Prenatal and postnatal growth failure associated with maternal heterodisomy for chromosome 7

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Abstract

The association of maternal uniparental disomy for chromosome 7 and postnatal growth failure has been reported in four cases and suggests the presence of genomic imprinting of one or more growth related genes on chromosome 7. However, in the reported cases, the possibility of homozygosity for a recessive mutation could not be excluded as the cause of the growth failure as in all cases isodisomy rather than heterodisomy for chromosome 7 was present. We report a case of prenatal and postnatal growth retardation associated with a prenatal diagnosis of mosaicism for trisomy 7 confined to the placenta. DNA typing of polymorphic markers on chromosome 7 has established that the zygote originated as a trisomy 7 with two maternal and one paternal chromosomes 7 with subsequent loss of the paternal chromosome resulting in a disomic child with maternal heterodisomy for chromosome 7. The growth failure seen in this child with heterodisomy 7 lends strong support to the hypothesis of imprinted gene(s) on chromosome 7.

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Uniparental disomy (UPD), the inheritance of two homologous chromosomes from one parent in a disomic cell, has been recognised for a number of chromosomes and its phenotypic effect appears to be chromosome specific. Maternal uniparental disomy for chromosome 15 is associated with Prader-Willi syndrome¹⁻³ and paternal uniparental disomy for chromosome 15 with Angelman syndrome.⁴ Maternal and paternal uniparental disomy for chromosome 14 have been reported. Paternal UPD 14 was found in a patient born with multiple congenital anomalies including bilateral subarachnoid hygromas, short neck with webbing, small thoracic cage, marked angulations of the ribs, bilateral simian creases, and facial dysmorphism who presented at 9 years of age with severe mental retardation, severe kyphoscoliosis, a seizure disorder, and coarse facial features.⁵ Maternal UPD 14 has been reported in three patients presenting with an abnormal phenotype.⁶⁻⁸ However, UPD is not always associated with an abnormal phenotype. Maternal UPD for chromosomes 13⁹ and 22¹⁰ and paternal UPD for chromosome 21¹¹ have been described in subjects with a normal phenotype.

It has been suggested that maternal UPD 7 is associated with significant postnatal growth

failure on the basis of three cases of maternal isodisomy for chromosome 7¹²⁻¹⁴ and one case of uniparental isodisomy for paternal 7p and maternal 7q.¹⁵ Paternal isodisomy for chromosome 7 has been reported in a child with a recessive condition, congenital chloride diarrhoea, but normal growth.¹⁶ These cases raised the possibility of genomic imprinting of one or more growth related genes on the long arm of chromosome 7 with a paternal contribution being necessary for normal growth. However, because in all four cases of growth failure isodisomy rather than heterodisomy for chromosome 7q was present, homozygosity for a recessive mutation could not be excluded as the cause of the growth failure. In this report, a child who presented with intrauterine growth retardation and who has shown postnatal growth failure despite being otherwise completely normal is shown to have maternal heterodisomy for chromosome 7. This represents the first case of complete heterodisomy for chromosome 7 and supports the hypothesis of imprinted growth related genes on chromosome 7.

Materials and methods

CLINICAL INFORMATION

The proband was born to a 42 year old gravida 2, term 1, aborta 0, living 1 mother at 36 weeks' gestation. Chorionic villi sampling (CVS) was performed at 9 weeks 6 days for an indication of advanced maternal age. Cultured preparations of CVS showed complete non-mosaic trisomy 7. Amniotic fluid cell culture performed at 12 weeks 6 days of gestation showed a normal female karyotype. No evidence of trisomy 7 was found. A diagnosis of confined placental mosaicism (CPM) for trisomy 7 was made. At 17 weeks' gestation, the maternal serum alphafetoprotein level was found to be raised at 2.75 MOM. A repeat amniocentesis was done following discussion of neural tube defect risks and the amniotic alphafetoprotein level was normal. At 29 weeks' gestation, an ultrasound identified intrauterine growth retardation (IUGR) (table 1). At 31 weeks oligohydramnios was diagnosed. There were no other complications such as placenta abruptio or premature rupture of membranes, but at 36 weeks' gestation, a caesarian section was performed for indications of IUGR, oligohydramnios, and breech presentation. A female infant was delivered with Apgar scores of 7 at one minute and 9 at five minutes. Her birth weight was 1560 g (−3 SD), length 41.5 cm (−3 SD), and head circumference 31.3 cm (−1 SD). There were no dysmorphic features

Table 1 Serial ultrasound assessment

Estimated gestational age	U/S findings	Centile
7 ⁺⁶	CRL 17 mm	50th
9 ^{+6*}	CRL 26 mm	10th
11 ⁺⁶	CRL 46 mm	25th
12 ^{+6,†}	CRL 59 mm	25th
18 ⁺⁶	BPD 39 mm F 23 mm	10th
26 ⁺⁵	BPD 65 mm AC 215 mm F 43 mm	10th
29 ⁺⁵	BPD 73 mm AC 222 mm F 51 mm	<10th
31 ⁺⁵	BPD 76 mm AC 230 mm F 52 mm	<10th
	oligohydramnios	
35 ⁺⁴	AC 250 mm oligohydramnios	<10th

CRL=crown rump length, BPD=biparietal diameter, AC=abdominal circumference.
* Chorionic villi sampling. † Amniocentesis.

noted and the remainder of her physical examination was normal. The placenta weighed 380 g.
At 2 years of age, she had a weight of 9 kg (−3 SD), length 80.4 cm (−2 SD), and head circumference 49 cm (50th centile). Her developmental milestones were normal as assessed by the Denver Developmental Screening Test. At 3 years 9 months of age, her height is 91 cm (−2 SD to −3 SD), her weight 12.2 kg (−2 SD to −3 SD). Her father and mother measure 183 cm and 178 cm respectively. Therefore her height is −4 SD for her expected height for her midparental height.¹⁷

CYTOGENETIC STUDIES
Heparinised umbilical cord blood taken at birth was cultured for 72 hours at 37°C in RPMI 1640 supplemented with 50% FBS and antibiotics. T cell mitosis was stimulated with phytohaemagglutinin. After harvesting, slides were banded and stained with 0.25% trypsin and Giemsa stain. One hundred metaphases were examined for the presence of trisomy 7.
Amnion, chorion, and villi from various areas of the placenta were separately digested in a 0.1% collagenase solution, centrifuged, and cells plated in T-25 flasks. After growth in supplemented F-10 medium, cells were harvested, banded, stained, and examined for the

presence of trisomy 7. Thirty metaphases from each placental tissue were examined. Cultured cells from amnion were examined by fluorescence in situ using alpha satellite centromeric probe D7Z1 and D7Z2 (Oncor).

MOLECULAR STUDIES
DNA was extracted from blood from both parents and the child and from chorion and amnion using a technique described elsewhere.¹⁸ Ten dinucleotide repeat polymorphisms were typed on all DNA samples. Primers for markers D7S513, D7S516, EGF, ELN, D7S482, D7S440, D7S515, D7S495, and D7S483 were obtained from Research Genetics (Huntsville, AL). All markers were typed following a similar protocol: 0.5 µl of 20 µmol/l of each primer, 2 µl of 10 × Taq polymerase buffer, 2 µl of dNTP mix of dATP, dCTP, dGTP, and dTTP at 20 µmol/l each, 0.6 µl of MgCl₂, 0.1 µl of α³²P-dCTP at 10 mCi/ml, 0.2 µl of Taq polymerase at 5 U/µl, and 100 ng of DNA were amplified through 35 cycles consisting of 40 seconds at 94°C, 30 seconds at 55°C, followed by an extension period of two minutes at 72°C. The alleles were separated by electrophoresis in an 8% denaturing polyacrylamide DNA sequencing gel. All gels were fixed and dried and were exposed to x ray film for 24 hours at room temperature. The polymorphic dinucleotide repeat in intron 17b of the cystic fibrosis transmembrane conductance regulator gene was analysed as previously described.¹⁹

Results
Chromosomes from cord blood showed a normal 46,XX karyotype in 100 cells examined, while cultured cells from placental tissues confirmed the trisomy 7 diagnosed prenatally. In both chorion and villi, 100% of cells were trisomic (fig 1). Amnion cultures showed 10%

Figure 1 Karyotype of trisomy 7 seen in both chorionic villi and term placenta.

Figure 2 Interphase analysis of digested amnion showing two signals for chromosome 7 in all analysed cells.

trisomy 7 cells. This was considered to represent contamination from chorion as FISH analysis of 1000 cultured amnion cells showed complete disomy for chromosome 7 (fig 2), thus confirming the prenatal diagnosis of CPM for trisomy 7.

The map position of the ten dinucleotide repeat polymorphisms and results of the typing done on blood from the parents and child, chorion and amnion are shown in table 2. For the dinucleotide repeat polymorphisms at the ELN, D7S482, D7S440, D7S515, and CFTR loci, the proband does not share an allele in common with her father but has inherited both alleles from her mother. In addition, for the same markers and for marker D7S516, the chorion was found to have one allele which is not seen in the amnion or child and is in common with the father. These markers allow the diagnosis of maternal uniparental disomy for chromosome 7 to be made in the proband. In addition, for eight of the 10 (CA)_n polymorphisms typed, including EGF and D7S482 which flank the centromere, the mother is heterozygous and both maternal alleles are present in chorion, amnion, and child indicating that the conceptus began as a trisomy 7 secondary to maternal meiotic I non-disjunction error with a postzygotic loss of the paternal allele leading to maternal heterodisomy in this child.

Table 2 Molecular analysis

Marker	Mother	Father	Child/amnion	Chorion
p arm				
D7S513	2,3	1,2	2,3	2,3
D7S516	1,2	2,3	1,2	1,2,3
Pericentromeric				
EGF	1,2	2,3	1,2	1,2,3
ELN	2,2	1,3	2,2	2,3
D7S482	1,2	3,3	1,2	1,2,3
q arm				
D7S440	1,2	3,4	1,2	1,2,3
D7S515	2,4	1,3	2,4	1,2,4
CFTR-intron 17 CA	1,1	2,2	1,1	1,2
D7S495	1,2	1,1	1,2	1,2
D7S483	2,3	1,2	2,3	1,2,3

Discussion

The association of postnatal growth failure with uniparental disomy for chromosome 7 has previously been reported. In 1988, Spence *et al*¹² reported the first case of UPD in humans in a patient with cystic fibrosis (CF), short stature, and growth hormone deficiency. At the age of 16, despite growth hormone therapy, her height was noted to be 130 cm (−5 SD). This patient was found on molecular analysis to be isodisomic for her maternal chromosome 7.

In 1989, Voss *et al*¹³ reported the second case of maternal isodisomy 7 in another patient with CF who had severe growth retardation. This patient was small for gestational age with a birth weight at 38 weeks' gestation of 1770 (−4 SD) and continued to display poor growth postnatally. At the age of 4 years his height was 87 cm (−3.5 SD) which is at the 50th centile for age 2 years.

The third reported case of maternal UPD for chromosome 7 (partial isodisomy) was by Spotila *et al*.¹⁴ Their patient was ascertained through studies of the COL1A2 gene in patients with osteoporosis. He had a history of intrauterine growth retardation. He was born at term weighing 2048 g (−4 SD). At the age of 8½ years, he was enrolled in a growth hormone treatment study because of his significant short stature. At the age of 30 years, he measured 143.7 cm (−4 SD). Molecular analysis showed that although his mother was heterozygous for a mutation in the COL1A2 gene and his father was normal, he was found to be homozygous for the mutation found in his mother. Five polymorphic markers on chromosome 7 showed only maternal inheritance and at eight out of nine loci on chromosome 7 analysed the patient was homozygous whereas his mother was heterozygous. Taken together these results indicate that the patient has partial isodisomy for chromosome 7 and heterodisomy for a portion of the short arm of chromosome 7 owing to a double crossover event involving the short arm.

The fourth case of maternal isodisomy 7q was reported in a child who was shown prenatally to have an unusual karyotype of 46, XX, t(7;7) (7p7p;7q7q) in 18/18 clones examined. Her birth weight was 3336 g (25th–50th centile), her length was 48 cm (10th–25th centile), and her head circumference was 35 cm (75th–90th centile). She presented with postnatal growth retardation and at 6 months her weight was 5.11 kg (−3.5 SD to −4 SD), her length 58.5 cm (−2 SD), and her head circumference 76 cm (−4 SD). Her developmental milestones were normal. Molecular studies showed isodisomy for paternal 7p and maternal isodisomy for 7q.¹⁵

The degree of short stature (−3.5 to −5.5 SD) described in these reported UPD 7 cases is too severe to be accounted for exclusively by the underlying medical condition that led to the ascertainment of three of the four cases. The fourth case, the patient with maternal isodisomy 7q, has no underlying illness to account for her short stature. Furthermore, the information provided in two of the four reported cases indicated the presence

of prenatal growth failure as both children were reported to be small for gestational age. In the present report, the maternal UPD for chromosome 7 was ascertained through a diagnosis of CPM for trisomy 7 on analysis of chorionic villi and amniocytes. The pregnancy was closely monitored by serial ultrasound assessments because of the increase risk of obstetrical complications associated with CPM. In the first trimester, fetal biometry was at the 25th centile. Serial assessments in the second and third trimester showed decreased fetal growth and at 29 to 36 weeks' gestation, fetal measurements were below the 10th centile for biparietal diameter, abdominal circumference, and femur length (table 1). At birth the baby was -3 SD for weight and length. The infant has shown normal developmental milestones and has had no medical problems. However, there has been poor postnatal catch up growth and at 3 years 9 months of age her weight and height are between -2 and -3 SD for her age and -4 SD for her expected height in view of mid-parental height. The absence of postnatal catch up growth in this patient suggests that placental dysfunction secondary to CPM for trisomy 7 is unlikely to be the only cause of her growth failure at birth. Other cases of growth failure associated with placental mosaicism have shown catch up growth postnatally.^{20,21} The present case together with the four previously reported cases indicates a causal association between maternal uniparental disomy for chromosome 7 and prenatal and postnatal growth failure. The degree of postnatal growth failure appears to be variable. The heights of the four previously reported patients ranged from -3 SD to -4 SD from the mean. However, mean parental heights were not reported. It is possible that the variability would be less if all heights were expressed in terms of deviation from expected height for midparental height. Certainly, the height of the patient presented in this report has been between -2 and -3 SD, which on its own suggests less severe growth failure. However, her height is -4 SD when compared to her expected height for her mid-parental height.

Two different mechanisms can be postulated to explain the association of maternal UPD and growth failure. (1) The presence of UPD increases the risk for homozygosity for a recessive gene causing growth failure. (2) Gene(s) that regulate growth on chromosome 7 are imprinted with a paternal contribution required for normal expression. Both mechanisms are plausible for the first four cases of UPD reported, as two cases represented total maternal isodisomy and two maternal isodisomy for the long arm of chromosome 7. The present case of maternal heterodisomy for chromosome 7 suggests that imprinting is the most likely cause of the growth failure. Homozygosity for a recessive gene is much less likely as a double recombination between two markers tested would be required for a portion of chromosome 7 to be isodisomic. The presence of growth related genes that have been mapped to human chromosome 7q and are syntenic with regions in the mouse genome that are known to be

imprinted lend further support to the causal role of imprinting in the growth failure seen in cases of maternal UPD 7.¹⁴

Uniparental heterodisomy can result from several types of errors in chromosome segregation.¹² The molecular analysis in this case has established that the maternal heterodisomy occurred owing to loss of the paternal chromosome 7 in early cell division in a trisomic 7 zygote, which resulted from a maternal meiotic error. Molecular studies of non-disjunction in trisomy 7 have not been reported. Studies of trisomy 16,²² trisomy 18,²³ and trisomy 21²⁴ have shown that the majority of trisomies are attributable to maternal meiosis I non-disjunction error. Our findings suggest that the same mechanism has occurred in this conceptus. Trisomy 7 is a lethal aneuploidy which accounts for 0.9% of spontaneous abortions.²⁵ Trisomy 7 zygotes rescued by a postzygotic mutation leading to a diploid embryo/fetus would have a 1/3 risk of maternal heterodisomy for chromosome 7.

In conclusion, maternal heterodisomy and isodisomy for chromosome 7 have been associated with intrauterine growth retardation and postnatal growth failure. Although identification of additional cases and long term follow up is needed to define the clinical spectrum of this genotype better, it is important to consider testing the UPD 7 in cases of CPM for trisomy 7 and in patients with a history of unexplained IUGR and postnatal growth failure.

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