenoteratozoospermia (OAT)**

Semen parameters	Semen analysis 1	Semen analysis 2	Normal Range (WHO)
Volume	3 ml	4 ml	≥2 ml
Concentration	$12 \cdot 10^6$/ml	$10 \cdot 10^6$/ml	$\geq 20 \cdot 10^6$/ml
Motility	40%	20%	>50%
Normal morphology	<5%	<5%	>30%

Figure 1. **a) Karyotype showing the two normal chromosomes 15 and the der(15): 47,XY,+psu dic(15;15) GTG; b) Ideogram of the SMC(15)**



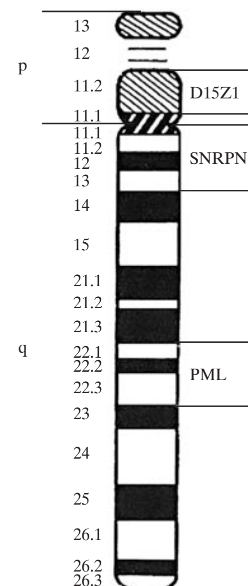
had a normal female karyotype (46,XX). The husband showed a supernumerary marker chromosome (SMC) in all metaphases (n=50). The marker was bisatellited, dicentric and smaller than chromosome 21 (*Fig. 1*). Analysis of the structure of the short arm regions showed, that the marker was not isodicentric, but originated from chromatid exchanges from two homologous chromosomes 15 (15p11.2, p12, p13 were of different size). The karyotype could be delineated as 47,XY,+der(15).

Family investigation The patient had two siblings, a brother and a sister, both of them healthy. The sister had 3 healthy children whereas in the brother an OAT syndrome was diagnosed as in our patient. Chromosomes were investigated in the siblings and the parents of the patient. While the sister and the father showed normal karyotypes, the brother and the mother had the same supernumerary marker chromosome as the patient. The 38 year-old brother who showed an OAT syndrome received chemotherapy 10 years ago after the diagnosis of a carcinoma testis. No data were available on his chemotherapy. Therefore, today the role of the marker chromosome and the chemotherapy as causes of the OAT syndrome cannot be determined.

The five siblings of the mother could not be examined cytogenetically, but the following peculiarities were noted: one of the brothers had no children in two marriages and one of the sisters none in one marriage. Moreover, one maternal brother was mentally retarded, and one sister died in early infancy.

FISH analyses For further analysis and for exclusion of euchromatic material in the marker chromosome, fluorescence in situ hybridization (FISH) was performed with the commercially available SNRPN/D15Z1/PML probe (Vysis, Downers Grove, IL, USA; *Fig. 2*). The FISH procedure was performed according to manufacturer's instructions. The slides were coun-

Figure 2. **Localisation of the FISH DNA probe set SNRPN/ D15Z1/PML**



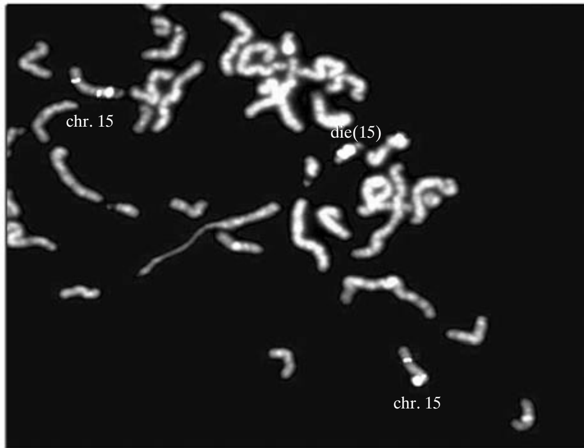


Figure 3. **FISH analysis of lymphocytes with the probe SNRPN/ /D15Z1/PML. Karyotype: 47,XY,+psu dic(15;15).ish psu die(15) (q11.1; q11.1)(D15Z1++,SNRPN-,PML-)**

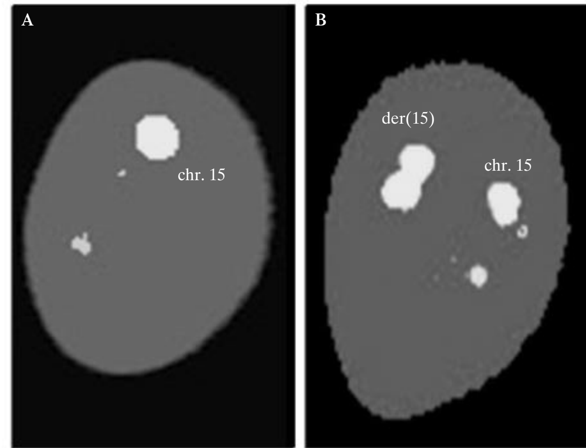


Figure 4. **Sperm nuclei of the patient with der(15) after FISH: A) one chromosome 15; B) one chromosome 15 and one der(15)**

terstained with DAPI/antifade. The probe SNRPN/D15Z1/PML labelled on the 2 normal chromosomes 15 in the three specific regions. The derivate showed two green signals for the D15Z1 region (*Fig. 3*). A euchromatic signal was absent. Thus, the SMC was determined as a heterochromatic dicentric marker derived from chromosome 15 [Karyotype: 47,XY,+psu dic(15;15).ish psu dic(15)(q11.1;q11.1)(D15Z1++,SNRPN-,PML-)].

Sperm preparation and interphase FISH

Methods Semen samples of the consultant and 10 control persons were analysed. The controls were fertile men of a matched age group [4]. The samples were washed three times in phosphate-buffered saline (PBS) and spinned at 1200 rpm for 10 min. The sediments then were fixed in methanol/acetic acid (3:1) and stored at -20°C until further processing. After thawing, the fixed semen samples were washed twice in fresh methanol/acetic acid (3:1), spread on clean glass slides and allowed to dry on air. To render the sperm chromatin accessible to DNA probes, the slides were treated with dithiotreitol (DTT). They were incubated for 3 min in 25 mM DTT/1 M Tris (pH 9.5) at room temperature. After decondensation, they were washed once for five minutes in 2x standard saline citrate solution (SSC), dehydrated through an ethanol series (70-90-100%) and air-dried. Each slide was denatured with 3M NaOH solution at room temperature for 3 min. They were then immersed in a 70, 90, and 100% ethanol series for 3 min each and air-dried. In the semen sample of the patient, interphase FISH was performed with the SNRPN/D15Z1/PML probe (*Fig. 4*) according to the manufacturer's instructions. In the control persons' probes, the FISH was carried out with the CEP 15 probe (Qbiogene).

The slides were examined using a diaplan-fluorescence microscope (LEICA) with the appropriate filter sets: single band pass filter (Aqua, FITC, TRITC) and a triple band pass filter (DAPI/ FITC/TRITC). Only samples with a hybridization rate of >99% were analysed. 5000 sperm nuclei were scored in each control person and 1000 in the patient. Inclusion criteria were the intact spermatozoa with a significant decondensation, and the hybridization signals had to be clear.

Table 2. **Sperm analysis by interphase FISH with probes SNRPN/D15Z1/PML in the patient with additional SMC(15) and in controls with the probe CEP 15**

Signal combinations	Patient (%)	Controls (%)
Monosomy 15	82.70	99.83
Monosomy 15 and der(15)	17.00	-
Disomy 15 and der(15)	0.20	-
Disomy 15	0.10	0.13
Nullisomy 15	0.00	0.04

Statistical analysis The data were analysed with the χ^2-test. The Statistic Package for Social Sciences (SPSS) version 11.0 for Windows was used for statistical calculation. A significant statistical difference was assumed when the P-value was lower than 0.05.

Results

Controls

50000 sperm nuclei from 10 healthy men of the same age group with normal fertility as the patient were analysed by FISH with the CEP 15 α-satellite-DNA probe (Qbiogene). The disomy rate for chromosome 15 was 0.13% and the nullisomy rate was 0.04% (*Tab. 2*).

Patient

1000 sperm nuclei were analysed after hybridization with the chromosome 15 probes SNRPN/D15Z1/PML (*Fig. 3*). In 82.7% of the sperm, there was the combination of three signals for one chromosome 15 per nucleus corresponding to a monosomy 15 (*Fig. 4a*); 17.0% showed one chromosome 15 and the additional marker chromosome (*Fig. 4b*); 0.2% of the nuclei had a disomy 15 and the additional marker, and finally 0.1% showed a disomy

Table 3. **Genetic risk estimation of fetal chromosome syndrome for the consultant with additional marker chromosome 15 and for his wife with increased age at pregnancy**

Maternal:	Increased age of 37/38 years at time of ICSI therapy Risk of aneuploidy 1:100
Paternal:	The SMC has a higher risk of meiotic error. This can lead to: • Uniparental disomy (UPD) 15 following trisomy 15 rescue Phenotype: Angelman syndrome • Trisomy 15 mosaicism due to incomplete trisomy rescue of the aneuploid zygote • Unequal crossing over in paternal meiosis can lead to: – duplication 15 q11.2 => trisomy 15 q11.2 syndrome – deletion 15 q11.2 => Prader-Willi syndrome
	Low risk estimation (less than 1%) for a pathologic development of the embryo due to the marker chromosome
Recommendation:	Prenatal diagnosis

for chromosome 15. Nuclei with a nullisomy for chromosome 15 were not observed (*Tab. 2*).

Comparing the data of our patient with those of the control group, there was a highly significant increase of sperm nuclei with disomy 15 (0.3 vs 0.13%; $p<0.001$), whereas there was no difference for the nullisomy rate between the patient and controls.

In the spermatozoa examined, the additional marker was only observed in combination with one or two normal chromosomes 15.

Genetic counselling and risk estimation

The couple asked for genetic counselling and interpretation of the chromosome findings. Different risk factors had to be taken into account (*Tab. 3*).

The increased maternal age of 38 years bears a higher aneuploidy risk of 1:100 compared to 1:500 in a 25 year-old woman. On the paternal side, the der(15) raises the risk for nondisjunction of chromosome 15. After fertilization, a trisomy rescue mechanism can lead to uniparental disomy (UPD) 15 with imprinting defects or, in the case of incomplete trisomy rescue, to a mosaic trisomy 15. Moreover, unequal crossing over of a normal chromosome 15 with the SMC(15) during male meiosis can result in a duplication or deletion of 15q11.2 in the normal chromosome 15 or in an insertion of euchromatic material in the marker. The duplication is associated with a trisomy 15q11.2 and the deletion with a Prader-Willi syndrome (PWS), but both types of chromosome aberrations are rare events. Despite the combination of maternal (maternal age) and paternal risk factors [SMC(15)], the genetic risk for the offspring in case of a successfully induced pregnancy could be regarded as low (*Tab. 3*).

Infertility treatment

The couple underwent two in vitro fertilization treatments which were combined with intracytoplasmic sperm injection because of reduced sperm quality. In the first ICSI-attempt, two oocytes were retrieved after ovarian hyperstimulation, but due to fertilization failure, no embryos could be transferred. In the second treatment cycle, sixteen oocytes could be obtained and four oocytes were fertilized. Two embryos were transferred to the uterus but no pregnancy could be achieved. The remaining two oocytes were frozen in the two-pronuclear stage, thawed after nine months, cultured and then transferred, again without resulting in a pregnancy.

The patient's brother and his wife were also treated with ICSI. Ten oocytes were obtained after ovarian stimulation, eight of them could be fertilized, six were cryopreserved, and two embryos were transferred without inducing a pregnancy. Three oocytes in the two-pronuclear stage were then thawed and transferred, but again failed to induce a pregnancy. The second transfer with three cryopreserved two-pronuclear stages resulted in an implantation, and a healthy boy was born after an uneventful pregnancy.

Discussion

Supernumerary marker chromosomes (SMC) show a frequency of 1:1 000 in unselected populations. About 50% of them are heterochromatic and thus harmless for all carriers. Derivates of chromosome 15 are the most frequent marker chromosomes with about 40% of all SMC. About 50% of the heterochromatic markers are familial in origin [5,6]. The inheritance occurs preferentially in females. In males the either get lost in the majority of germ cells or lead to infertility as in the family presented here. Several studies reported a higher incidence of SMC(15) in infertile males with oligo- or azoospermia. In contrast, females usually show a normal fertility, which is the reason for the prevalence of maternal inheritance of the familial SMC [7-9].

The patient described here is a carrier of a familial heterochromatic der(15), which is associated with an OAT-syndrome and infertility. We performed dual-colour FISH analysis of sperm to determine the segregation ratio of the SMC(15) during spermatogenesis. Theoretically, there should be a 1:2 ratio for the SMC, but only 17.2% of 1 000 sperm nuclei analysed carried the SMC(15). This segregation ratio of almost 1:5 reflects a selection process against the marker during sperm development. Spermatogenetic failure occurs during prophase of meiosis I, where the marker chromosome preferentially associates with the XY-bivalent, leading to an early disruption of spermatogenesis [10]. This partial spermatogenetic arrest may be the reason for the reduced fertility found in our patient.

The lack of larger study data on segregation ratios of SMC during male meiosis does not allow the generalization of our observations of meiotic inheritance.

The difference in the success of the ICSI treatment in the 2 brothers can be caused by different transmission factors.

First, the age of the wife of our index patient was significantly increased compared to his sister in law.

Secondly, the genetic background in the 2 couples is different and may thus lead to differences in the success of the ICSI-therapy.

Conclusions

A familial supernumerary marker chromosome in 2 brothers with OAT syndrome and maternal inheritance could be delineated by the combination of chromosome and FISH-analyses as a constitutive heterochromatic derivate of chromosome 15. The marker was dicentric with stable inactivation of one centromere and originated from an exchange between two homologue chromosomes 15. Sperm analyses by interphase FISH showed a normal disomy rate for chromosome 15 and a proportion of the additional marker of 17.2%. ICSI-therapy was unsuccessful in the patient but lead to the birth of a healthy boy in his brother.

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