

Longitudinal Study in a Patient with Trisomy 8 Mosaicism: Cytogenetic and Molecular-Genetic Investigations over a Period of Eleven Years

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ABSTRACT We report on the cytogenetic and molecular-genetic investigations of a child with mosaic trisomy 8, analysed over a period of eleven years. The female patient showed clinical features and facial dysmorphisms characteristic of the syndrome as well as mental impairment. The mosaic trisomy 8 was diagnosed prenatally in amniotic fluid cells (38%) and fetal lymphocytes (78%) and was confirmed postnatally in the umbilical cord (52%), placental biopsy (40%), lymphocytes (between 55% and 70%) and buccal mucosa cells (between 30% and 42%), demonstrating the overall prevalence of the trisomy 8 cell line in this patient was quite high. We found no evidence of an appreciable increase or decrease in the frequency of the trisomic cell line over a period of eleven years. Molecular genetic investigation demonstrated the maternal origin of the additional chromosome 8.

INTRODUCTION

Trisomy 8, also known as Warkany syndrome, is a rare condition in man, comprising 0.8% of spontaneous abortions, and is estimated to occur in about 0.1% of all clinically recognised pregnancies (James and Jacobs 1996). In the liveborn population, trisomy 8 is almost always associated with mosaicism, and more than 100 cases have been reported so far (Jordan et al. 1998). Individuals with trisomy 8 mosaicism may present with a variety of features. These include moderate mental retardation, multiple skeletal and joint anomalies (Beighton et al. 1999), urogenital malformations, congenital heart defects, deep palmar and plantar furrows, distinct facies (especially prominent lower lip, characteristic morphology of the ears and nose), and agenesis of the corpus callosum (Karadima et al. 1998). There is a great phenotypical variability, and several patients with a significant percentage of trisomic cells in one or several tissue types displaying normal intelligence and development and normal or near-normal phenotype have also been described (Jordan et al. 1998; Chandley et al. 1980; Habecker-Green et al. 1998). The un-

equal distribution of trisomic cells in different tissues (Jay et al. 1999; Klein et al. 1994) and the variability of these phenotypic features, therefore, make this syndrome so difficult to counsel for when it is detected prenatally. When a mosaic trisomy 8 is found in amniocytes, fetal blood sampling (FBS) may be considered as a means of gaining further confirmation. However, fetal cord blood and amniocytes show diverging numbers of aberrant cells, and so a finding of exclusively normal cells in one of these systems cannot rule out trisomy 8 mosaicism (de Pater et al. 2000; Webb et al. 1998; Hsu et al. 1997). Furthermore, minor clinical features are not easily detected through prenatal ultrasound (Miller et al. 2001). The diagnostic and counselling situations are further complicated by the possibility of confined placental mosaicism (van Haelst et al. 2001; Hsu et al. 1997).

To date, relatively few long-term studies of probands with trisomy 8 mosaicism in different tissues have been published (Camurri et al. 1994). The existing studies have demonstrated that the phenotypic severity does not seem to be related to the degree of mosaicism in the analysed cell systems (Jordan et al. 1998). A decrease in the percentage of trisomic cells was observed in some patients who were examined repeatedly, which may be due to the known replicative disadvantage of the abnormal cell line (Jordan et al. 1998). Furthermore, molecular genetic studies have demonstrated that trisomy 8 mosaicism in liveborns can almost always be traced to a post-zygotic mitotic error (Karadima et al. 1998; James and Jacobs 1996; Webb et al. 1998).

The description of further cases, their phenotype and development should be helpful in this respect. In this paper we report on the case of a girl with mosaic trisomy 8 who was diagnosed prenatally and then followed up for a period of eleven years. Her development and neuropsychological behavior up to the age of seven years has previously been published by Kautza et al. (1991) and Floß et al. (2001).

PATIENT AND METHODS

Case Report

The proband was the first child of healthy parents (mother 26 years, father 37 years old). At 34 weeks gestational age a moderate fetal hydrocephalus and hydronephrosis were diagnosed by ultrasound, followed by diagnosis of double kidneys at 36 weeks. A girl was delivered via sectio cesarian at 38 weeks gestational age because of premature rupture of the amniotic membrane. Length, weight and head circumference were all slightly above the 90th percentile from birth up to the age of 10 years, when adipositas was diagnosed. Facial dysmorphisms included slight strabismus convergens on her right eye, hypertelorism, a broad nasal root, anteverted nares, broad philtrum, low-set ears, high arched palate, full lips and short broad neck (Fig. 1-a, b, c). Other clinical features are increased epidermal thickness, hypo- and aplasia of finger and toe nails, deep palmar and plantar furrows, a missing patella on the right side, low-grade lordosis which seemed to stem from divergence in the lumbar vertebrae, muscular hypotonia, hydronephrosis and hypertrophy of the clitoris. A diagnosis of agenesis of the corpus callosum, which was suspected at the age of 2 years because of grand mal attacks, was later retracted upon more detailed examination.

Developmental tests according to Griffiths performed at the age of 11 months showed her to be in the lower average range, with a developmental age of 9 months. The same tests performed at the age of 24 months confirmed this trend (de-

velopmental age 19 months). Overall she had a very friendly, sociable personality. Her motorical coordination was clumsy. The girl received intense and regular support in the gross and fine motor area. She was enrolled in a normal elementary school at the age of almost 8 years, where she has since remained.

Now, at the age of almost eleven years, she is tall and tends to overweight. She continues to be uncoordinated and one leg is shorter than the other. Her ankles are stiff and she has bilateral hammer toes. The kyphoscoliosis first suspected at the age of 23 months was confirmed. She can not fully extend her fingers, so physiotherapy has been continued. Her achievements at school, except in mathematics, where she can not be graded, are sufficient to allow her to continue to attend. She seems generally slow and requires about 3-4 hours daily in order to complete her school work. She has been receiving psychological treatment. In general, the girl is socially well-adapted, friendly and open. She is cooperative and willing to accept help when she realises she has reached her limits.

Cytogenetic and Molecular-Genetic Investigations

Semi-direct preparations of chorionic villi (12 h incubation) and long-term cultures of fibroblasts, PHA-stimulated 72 h lymphocyte cultures and preparations of buccal smears were done according to standard cytogenetic procedures. FISH was performed following the method described by Lichter et al. 1988, using a biotin-labelled chromosome 8 a-satellite DNA probe.



Fig. 1: The proband at different stages of age:

a) 10 months

b) 3 3/4 years

c) 10 3/4 years

DNA of the patient and her parents was isolated from lymphocytes by a simple salting out procedure. The parental origin of the supernumerary chromosome 8 was determined by PCR-based microsatellite typing, using the following markers: D8S264, D8S262, D8S532, D8S601, D8S1828, D8S133 and D8S166, which span the whole chromosome 8.

RESULTS

A number of tissue systems from the patient were examined by GTG- and QFQ-banding and through fluorescence in situ hybridisation (FISH) with a centromeric DNA probe for chromosome 8. This characterisation included analyses conducted prenatally (fetal lymphocytes after cordocentesis), perinatally (of placenta, amniotic membrane and umbilical cord) and postnatally over a period of eleven years (buccal smears and peripheral lymphocytes). As summarised in table 1, all tissues showed a mosaic of both cell lines, 46,XX and 47,XX,+8. The percentage of trisomic cells in the analysed tissues differed, but it is consistently higher in blood than in buccal smears or in fibroblasts examined at birth. The analysis of the fibroblasts shows a distinct difference between placental tissues and umbilical cord, with a higher percentage of trisomic cells in the latter.

The trisomy level in lymphocytes appears to be variable at every age from birth up to 5 years of age, showing an appreciable fluctuation in the actual numbers from year to year, ranging from 52% to 78%. The results obtained by FISH on

non-dividing lymphocytes / interphases are completely compatible with those signals enumerated in dividing cells / metaphases (see age 23 months). The proportion of trisomic cells in the buccal smears is lower than in the lymphocytes (between 42% at five and 30% at ten years of age).

Molecular genetic investigation on the origin of the trisomic cell line was performed by PCR-analysis of microsatellite markers in the DNA of parents and the patient herself. The results are shown in table 2, demonstrating the maternal origin of the additional chromosome 8 by increased dosage of a unique maternal allele at informative loci. Unfortunately, we were not able to distinguish between a meiotic or mitotic non-disjunction event, because only three of the seven markers used are informative.

DISCUSSION

The long-term evaluation of this patient allowed us to study tissue-specific distribution and stability of the trisomic cell line and to monitor the girl's development from before birth over the course of eleven years. In fact, this time frame was apparently crucial for the diagnostic process, since some important clinical details were not diagnosed at birth but rather later during her infancy and childhood, like the missing patella (5 years of age) and the corrected diagnosis concerning the corpus callosum (almost 7 years). The extended evaluation period allowed a comprehensive insight into the child's psychomotoric development.

Table 1: Confirmation of trisomy 8 mosaicism in different extrafetal tissues and in two cell systems of the proband over a period of 11 years

<i>Tissue type</i>	<i>Time of investigation</i>	<i>46, XX (%)</i>	<i>47, XX, +8 (%)</i>	<i>Total cells</i>
Fetal blood	prenatal	40	60	15
Fetal blood	perinatal	22	78	50
placenta biopsy	perinatal	60	40	10
Amniotic membrane	perinatal	62	38	50
Umbilical cord	perinatal	48	52	50
Lymphocytes	10 month	48	52	50
Lymphocytes	23 month	33 ^a	67 ^a	121
		36 ^b	64 ^b	153
Lymphocytes	3 years	30	70	50
Lymphocytes	5¼ years	45 ^a	55 ^a	38
Buccal smears	5¼ years	58 ^b	42 ^b	186
Buccal smears	10¼ years	70 ^b	30 ^b	200

^a metaphases analysed by FISH

^b interphases analysed by FISH

Cytogenetic analysis performed prenatally, perinatally and at five different times after birth showed that the girl carries a mosaic of a normal and a trisomic cell line in all examined tissues. The fact that fetal and extra-fetal tissues carry both cell lines points to a non-disjunction event occurring very early during embryonic development.

Lymphocytes cultivated after cordocentesis contained a high proportion of trisomy 8 cells (60%). Although some authors investigating prenatal cases of trisomy 8 do not find the trisomic cell line in cord blood, still a comparison with other trisomies shows that these are yet more prone to be missed when cytogenetic analysis after cordocentesis is chosen as a means of confirmation of CVS-results (overall, confirmation rates for trisomic cells compiled by Hsu et al. (1997) are 15% for lymphocytes and 74% for other cell systems). However, even for trisomy 8 a confirmation rate of only 40% after FBS is described, as compared to a confirmation rate of 75% for analysis of tissues. In the present case there was no significant difference between results obtained from (semi) direct-preparations of CVS and those stemming from long-term cell culture of the amniotic membrane (40% and 38%, respectively). This is in contrast to studies by other authors, who find that direct preparations (deriving from the cytotrophoblast) often exhibit fewer or no trisomy 8 cells as compared to long-term cultures deriving from extra-embryonic mesoderm (Karadima et al. 1998; Webb et al. 1998). In the present case, fibroblasts from umbilical cord had a higher proportion of trisomic cells (52%) than did either placental tissue or amniotic membranes.

Lymphocytes from peripheral blood consistently showed a large proportion of trisomic cells, ranging from 52% to 78%, with no

evidence of a decrease over the years from birth to 5 $\frac{3}{4}$ years. There is a distinct fluctuation in these values which is especially noticeable because the child was examined frequently over such a long period of time. We can not, however, confirm decreases of the trisomic cell line which some authors have found both in patients' lymphocytes and in fibroblast cell culture over several passages (Jordan et al. 1998; Miller et al. 1997). The cytogenetic evaluation at the age of 23 months was performed via interphase and metaphase FISH using an α -satellite probe specific for chromosome 8. As has been described by other authors (Jordan et al. 1998), the percentage of trisomic cells identified by FISH on interphases correlates precisely with those using metaphases: 67% and 64%, respectively. This correspondence made the non-invasive examination of a further tissue system feasible via interphase FISH, namely buccal cells. The results obtained at age 5 $\frac{3}{4}$ and 10 $\frac{3}{4}$ show a lower proportion of trisomic cells (42% and 30%, respectively) than found in lymphocytes. The variation in frequency of the trisomic cell line observed in lymphocytes and buccal smears indicates an unequal distribution.

The overall prevalence of the trisomy 8 cell line in various tissues in this girl is quite high. A particular problem in the diagnosis and counselling of cases of trisomy 8 mosaicism is the fact that there is no apparent correlation between the extent of the trisomic cells, its tissue distribution and the expected phenotype. Clinical descriptions range from normal or near-normal adults presenting solely with reduced fertility despite mosaicism even total trisomy 8 in lymphocytes (Jordan et al. 1998; Chandley et al. 1980) to individuals with a relatively low percentage of trisomic cells in a restricted number of tissues who evidence a number of symptoms

Table 2: Results of microsatellite typing in the presented family with trisomy 8. The genetic order of markers corresponds to that of the genethon link map (<http://www.genethon.fr>). (n.i. = not informative)

Locus	Polymorphic marker genotype			
	Maternal	Paternal	Proband	Origin
D8S264	2-3	1-2	2-3-3	maternal
D8S262	1	1	1	n.i.
D8S532	1	1	1	n.i.
D8S601	1	1	1	n.i.
D8S1828	1-2	2-3	n.i.	n.i.
D8S133	1-1	2-2	1-1-2	maternal
D8S166	1-3	1-2	2-3-3	maternal

typical of the syndrome (Miller et al. 1997). In the present case, the high percentage of trisomic cells in lymphocytes (mean value of 64%) and the significant percentage in buccal cells (42% and 30% at ages 5 $\frac{3}{4}$ and 10 $\frac{3}{4}$) has not resulted in an extremely severe phenotype: the girl has some physical symptoms and is still able, at eleven years of age, to attend a normal elementary school.

The majority of both non-mosaic and mosaic autosomal trisomies appear to be due to a meiotic non-disjunction event, with the latter resulting from a post-zygotic loss of the additional chromosome (James and Jacobs 1996). In the case of trisomy 8, however, meiotic errors appear almost always to lead to spontaneous abortions (Nicolaidis et al. 1998). Disomic embryos who later develop a mosaic trisomy 8 through mitotic error seem to have a significantly higher viability than trisomic embryos resulting from a meiotic error (Robinson et al. 1995; Karadima et al. 1998). In the present case, molecular-genetic results could only demonstrate that the additional chromosome 8 was maternal in origin, but they did not provide conclusive evidence as to whether the non-disjunction event occurred in meiosis or mitosis. In contrast to spontaneous abortions with a trisomy 8 resulting from a meiotic error, the mean parental ages in liveborn cases of trisomy 8 are usually not increased (James and Jacobs 1996), and in the present case the mother was only 26 years of age. Uniparental disomies (UPD) of chromosome 8 have only rarely been observed to date, as they do not occur as a result of mitotic non-disjunction, but apparently UPD 8 does not cause a phenotype (Karanjawala et al. 2000; Kotzot 1999; Piantanida et al. 1997).

An important aspect resulting from the presence of a constitutional trisomy 8 mosaicism is the risk of the affected person developing a neoplastic disease (Brady et al. 2000; Danessino et al. 1998). Possibly the presence of the oncogenes c-myc and c-mos on chromosome 8 may help to explain the increased risk of malignancy in cells carrying three copies of the chromosome, perhaps through an enhanced proliferation (James and Jacobs 1996; Seghezzi et al. 1996; Habecker-Green et al. 1998). Also one case of common variable immunodeficiency is described in the literature so far (Yu et al. 2000).

In conclusion, the diagnosis of a mosaic trisomy 8, especially in prenatal cases, calls for comprehensive additional investigations. CVS,

amniocentesis and fetal blood sampling may be necessary to exclude the possibility of confined placental mosaicism, which is not uncommon for trisomy 8 and may result in intra-uterine growth retardation (Saks et al. 1998). Further analysis of a variety of tissues is of the essence, as there is a strong tendency to unequal distribution of cell lines. An awareness of the risk of cancerogeny in probands with constitutional trisomy 8 mosaicism is important. The postnatal investigation of several tissues in a long-term study should be of use to the research community, who can hope to gain insight into mechanisms and karyotype-phenotype correlations in these cases.

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