Supernumerary Asymmetric Dic(15;15) With Secondary Mosaic Formation in One of Two Developmentally Retarded Twins[*]

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ABSTRACT Two male twins born after IVF therapy were investigated for 9 years. Lymphocyte culture at the age of 1 and 9 years revealed that one twin (P1) carried a supernumerary asymmetric dic(15;15) which was present in 100% of cells. In buccal mucosa, however, the derivative 15 (der(15)) was lost in 12% of cells. The der(15) was maternal in origin and was determined to be a meiosis I abnormality. The asymmetric structure of the der(15), as demonstrated by GTG banding and microarray analysis, resulted in a tetrasomy for the region 15q11q13.2 and trisomy for 15q13.2q13.3. Both regions were interrupted by a disomic fragment of 514 kb; this region has been published as a CNV. Monosomy for this CNV was confirmed in the mother, along with a second CNV in the distal breakpoint region in 15q13.3. The patient had a second structural aberration, namely an almost complete deletion of one of the two centromeres in the der(15), such that it was not detected by metaphase or interphase FISH with the DNA probe D15Z4. The conventional karyotypes of the parents and the second twin (P2) were normal. The second twins retardation was obviously caused by an infection at the age of 4 weeks, followed by multiple organ failure. Twin P1 received special support from birth to the age of 9 years and has developed better than was to be expected from findings in the literature, while his twin brother failed to show developmental progress.

INTRODUCTION

Supernumerary marker chromosomes occur with a frequency of 1/1000 in the general population. However, the incidence is 2.88-3.3 per thousand among patients with mental retardation (Buckton et al. 1985; Liehr and Weise 2007). The majority of these markers are derivatives of the acrocentrics, and of these, more than 40 percent are derived from chromosome 15 (for review: Eggermann et al. 2002). The distribution of supernumerary marker chromosomes is similar in familial and de-novo cases (Pätzold et al. 2006).

About 70 percent of markers are stably main-tained in mitosis and thus are present in all cells in somatic tissues, whereas 30 percent are subject to nondisjunction errors and may be lost in mitosis, resulting in mosaicism (Crolla et al. 2000).

Marker chromosomes often arise through inverse duplications, and this may also be the causing principle of dicentric derivatives of chromosome 15 (Battaglia 2005; Wang et al. 2008). They are usually bi-satellited with one inactivated centromere. The phenotype and the developmental prognosis for the carrier depend on the amount of euchromatic material between the two centromeres.

Here we present a pair of twins born after IVF. Both boys showed evidence of developmental retardation, but only one of them carried a chromosomal aberration. Clinical, cytogenetic and molecular findings are given and the childrens' development is documented over a period of 9 years. We describe the consecutive investigation steps, from chromosome analysis to microarray, which enabled us to precisely characterise the additional euchromatic mate-

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rial, the breakpoints and their partial associa-
tion with a maternal CNV.

CLINICAL REPORT

The two boys presented here were born after
IVF treatment because of tubal ligation. Three
elder sibs, two boys and a girl (now 15, 17 and 18
years old, respectively) were born as a result of
spontaneous pregnancies and show normal, age
appropriate development. The parental age was
30 years for the mother and 31 years for the fa-
ther at the time of the IVF-induced pregnancy.

Pregnancy

Normal pregnancy course, ultrasound exams
in pregnancy weeks 12+6, 16+4, 20+1. The twins
were dichorionic and diamniotic.

Birth and Postnatal Findings

Delivery: Caesarean section in week 39, twin
P2 was born first. Apgar 9/10 for both boys.

Biometrics

Data for twin 1 with the chromosomal aberra-
tion is shown in Table 1. The biometric data were
in the lower normal range at birth, later falling
below the 3rd percentile for length, weight and
head circumference. Maternal height was 174 cm,
paternal height was 178 cm. The three sibs were
within the normal range for their ages (male, 15
years, 168 cm; male, 17 years, 185cm; female, 18
years: 169 cm).

CLINICAL FINDINGS

Malformations, dysmorphisms and develop-
mental peculiarities are documented for the pa-
tient P1 and compared to findings for tetrasomy
15q12 in the literature (Tables 2, 3). At the age of
1 and 2.5 years, the patient P1 exhibited the fol-
lowing symptoms which are in good accordance
with the literature: Down-slanting palpebral fis-
sures, epicanthus, high arched palate, frontal
bossing, broad nose, crowded teeth, growth re-
tardation, short attention span (Table 3, Fig. 1).
In addition to these abnormalities, both twins
showed further clinical symptoms:
Twin P1: Hypotonia at birth, hyperflexation

**Table 1: Biometrics of twin carrying the supernume-
rary dic (15;15) from birth up to the age of 9 years**

Age (years)	Twin P1 Length (cm)	Weight (kg)	OFC (cm)
At birth	50.0 (P25)	3.3 (P25)	35.5 (P50)
1	73.0 (P10)	8.3 (<P10)	43.0 (<P3)
2	80.0 (<P3)	9.7 (P3)	47.0 (<P3)
4	93.0 (P10)	12.5 (<P3)	49.0 (<P3)
5	98.0 (<P3)	14.0 (<P3)	49.0 (<P3)
6	101.0 (<P3)	16.5 (<P3	50.0 (<P3)
7	103.5 (<P3)	18.5 (<P3)	50.5 (<P3)
8	113.5 (<P3)	21.0 (<P3)	51.0 (<P3)
9	120.0 (<P3)	23.0 (<P3)	51.0 (<P3)

of joints, dolichocephaly. His mental and motor
development is better than was to be expected
according to the literature.
Twin P2: Normal findings at birth. Following
an infection at the age of 4 weeks there was a
complete failure of further development. His cli-
nical symptoms were also compared to findings
for triplication 15q12 in order to exclude an ad-
ditional chromosome aberration in twin P2.

Table 2: Development of twin P1.

Age	Twin P1
at birth	muscular hypotonia, sucking weakness
1 year	muscular hypotonia, severe motor and developmental delay (DQ 64), macroce-phaly
2.5 years	hyperactive, autoagression, short atten-tion span, sleeping disorder, 3 words other than "dad" and "mama", needs perma-nent supervision, short stature, mental retardation with autistic behavior, inse-curity when walking
5 years	ataxia, restlessness (therefore DQ not determinable)
7 years	special logopedic and ergometric thera-py; special school: needs permanent in-tegration help. Not interested in teach-ing, no concentration, no motivation
9 years	friendly attention, insists on constant course of actions, no recognition of dan-ger in daily life, no social contacts, visits special school where he must be indivi-dually attended at all times (DQ ~45)

Diagnosis of a well-defined chromosome
syndrome during early infancy in twin P1 resulted
in a specific program of permanent support for
the boy, monitored by regular developmental
tests. The success of these measures became
possible only by the unique support and ac-
ceptance of the child in his family. The special
school the child attends also affords him very
specific and intensive attention. This advanta-
geous combination of circumstances has obvi-
ously promoted the childs development.

Table 3: Clinical symptoms in tetrasomy 15q12 from the literature (Schinzel et al. 2001) compared to the findings in our patient P1. (() symptom only slightly expressed)

Feature	Twin P1
Pregnancy	normal
Birth biometry	normal
Facial Dysmorphisms	
Downslanting palpebral fissures	(+)
Enophthalmus	(+)
Epicanthic folds	(+)
Strabism	-
High arched palate	+
Cleft palate	-
Dysmorphic ears	-
Limbs	
Partial syndactyly of toe II/III	-
Clinodactyly V	-
Brachydactyly	-
Hip luxation	-
Talipes	-
Simian crease	-
Rare Symptoms	
Brachycephalia	-
Frontal bossing	+
Facial asymmetry	-
Broad nose	(+)
Synophris	-
Short philtrum	+
Crowded teeth	+
Prominent mandibula in adults	small chin
Malformations of Internal Organs	
VSD	-
Umbilical and inguinal hernia	-
Unilateral renal agenesis	-
Genital Anomalies	
Hypospadia	-
Cryptorchism	-
General Abnormalities	
Growth retardation	+
Macrocephaly	
Severe to profound mental retardation	DQ~45 (9 years)
Personality Disorders and Behavioural Abnormalities	
Hyperactivity	+
Aggresivity	-
Self mutilation	-
Short attention span	+
Tremor	-
Ataxia	-
Stereotypic movements	-
"Parroted speech"	-
Autism	-
Seizures (resistent to antiepileptic treatment)	-
Spasms	-

Developmental Peculiarities

EEG and Cranial MRI

The patient was 4 years old at the time of assessment. A sleep EEG was performed and showed normal results with age-appropriate theta activity, normal sleep changes (???), no signs of increased propensity for seizures.

The MRI revealed an asymmetrical brain configuration, particularly of the cerebellum and favoring the right hemisphere. Apart from this asymmetry there were no irregularities, specifically no signs of a tumour or larger malformations.

Assessment at 10 Months of Age

Griffith´s developmental test scores were as follows:
Locomotor: DQ 65; Personal-Social: DQ 77; Language: DQ 77; Eye and Hand Co-ordination: DQ 61; Performance: DQ 77. The total developmental score was 71.

Assessment at 4 Years of Age

Social Behaviour: very restless; lacked concentration; insisted on unvarying sequences of activities; acted in an "infantile" manner when refusing to perform given tasks

Locomotor Skills: conspicuous tip-toe gait with satisfactory posture and straight torso; motor uncertainty and jerky movements, particularly leading into a motion or when modifying a movement in mid-stride

Muscle reflexes are normal, reduced muscle tone is evident; hyperextensible joints

Assessment at 8 Years of Age

Social Behaviour: complete lack of independent initiation of contact; little impetus; lack of stamina; easily distracted; not yet dry
Locomotor Skills: awkward movements

Assessment at 9 Years of Age

Social Behaviour: relatively long co-operative phases; expressed interest in various learning offers
Locomotor Skills: continuously improving; used bathroom independently
Language Skills: The patient began to develop language skills at age 4, and at age 5 was able to speak 5 words. At 6 years it was ascertained that the patient has a phonetic-phonological disorder.

During logopedic testing the following anomalies were observed: omission of unstressed syllables; omission of final consonants; reduced

Fig. 1a) **Twin 1**

Fig. 1c) **Twin 2**

1 year

2.5 years

Fig. 1b)
Twin 1

5 years

7 years

9 years

Fig. 1. Phenotypes of the two handicapped twin boys at ages 1 and 2.5 years (Fig. 1a, 1c). Clinical picture of twin 1 at 5, 7, and 9 years of age (Fig. 1b). (twin 1: dic(15;15) carrier, twin 2: multiple organ failures at the age of 4 weeks)

multiple consonants. Some instances of non-physiological replacement processes were observed, such as alveolarisation, velarisation, plosivation, deaffrication and lenition. Automatic mouth closure was not present and the tongue generally was in a non-physiological interdental resting position.

At 9 years the patient's speech had improved and he was able to recognize individual letters.

CYTOGENETIC AND MOLECULAR INVESTIGATIONS

Materials and Methods

Peripheral blood samples were obtained from the patient (aged 1 and 9 years), from his twin brother and from his parents. Chromosomes were prepared by standard techniques. Cytogenetic analyses included conventional GTG, QFQ and CBG banding and NOR staining. Chromosome investigations were performed on 50 mitoses per proband and at a banding of at least 550 bands per genome.

In addition, buccal mucosa was prepared from the patient and his twin brother. FISH investigations were performed on metaphase spreads and interphase cells and on cells from buccal mucosa after direct preparation.

The following FISH probes were applied: wcp15, D15Z1(p11), D15Z4(15cen/q10), D13Z1/D21Z1, D14Z1/D22Z1, SNRPN (Abbott/Des Plaines, USA; Kreatech/Amsterdam, NL).

The parental origin of the der(15) in the patient was analysed by short tandem repeat (STR) typing.

The DNA of twin P1 was further investigated for copy number changes using a high-resolution genomic array containing approximately 1.8 Mio. DNA oligonucleotide probes der(15) (GenomeWideSNP_6, Affymetrix, High Wycombe, UK). DNA processing, hybridisation and washing was performed according to the manufacturer's instructions. Arrays were recorded using an Affymetrix GeneChip®Scanner 3000 7G. Data processing including quality assessment was performed using the "R" statistical framework (http://www.r-project.org) with dedicated extensions from the "aroma.affymetrix" project (Bengtsson et al., 2008)[http://aroma-project.org]. Copy number segmentation results were visualized using tools developed for the Progenetix project [http://www.progenetix.net] (Baudis et al. 2001).

RESULTS

Patient

Conventional chromosome analysis revealed a supernumerary marker in 100% of the mitoses. It was characterized as dicentric with one inactivated centromere, asymmetric and containing 2 short arm regions by different banding techniques. Thus, the marker exhibited the typical morphology of an acrocentric chromosome.

Hybridisation with wcp15 resulted in positive staining in the euchromatic long arms of both normal chromosomes 15 and in the region between the two centromeres of the marker. The marker was thus defined as a dicentric derivative of chromosome 15 (Fig. 2).

Additional hybridization with D15Z1 (15p11) and D15Z4 (15q10) exposed unexpected peculiarities. While both probes showed the usual number of signals for the normal chromosomes 15, the derivative 15 had only 1 signal for each probe.

Probes D13Z1/D21Z1 and D14Z1/D22Z1 were used in order to exclude a translocation with a heterologous acrocentric chromosome. The normal chromosomes 13, 14, 21 and 22 each showed the expected signals, while the der(15) was negative for these probes (Fig. 2).

These findings confirmed that the marker consisted of DNA from chromosome 15. Interphase analyses of the lymphocytes showed a fraction of cells with 3 distinct and one very small fourth signal after hybridization with D15Z4. In this situation, a deleted region cannot be differentiated from cross hybridization. Another 150 nuclei from buccal mucosa were analyzed. A mosaic was detected, with 12% (18/150) of the cells showing only the 2 FISH signals expected in normal cells. Here also, in the cells with the marker, just as in the lymphocytes, only 3 signals were detected.

Short tandem repeat (STR) typing confirmed the maternal origin of the marker. The allelic distribution pointed to a maternal meiosis I error as the initial event in marker formation. Furthermore, we were able to show dizygosity of the twins by typing of four STRs from other chromosomes than 15.

Microarray typing of the patients´ DNA confirmed the asymmetric structure of the marker. It gave rise to tetrasomy of 15q for the region 15q11.1q13.2 (18,276,242-28,173,704; 9.9 Mb) and to trisomy for the region 15q13.2q13.3 (28,688,171-30,299,513; 1,6 Mb) (Fig. 3). Interestingly, both

a)

Chr.15   der(15)

b)

c)
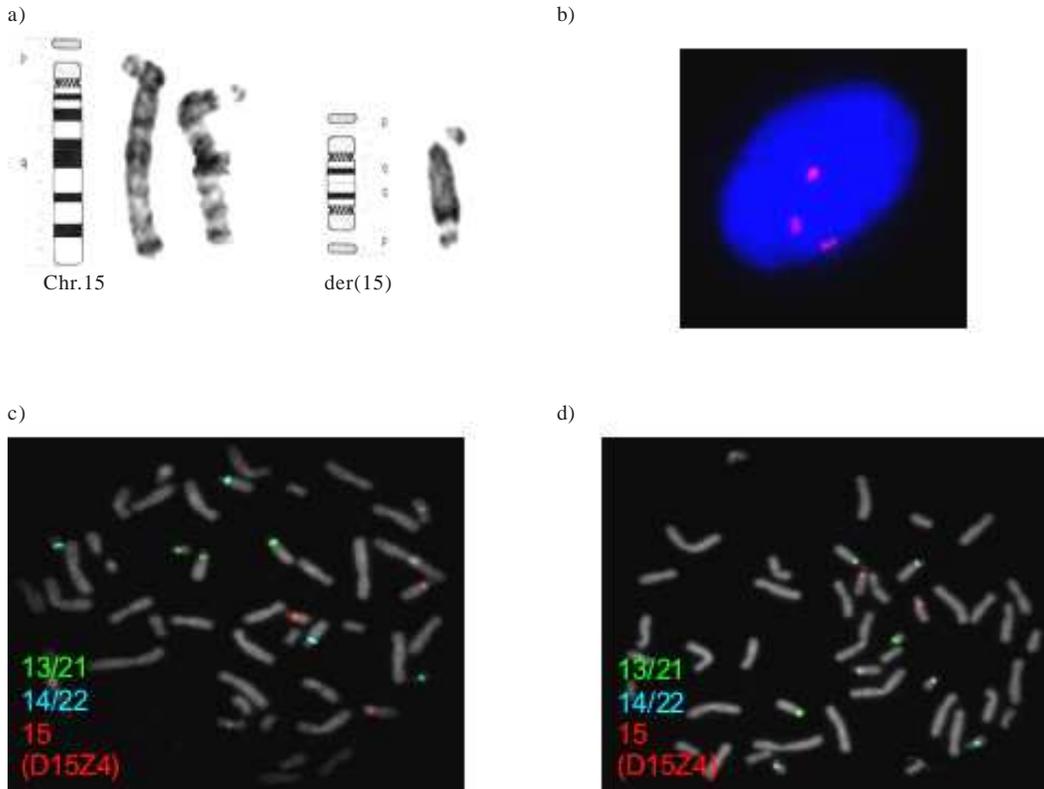

13/21
14/22
15
(D15Z4)

d)

13/21
14/22
15
(D15Z4)

Fig. 2. a) Derivative chomosome 15 after GTG banding, compared to the normal chromosomes 15 of patient P1. b) Interphase FISH in buccal mucosa cell of patient P1 with chromosome 15 á-satellite DNA-probe (D15Z4) showing three signals due to the deletion of á-satellite DNA in one centromeric region of the dicentric marker. c) Metaphase FISH with á-satellite DNA-probes of the acrocentric chromosomes in the patient P1, and d) his mother.

regions were interrupted by a disomic fragment of 514 kb; this region has been published in the databases as a copy number variation. Monosomy for this CNV was confirmed for the mother (Fig. 3), along with a second copy number variation determined in the distal breakpoint region (15q13.3; 30,318,609-30,690,276; 372 kb).

Through combination of cytogenetic and molecular genetic findings, the patient's karyotype has thus been delineated as follows:

47,XY,+mar.ish dic(15;15)(D15Z4+,D15Z1, wcp15+);arr15q11.1q13.2(18,276,242-28,173,704)x4/15q13.2q13.3(28,688,171-30,299,513)x3.

Twin Brother of the Patient

Chromosome analysis revealed a normal karyotype (46,XY). FISH with probes D15Z1 and

D15Z4 confirmed that both chromosomes 15 had the normal number of hybridisation signals.

Because of the child's severe developmental retardation the probe SNRPN was used to examine the Angelman region. Two normal-sized signals were diagnosed in 20 mitoses, thus permitting us to exclude a microdeletion.

FISH analyses of buccal mucosa cells with the probe D15Z4 confirmed the normal number of signals in all interphases. There were no signs of a mosaic in this second cell system (145 nuclei analysed: 143 with 2 signals, 2 with 1 signal).

Mother of the Patient

The mother's karyotype was normal as analysed in 50 mitoses (46,XX). FISH with the DNA probe D15Z4 showed 2 normal signals in both chromosomes 15. STR typing proved the
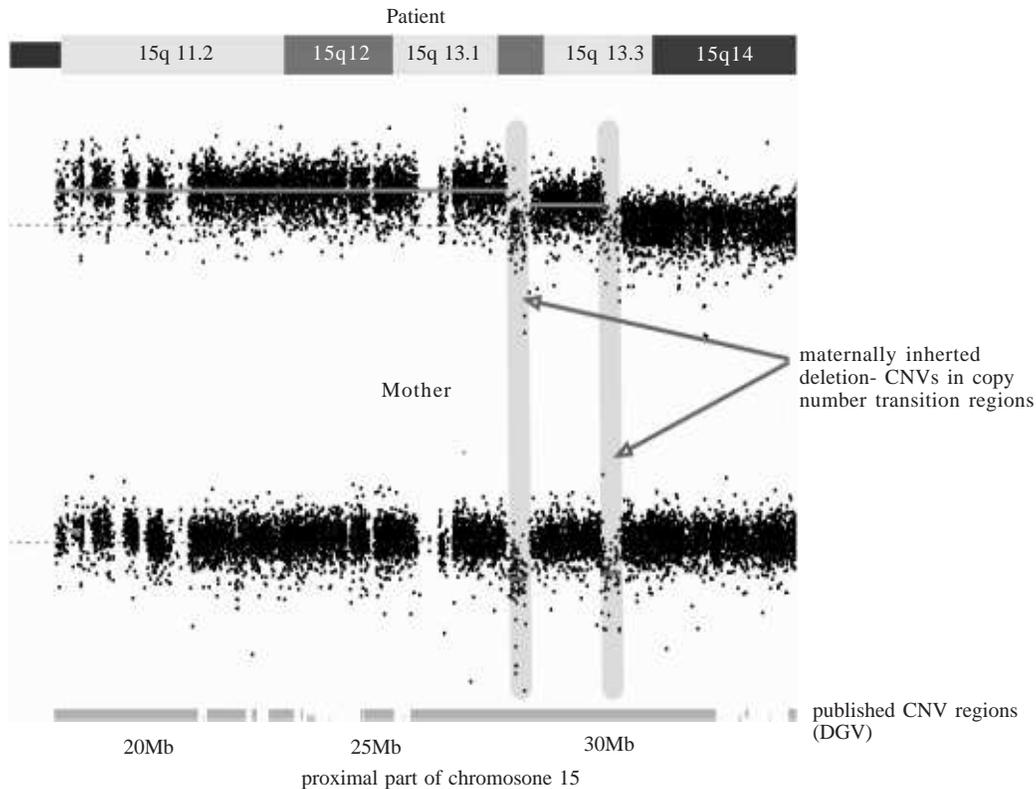
Fig. 3. Local Affymetrix Genotyping 6 signal distribution pattern and segmentation result in patient P1. Tetrasomy of proximal 15q11.2 to 15q13.2 and triplication of 15q13.2 to 15q13.3 was observed in the sample. Both regions are interrupted by a disomic fragment. This region is known as a CNV. This CNV was confirmed in the mother.

maternal origin of the marker. High resolution array analysis demonstrated a copy number variation at the distal breakpoint region of the marker. The monosomy of his CNV has been published in the data bases (514 kb).

Father of the Patient

The father´s karyotype was normal (46,XY) in 50 analysed mitosis. A paternal contribution to the formation of the marker was excluded through STR typing.

DISCUSSION

Cytogenetic investigations were performed in two dizygotic twin brothers at the age of one year, one with a moderate (P1), the other with a severe global retardation (P2). Chromosome analysis of twin P1 revealed an additional der(15)

in all cells of a lymphocyte culture and in 88% of buccal mucosa cells. Twin P2 had a normal karyotype. His retardation was traced to an exogenous event in the newborn period.

Further analyses of the marker chromosome showed that it was asymmetric. The genome showed tetrasomic, trisomic and disomic elements. Additionally, one of the 2 centromeres in the marker was so excessively deleted that it was not detectable by the 15cen (D15Z4) and 15p11 (D15Z1) probe. The distal breakpoint of the maternally inherited marker in 15q13.3 was identical to a maternal CNV. The development of twin P1 up to the age of nine years was better than it could have been expected from comparisons with reports in the literature. Two factors might be responsible: (1.) the der(15) was not tetrasomic in all segments and a low-grade mosaic with a normal cell line was present in the second cell system investigated; (2.) the child

experienced consistent and intensive support through his family.

Different genomic peculiarities affecting the maternal chromosomes 15 lead to the complex rearrangements of chromosome 15 in the patient. They involve different euchromatic and hetero-chromatic regions. In the maternal meiosis I an abnormal pairing of the two homologous chromosomes 15 occurred with U-type fusion of two chromatids and an exchange in the CNV in 15q13.3. 3:1 malsegregation resulted in a germ cell containing one normal chromosome 15 and the derivative 15. The karyotype of the zygote was then 47,XY,+dic(15;15). In the course of postzygotic cell divisions the marker was lost in a small proportion of cells.

Interestingly, the tetrasomic, the trisomic, and the disomic segments of chromosomes 15 were interrupted by polymorphic CNVs. Both these CNVs and the imbalances were assigned to the maternal genome, making a functional relation feasible. Indeed, CNVs can contribute significantly to genome instability and can lead to deletions, duplications and inversions (reviewed by Stankiewicz and Lupski, 2010). In our patient, the second de-novo structural aberration (deletion of satellite DNA) occured independently of the CNV and the formation of the derivative 15. The process leading to the interstitial deletion of satellite DNA remains unclear.

ACKNOWLEDGEMENTS

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