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Mosaic Trisomy 16 Ascertained Through Amniocentesis: Evaluation of 11 New Cases

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Trisomy 16, once thought to result uniformly in early pregnancy loss, has been detected in chorionic villus samples (CVS) from on-going pregnancies and was initially ascribed to a second, nonviable pregnancy. Prenatally detected trisomy 16 in CVS and its resolution to disomy has led to the reexamination of the viability of trisomy 16. This study evaluates 11 cases of mosaic trisomy 16 detected through second trimester amniocentesis. In 9 of the 11 cases, amniocenteses were performed in women under the age of 35 because of abnormal levels of maternal serum alpha-fetoprotein (MSAFP) or maternal serum human chorionic gonadotropin (MShCG). The other two amniocenteses were performed for advanced maternal age. Five of the 11 pregnancies resulted in liveborn infants, and six pregnancies were electively terminated. The liveborn infants all had some combination of intrauterine growth retardation (IUGR), congenital heart defects (CHD), or minor anomalies. Two of them died neonatally because of complications of severe congenital heart defects. The three surviving children have variable growth retardation, developmental delay, congenital anomalies, and/or minor anomalies. In the terminated pregnancies, the four fetuses evaluated by ultrasound or autopsy demonstrated various congenital anomalies and/or IUGR. Cytogenetic and fluorescent in situ hybridization studies identified true mosaicism in 5 of 10 cases examined, although the abnormal cell line was never seen in more than 1% of cultured lymphocytes. Placental mosaicism was seen in all placentas examined and was associated with IUGR in four of seven cases. Maternal uniparental disomy was identified in three cases. Mosaic trisomy 16 detected through amniocentesis is not a benign finding but associated with a high risk of abnormal outcome, most commonly IUGR, CHD, developmental delay, and minor anomalies. The various outcomes may reflect the diversity of mechanisms involved in the resolution of this abnormality. As 80% of these patients were ascertained because of the presence of abnormal levels of MSAFP or MShCG, the increased use of maternal serum screening should bring more such cases to clinical attention. Am. J. Med. Genet. 80:473–480, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: amniocentesis; trisomy 16;

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uniparental disomy; confined placental mosaicism; multiple congenital anomalies

INTRODUCTION

Approximately 15 to 20% of recognized pregnancies abort spontaneously, and approximately 50% of these have major chromosome abnormalities [Hassold et al., 1984]. Previous studies [Boue et al., 1975; 1985; Warburton et al., 1991] indicate that trisomy 16 is the most commonly observed trisomy in spontaneous abortuses, comprising 31% of all autosomal trisomies, and occurring in at least 1.5% of all clinically recognized pregnancies [Wolstenholme, 1995]. In these pregnancies, only minimal embryonic development occurs, and almost half show cystic or hypoplastic changes in the placental villi [Warburton et al., 1991]. Molecular studies showed that in spontaneous abortions with trisomy 16, the extra copy of 16 was always maternal in origin, and in virtually all cases the error occurred at meiosis I in which a significant reduction in recombination was observed [Hassold et al., 1995].

Trisomy 16, once thought to result uniformly in early pregnancy loss, has been detected in chorionic villus samples (CVS) from on-going pregnancies [Tharapel et al., 1989]. The clinical outcomes associated with mosaic trisomy 16 or full trisomy 16 detected by CVS have been variable [Dworniezak et al., 1992; Hashish et al., 1989; Tharapel et al., 1989; Verp et al., 1989; Kennerknecht et al., 1990; Williams et al., 1992; Adam et al., 1993; Johnson et al., 1993; Kalousek et al., 1993; Sutcliffe et al., 1993; Vaughan et al., 1994; Whiteford et al., 1995; Zimmermann et al., 1995; Groli et al., 1996; Morssink et al., 1996; Robinson et al., 1997]. When mosaic trisomy 16 or full trisomy 16 is encountered in CVS specimens, it is often the result of confined placental mosaicism (CPM), which represents a dichotomy between the chromosomal constitution of placental tissues and fetal tissues. Kalousek et al. [1993] studied nine pregnancies with trisomy 16 ascertained through CVS and demonstrated uniparental disomy for a maternally derived chromosome 16 in four of five with intrauterine growth retardation (IUGR) or fetal death. These authors suggested that pregnancies that demonstrate high levels of trisomy 16 in the placenta, with diploid fetal karyotypes, are likely to have originated from a trisomic zygote that lost one copy of chromosome 16 in early embryogenesis, a phenomenon known as trisomy rescue [Engel, 1993].

Although trisomy 16 has been reported in first trimester CVS, it has rarely been observed in second trimester amniocentesis. A single case of trisomy 16 mosaicism was reported in over 118,000 amniocenteses in three large collaborative studies [Bui et al., 1984; Hsu and Perlis, 1984; Worton and Stern, 1984]. Hence, the more recent reports of cases of mosaic trisomy 16 detected by amniocentesis are somewhat surprising. These reports indicate that trisomy 16 mosaicism can be associated with neonatal death [Watson et al., 1988; Devi et al., 1993], live births with multiple congenital anomalies [Pletcher et al., 1994; Paulyson et al., 1996; Devi et al., 1997; Smith et al., 1997; Wang et al., 1997], live births with relatively mild but still abnormal phenotypes [Lindor et al., 1993], or live births with normal phenotype [Hsu et al., 1997]. Six electively terminated fetuses with mosaic trisomy 16 detected by amniocentesis were abnormal showing IUGR, congenital cardiac anomalies (atrial septal defect, ventricular septal defect, and tetralogy of Fallot), severe pulmonary hypoplasia, horseshoe or ectopic kidney, thymic and adrenal atrophy, single umbilical artery, and minor facial anomalies [Huff et al., 1991; Davies et al., 1995; Tantravahi et al., 1996; Hsu et al., 1997].

In a previous publication [Garber et al., 1994], we reported on two cases of trisomy 16 detected by amniocentesis with divergent outcomes. This report expands our series to 11 cases. The clinical findings are compared with cytogenetic and molecular studies on a variety of tissues to evaluate the presence and influence of tissue specific mosaicism and uniparental disomy on pregnancy outcome.

MATERIALS AND METHODS

Clinical Evaluation

Patient information collected included pregnancy history, family history, ultrasound findings, maternal serum screening results (MSAFP or triple marker studies), and pregnancy outcomes.

Cytogenetic Analysis

To assess the level of trisomy 16 cells in multiple fetal tissues, primary cultures were established from various organs (i.e., skin, liver, lung, kidney, amnion, chorion, and placenta) using standard culture techniques after treatment with collagenase to disaggregate the tissue (final concentration 0.83 mg/ml; Sigma, St. Louis, MO). Peripheral blood or cord blood samples were cultured after phytohemagglutinin stimulation. Metaphase chromosome preparations were made from each culture and evaluated using GTG banding performed by standard procedures. Twenty to 100 metaphase cells were analyzed from each tissue.

Fluorescent In Situ Hybridization (FISH)

For analysis of large numbers of cells, including interphase cells from cultures with low mitotic indices, FISH was performed using the commercially available chromosome 16 alpha-satellite probe (D16Z2) labeled with either biotin or digoxigenin (Oncor, Inc., Gaithersburg, MD). Hybridization was performed according to the manufacturer's instructions (Oncor, Inc.). Samples from cytogenetically normal patients were used as controls. The number of signals observed in a minimum of 200 metaphase and interphase cells was analyzed for each hybridization.

Molecular Analysis

To identify the parental origin of existing copies of chromosome 16, genomic DNA was extracted from fresh tissues, lymphoblastoid cell lines, fibroblast cultures or formalin-fixed tissues of patients, or parental blood. Multiple polymorphic markers on chromosome 16 were used to determine the parental origin. These

included microsatellite markers SM7 (D16S283), AC2.5 (D16S291), AC2.3 (D16S292), D16S265, D16S266, D16S402, D16S403, D16S404, D16S405, D16S408, D16S410, D16S411, D14S413, D16S419, D16S422, D16S423 (Research Genetics, Inc., Huntsville, AL), and a VNTR marker 3′HVR (HBA).

For the three dinucleotide (CA) repeats SM7, AC2.5, AC2.3, the following experimental approaches were used. The CA-repeat SM7 was amplified by 35 cycles of polymerase chain reaction (PCR) amplification, using 94°C denaturation, 55°C annealing, and 72°C extension [Harris et al., 1991]. The PCR products were separated on a 15% polyacrylamide gel and stained with BioRad (Hercules, CA) silver stain. To improve resolution of the alleles, one family was also studied by using a radioactively labeled primer and denaturing gel electrophoresis. For the CA-repeats AC2.5 and AC2.3, the PCR amplification included 10 cycles with 94°C denaturation, 65°C annealing, and 72°C extension and 25 cycles using 94°C denaturation, 60°C annealing, and 72°C extension [Thompson, 1992]. PCR products were electrophoresed in a nondenaturing 15% polyacrylamide gel, and the BioRad silver staining procedure was performed. For the microsatellite markers D16S265, D16S266, D16S402, D16S403, D16S404, D16S405, D16S408, D16S410, D16S411, D16S413, D16S419, D16S422, and D16S423 (Research Genetics, Inc.), the primers were labeled with ^{32}P. The PCR amplification was performed according to the manufacturer's suggestions (Research Genetics, Inc). The products were separated on 5% polyacrylamide gel. All gels were dried and exposed to X-ray film overnight at –70°C.

For VNTR marker 3′HVR, 5 μg of genomic DNA was digested with 3 units of PVU II (Promega, Madison, WI), electrophoresed in a 0.8 to 1% agarose gel and transferred to a nylon membrane. The probe 3′HVR was multiprime labeled with ^{32}P and after hybridization (using 10^6 CPM/ml probe) at 65°C, the membranes were washed at 65°C with 0.1 × SSC, 0.1% sodium dodecyl sulfate (SDS). All gels were dried and exposed to X-ray film overnight at –70°C.

Paternity was established in each cases using three informative microsatellite probes from chromosomes 8, 11, 15, 17, 18, and X.

RESULTS

The clinical findings, including the indications for prenatal diagnosis, the maternal ages, ultrasound findings, pregnancy outcomes, and fetal/newborn findings of each patient are summarized in Table I, and cytogenetic and molecular studies in each patient are summarized in Tables II and III, respectively.

In this study, 11 pregnancies with mosaic trisomy 16 ascertained through amniocentesis have been evaluated in order to gain insight into the clinical implications of this occurrence. Of the 11 pregnancies with mosaic trisomy 16 detected in amniocentesis, nine occurred in women between the ages of 23 to 32 (mean maternal age of 28) who underwent amniocentesis because of abnormal levels of MSAFP or MShCG. Five pregnancies (42%) were continued and all showed IUGR on prenatal ultrasound. These pregnancies resulted in liveborn infants with IUGR and congenital anomalies, most commonly congenital heart defects (CHD), mild development delay, and minor anomalies (case 2, 3, 5, 9, and 10) (Table I); Figs. 1 and 2. Two liveborn infants died of congenital heart anomalies as

TABLE I. Summary of Clinical Findings

Case	Indication	MA (years)	Placenta	Fetal growth	Outcome	Phenotype
1	AMA	37	Normal	Normal	EAB at 22 weeks	Short femora, right single transverse palmar crease, clinodactyly
2	↑ MShCG	27	Enlarged, cystic	IUGR	Delivered at 36 weeks, neonatal death	ASD, VSD, single coronary artery, perineal groove, Meckels diverticulum
3	↑ MSAFP	25	Enlarged, cystic	IUGR	Delivered at 29 weeks, neonatal death	Coarctation of aorta, VSD
4	↑ MSAFP	30	Normal	Normal	EAB at 22 weeks	Normal
5[a]	↑ MSAFP	23	Normal	IUGR	Delivered at 35 weeks	At 3 9/12 years of age: minor facial anomalies, craniofacial asymmetry syndactyly, hyperpigmented skin lesion, developmental and speech delay
6	AMA	35	Normal	Normal	EAB at 21 weeks	Horseshoe kidney, two-vessel umbilical cord
7	↓ MSAFP	28	Normal	Normal	EAB at 21 weeks	Normal
8	↑ MShCG	31	Normal	IUGR	EAB at 22 weeks	Normal
9	↑ MSAFP	26	Normal	IUGR	Delivered at 34 weeks	At 2 years of age: minor facial anomalies, facial and chest wall asymmetry, hearing loss, VSD, anteriorly placed anus, congenital abnormality of cervical vertebrae, short left forearm, hypoplastic left thumb, developmental delay
10	↑ MSAFP ↑ MShCG	32	Normal	IUGR	Delivered at 29 weeks	At 20 months of age: IUGR, ASD repaired, several suspected seizure episodes treated with Tegretol
11	↑ MShCG	32	Normal	IUGR	EAB at 20 weeks	Abnormal ear, wide mouth, clinodactyly, cutaneous syndactyly

Abbreviations: MA, maternal age; EAB, elective abortion.
[a]Hajianpour, 1995.

Table II. Frequency of Trisomy 16 Cells

Prenatal diagnosis				Postnatal confirmation			
Case	Amniocytes	PB	PUBS	Blood	Tissue	EET	Interpretation
1	20/25	–	–	–	Skin 2/50, umbilical artery 0/50, fascia 0/50	Placenta 32/50	TM
2	3/31	17/17	–	–	Skin 0/50	Placenta 0/24, Chorion 0/24, amnion 1/26,	CPM
3	14/15	–	–	0/30	Skin 0/36	–	–
4	18/50	–	–	0/20	Intestine 6/31, liver 6/20, lung 14/20, skin 5/20, 0/25–40	Villi 9/37, chorion 14/25, amnion 4/18	TM
5	6/20	–	1/100	0/200	Skin 0/200, 35/411[a]	–	TM
6	5/21	–	–	–	Lung 5/180, kidney 0/100, liver 0/100	–	TM
7	2/15	–	–	–	Thymus 0/50, kidney 0/58, 0/200[a], skin 0/60, 0/200[a]	Placenta 22/24	CPM
8	3/31 22/31r(16)	–	–	–	–	Placenta +16/+r(16)	–
9	2/20	–	–	0/50	Skin 14/40	Placenta 19/20	TM
10	2/53	–	–	0/100	Skin 0/150[a]	Placenta 20/20	CPM
11	6/38	–	–	0/100	Lung 0/200[a], kidney 0/200[a], heart 0/52[a], cartilage 0/200[a], skin 0/200[a]	–	–

[a]FISH was applied to each tissue.
Abbreviations: PB, placental biopsy; PUBS, percutaneous umbilical cord blood sampling; EET, extra embryonic tissue; TM, true mosaicism; CPM, confined placental mosaicism.

neonates (case 2 and 3), and interestingly, both were observed to have enlarged cystic placentas on ultrasound. Four of six pregnancies electively terminated demonstrated either IUGR or a variety of structural anomalies, including short femora, renal anomalies, and minor anomalies (case 1, 6, 8, and 11). Thus, at least 9 of 11 cases (82%) with trisomy 16 mosaicism had some abnormal findings.

Table III. Evaluation of Uniparental Disomy for Chromosome 16

Case	Tissue	Informative polymorphic marker	Parental origin of chromosome 16
1	Fascia	SM7, AC2.5	Maternal heterodisomy
2	Skin, heart tissue	SM7, AC2.5	Biparental
3	Skin	3′HVR	Biparental
4	Skin	3′HVR	Biparental
5	Blood	3′HVR	Biparental
	skin	D16S402, D16S404, D16S403	Biparental
6	Parental samples not available	—	—
7	Thymus, lung, skin, kidney, placenta	3′HVR	Maternal UPD
8	Lung	D16S403, D16S410	Biparental
9	Skin	D16S402, D16S410	Biparental
	blood	D16S265, D16S313, D16S402, D16S410	Biparental
10	Amniotic fluid	D16S265, D16S266, D16S410	Maternal heterodisomy
11	Lung	D16S404, D16S265	Biparental

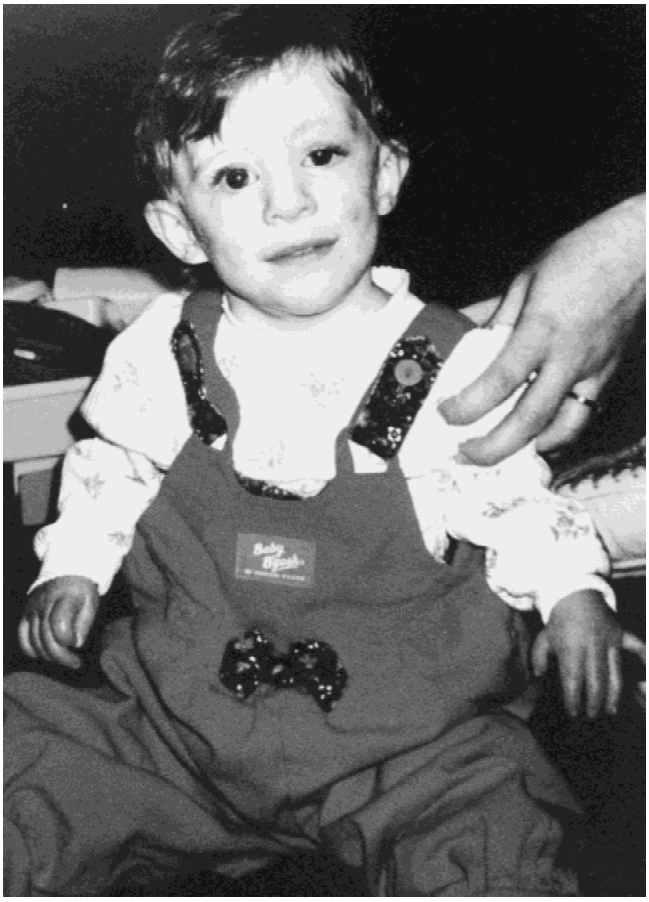

Fig. 1. Photograph of case 9 at age 16 months. Note facial asymmetry, mild left ptosis, and shortness of left forearm with hypoplastic thumb.

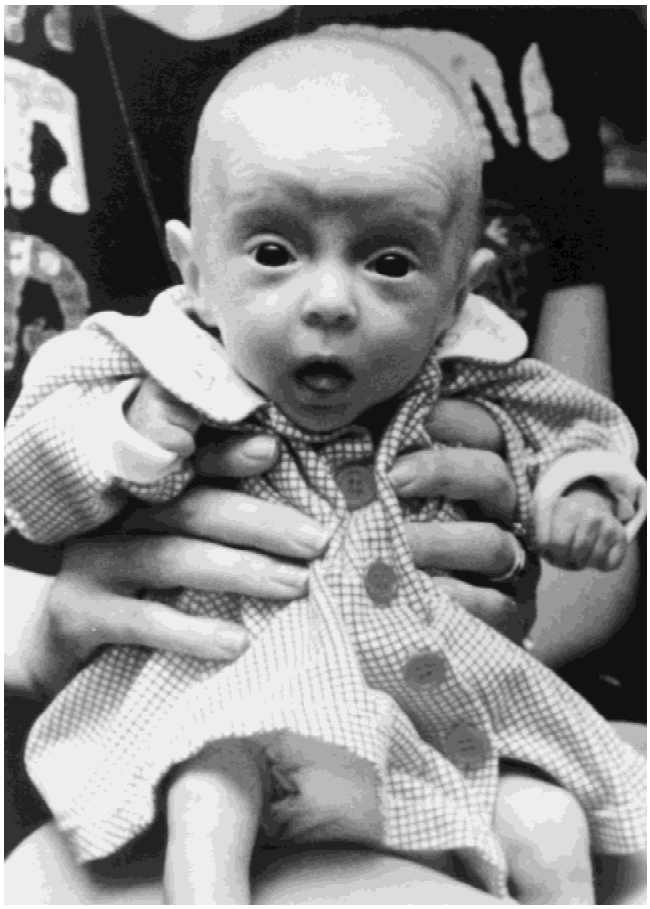

Fig. 2. Photograph of case 10 at age 6 months. No minor anomalies were noted besides growth retardation.

DISCUSSION

Including this series, 33 cases of mosaic trisomy 16 detected through amniocentesis have been reported to date. Of 21 continuing pregnancies, 16 pregnancies (77%) had abnormal outcomes, including neonatal death [Watson et al., 1988; Devi et al., 1993], or liveborns with some combination of IUGR, CHD, and minor anomalies [Lindor et al., 1993; Pletcher et al., 1994; Paulyson et al., 1996; Devi et al., 1997; Hsu et al., 1997; Smith et al., 1997; Wang et al., 1997]. The remaining five pregnancies produced infants with an apparently normal phenotype at birth [Hsu et al., 1997]. In 12 electively terminated pregnancies, 10 fetuses (83%) showed some combination of IUGR, congenital heart, lung, kidney, thymic, and adrenal, and facial anomalies [Huff et al., 1991; Davies et al., 1995; Tantravahi et al., 1996; Hsu et al., 1997]. The remaining two fetuses appeared normal. Thus, the prenatal detection of trisomy 16 in midtrimester is not a benign finding but is associated with a high risk of abnormal outcome.

The abnormalities, most commonly observed in these patients (IUGR, CHD, and minor anomalies) are most likely caused either directly or indirectly, by the existence of cells with trisomy 16. We propose different mechanisms that may explain the variability of outcomes. For each individual case, a different mechanism may be operative.

True fetal mosaicism is one of the mechanisms that can be associated with abnormal fetal morphogenesis [Pallister et al., 1976; Teschler-Nicola and Killian, 1981]. In our study, mosaicism for trisomy 16 was detected in 4 to 90% of the amniocytes. There was no direct correlation between the fraction of trisomic cells detected in amniocentesis and pregnancy outcome. In follow-up cytogenetic studies, fetal and newborn tissues were evaluated in all cases but one (case 8), and trisomy 16 cells, indicative of true mosaicism, were identified in 5 of the 10 patients (50%) with the proportion of trisomic cells ranging from 2 to 70% in various tissues (case 1, 4, 5, 6, and 9). This may be an underestimation of true mosaicism as only skin and blood were examined in three of the liveborns (case 2, 3, and 10). If trisomic cells are present in the fetus, morphogenesis in the involved tissue could be disturbed in different ways. The trisomic cells themselves can cause abnormal morphogenesis leading to abnormal development. Selective death of the trisomic cells may occur, and the loss of large numbers of cells in a developing organ may disrupt normal morphogenesis. In this latter situation, true mosaicism may have been present at some time during development but may not be detected at a later stage in pregnancies or after birth because of the loss of these cells or to limited access to the relevant tissues. Thus, the rate of true mosaicism may be even higher than has been confirmed. Interestingly, cells with trisomy 16 were seen only in one lymphocyte culture and at a very low level (1%), indicating that blood is not the optimal tissue for investigation of possible mosaicism. This is an important clinical observation because PUBS has significant risk to the pregnancy and it is unlikely to give valuable information. In addition to the five cases of true mosaicism in this series, true mosaicism of chromosome 16 has been reported previously in six liveborn infants [Gibertson et al., 1990; Greally et al., 1990; Devi et al., 1993; Lindor et al., 1993; Pletcher et al., 1994], all having abnormal phenotypes. Trisomy 16 detected at amniocentesis is often indicative of true fetal mosaicism that can lead to phenotypic abnormalities, unlike trisomy 16 detected by CVS most often represents confined placental mosaicism that can be associated with IUGR, but rarely with other phenotypic abnormalities [Kalousek et al., 1993; Wolstenholme, 1995], particularly if no trisomy 16 cells are seen in the amniocentesis sample.

Placental mosaicism, either CPM or placental mosaicism associated with fetal mosaicism, represents another explanation for some of the abnormal findings associated with mosaic trisomy 16 detected in amniocentesis. Previous reports indicated that perinatal outcome in cases with CPM might be compromised [Johnson et al., 1990; Kalousek and Barrett, 1994; Wolstenholme et al., 1994; Kalousek and Vekemens, 1996], with these cases demonstrating spontaneous abortions, IUGR, or morphological abnormalities in the fetus or newborn [Fryburg, 1993]. In some studies, however, a clear association between CPM and poor pregnancy outcome was not observed [Roland et al., 1994]. In this series, seven midtrimester or term placentas (case 1, 2, 4, 7, 8, 9, and 10) were available for analysis, and all showed evidence of trisomy 16 cells (24 to 100% abnor-

mal cells) (Table II), with three of these pregnancies also showing true fetal mosaicism (case 1, 4, and 9). Four cases demonstrated IUGR (case 2, 8, 9, and 10), whereas the others showed normal growth at the time of pregnancy termination in midtrimester (20 to 22 weeks). All three continuing pregnancies, in which cells with trisomy 16 were identified in the placenta, resulted in early delivery (28 to 34 weeks), perhaps a result of the malfunction of placenta caused by high levels of trisomic cells. Of the 20 reported cases of trisomy 16 mosaicism detected at amniocentesis, in which the placenta and/or other extra embryonic tissues were available for study [Watson et al., 1988; Huff et al., 1991; Lindor et al., 1993; Pletcher et al., 1994; Tantravahi et al., 1996; Paulyson et al., 1996; Hsu et al., 1997; Devi et al., 1997; Smith et al., 1997; Wang et al., 1997], 19 (95%) had cells with trisomy 16. Among the 13 continuing pregnancies, 11 (85%) resulted in premature delivery and/or IUGR. Thus, most pregnancies with mosaic trisomy 16 at amniocentesis had associated placental mosaicism and adverse perinatal outcomes.

Uniparental disomy (UPD) can provide a third possible explanation for the abnormalities observed in pregnancies with mosaic trisomy 16 detected through amniocentesis. UPD has been observed for most chromosomes, but frequency and outcome depend on the chromosome involved [Ledbetter and Engel, 1995]. Both uniparental heterodisomy and isodisomy can cause disruption of normal development if imprinted genes critical to growth and development are present on the chromosome involved or if deleterious recessive alleles become homozygous by this mechanism [Engel, 1993]. Three of 10 cases in this series (case 1, 7, and 10), or the predicted one third, displayed maternal UPD for chromosome 16 (Fig. 3). No paternal UPD 16 was observed. Two of these (including one case that also showed true mosaicism) had abnormal phenotypes, whereas one was reported as normal at 21 weeks of gestation. Previously reported cases of UPD for chromosome 16 [Lindor et al., 1993; Kalousek et al., 1993; Sutcliffe et al., 1993; Vaughan et al., 1994; Whiteford et al., 1995; Schneider et al., 1996; Hsu et al., 1997; Robinson et al., 1997; Woo et al., 1997; Wang et al., 1997] as well as the three cases reported here indicate that intrauterine growth retardation is a frequent consequence but that a diversity of congenital anomalies may occur as well. An extensive review of prenatally detected trisomy 16 [Wolstenholme, 1995] indicates that maternal UPD 16 has been associated with pregnancy loss in the second or early third trimester as well as early delivery with severe growth retardation. Recently, Robinson et al. [1997] reported a significant increase in adverse outcome for pregnancies demonstrating UPD 16. These authors suggested that imprinting may exist for genes located on chromosome 16, but that the effects of imprinting may be limited to the placental tissues. Such a tissue limited imprinting effect may still affect prenatal development. However, in three UPD cases reported here, high levels of trisomic cells in the placenta were detected as well. Therefore, the independent clinical effects of UPD 16 on IUGR and pregnancy outcome remain to be determined.

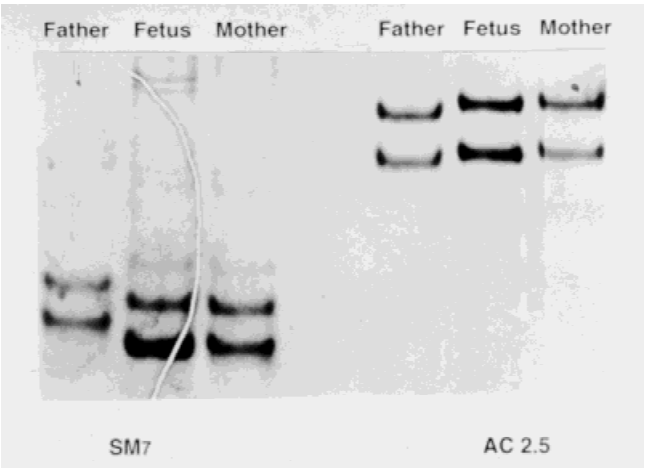

Fig. 3. Case 1: DNA from father, mother, and fetus amplified by PCR using two dinucleotide repeats: SM7(16S283) and AC (D16S291) on chromosome 16. The results show that the fetus inherited two maternally derived alleles and no paternally derived allele.

In a review of over 118,000 cases of amniocentesis from the early 1970's to 1984, only a single case of mosaic trisomy 16 was detected. Yet, in the past 9 years, 33 cases (including our series) have been reported. It is significant that 80% of patients were referred for amniocentesis because of abnormal levels of MSAFP or MShCG (an indication that was not used in the 1970's and early 1980's), suggesting that the increased use of maternal serum screening has brought these cases to our attention. Because all evaluated cases showed abnormal cells in the placenta as well, it could be speculated that placental abnormalities related to trisomy may either facilitate the transfer or result in increased leakage of fetal proteins into maternal serum. The increased use of maternal serum screening will likely reveal more such cases that will then require appropriate counseling. This counseling should indicate that pregnancies with trisomy 16 detected by amniocentesis often result in early delivery and that greater than 70% of such reported pregnancies result in abnormal outcomes including IUGR, CHD, and minor anomalies. True mosaicism, which was detected in a significant number of these cases, may explain some of these congenital anomalies. It is essential to monitor these pregnancies closely to evaluate the existence of abnormalities commonly associated with mosaic trisomy 16. Long-term follow-up of continued pregnancies will be necessary to determine overall outcome.

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