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Confined chorionic mosaicism in prenatal diagnosis

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Summary. Confined chorionic mosaicism, detected commonly on chorionic villus sampling (CVS) and occasionally in cultured amniotic fluid cells, is described in five pregnancies that showed confined chorionic mosaicism for trisomies 12, 13, 14, 17 and a marker chromosome. Cytogenetic findings in these pregnancies support the conclusion that within chorion some chromosomal mosaicism are confined to the trophectoderm derivatives while others to the extra-embryonic mesoderm. The etiology of confined chorionic mosaicism is discussed in relation to a significant role of multiple cell lineages contributing to the early development of placenta. The need is indicated for the use of both direct and long-term cultures in CVS prenatal diagnosis, and for the confirmatory testing of fetal blood or amniotic fluid in cases where mosaicism is detected in chorionic villi.

Introduction

Discrepancies between the chromosomal complement in fetal and placental tissues identified by chorionic villus sampling (CVS) have been reported (Eichenbaum et al. 1986; Martin et al. 1986; Mikkelsen 1985; Simoni et al. 1985, 1986; Pergament and Verlinsky 1986). In this paper such "discrepancies" are viewed from a developmental angle and a descriptive term "confined chorionic mosaicism" is coined for them. Chromosomal mosaicism implies the presence of two or more distinct cell lines containing different karyotypes in one individual. It may originate during early post-zygotic development through non-disjunction, anaphase lag or structural rearrangement. Chromosomal mosaicism has been documented in spontaneous abortions, newborns, children and adults (Warburton et al. 1978; Ford 1969) and is usually found in multiple tissues with varying clinical manifestations, and therefore considered to be generalized. When mosaicism is tissue limited or confined, its origin can be traced to specific cell lineage(s) and to a specific time at the pre-implantation and early post-implantation period. Confined chorionic mosaicism (CCM), representing a discrepancy between the chromosomal constitution of chorionic tissues and embryonic/fetal tissues is commonly detected on CVS (Mikkelsen 1985; Simoni et al. 1985, 1986; Eichenbaum et al. 1986). Its existence has also been demonstrated in term placenta of two infants with a significant intrauterine growth retardation (Kalousek and Dill 1983).

Detection of CCM would not normally be expected during prenatal diagnosis using amniocentesis at 16 weeks of gestation. Yet discordance between cytogenetic findings in cultured amniotic fluid cells and fetal cells, which cannot be explained by true fetal mosaicism, pseudomosaicism or maternal cell contamination of amniotic fluid, has been reported by several authors (Bui et al. 1984; Hoehn et al. 1978; Hsu and Perlis 1984; Greenberg et al. 1982; Jensen et al. 1984; Djalali et al. 1985; Pfeifer et al. 1984; Rudd et al. 1977). In these cases an accidental aspiration of chorionic cells by the needle passing through the anteriorly located placental disc is the most likely explanation for the presence of cells that differ from those of the fetus (Stetten and Meissner 1981).

We report two cases of CCM detected among 6,722 consecutive second trimester amniocenteses and three cases of CCM diagnosed by CVS prenatal diagnosis performed in 140 consecutive pregnancies.

Materials and methods

Case 1

A 32-year-old gravida I woman was referred for genetic counselling because of concerns regarding the effect of paternal chemotherapy on reproduction. The father had had an embryonal carcinoma of the left testis with inoperable retroperitoneal metastases diagnosed two years earlier. He received a 10-week course of chemotherapy with Cisplatinum 800 mg, Vinblastin 60 mg, and Bleomycin 300 mg. No radiotherapy was given. A laparotomy performed following the course of chemotherapy revealed no metastases. The interval between the completion of the therapy and conception was 20 months. The family history was non-contributory.

As the long term effects of intensive chemotherapy on reproduction in males are not well documented, cytogenetic prenatal diagnosis was offered to this couple as a means of reassurance. Amniocentesis with ultrasound was performed at 16 weeks gestation. The placenta was anteriorly placed. The first tap in the right fundal region was unsuccessful. The fundus was described as "mobile". Another ultrasound scan was done and the second tap was performed in the midline region, and 16 ml of clear amniotic fluid was removed. No red blood cells were identified in the amniotic fluid specimen. After cytogenetic results of chromosomal mosaicism were reported, the couple elected to terminate the pregnancy.

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Case 2

A 36-year-old woman was referred for amniocentesis for advanced maternal age. Her first pregnancy had resulted in a term liveborn male and the second had been ectopic. The present pregnancy was uncomplicated with appropriate fetal growth. Amniocentesis was performed at 16 weeks. By ultrasound, the placenta was anterior in location, and no fetal anomalies were detected. A single tap obtained 16 ml of clear amniotic fluid. Both the Kleihauer and amniotic fluid alpha fetoprotein measurements were normal.

She elected to continue the pregnancy after results of the karyotyping were discussed. Repeat ultrasound examination at 20 weeks was consistent with continued appropriate growth. The patient spontaneously delivered at term a liveborn male infant with Apgars of 9/1 and 10/5 minutes. The birth weight was 3940 g. Subsequent growth and development have been normal.

Case 3

This 30-year-old gravida 4, term 2, therapeutic abortion I presented for prenatal diagnosis with a history of a previous child with trisomy 21. Her third pregnancy had resulted in a female infant with trisomy 21 who died at 3 months of age due to cardiac anomalies. This pregnancy was uneventful until the time of the chorionic villus sampling at 10 weeks of gestation. The fetal crown–rump length was 30 mm and fetal movement and heart rate were observed. The placenta was in the posterior position. Two aspirations yielded 5 and 15 mg of tissue, respectively. There was no rupture of membranes and no bleeding following the aspiration.

Direct chromosome preparation from the trophoblastic tissue showed mosaicism. Amniocentesis was recommended and was undertaken (single tap) at 16 weeks gestation. Chromosomes were shown to be normal 46,XX and amniotic fluid alpha fetoprotein was also in the normal range.

The pregnancy continued uneventful until the patient was 41 weeks plus 6 days. Induction of labour was initiated, which resulted in a Cesarean section due to cephalopelvic disproportion. A female infant was delivered in a good condition with a birth weight of 4060 g. The post partum course for mother and infant was unremarkable.

Case 4

A 33-year-old gravida 5 was initially seen immediately after delivery of a small-for-dates (1480 g at 37 weeks) malformed female infant. The abnormalities included hypertelorism, vestigial ears, cleft palate, and an endocardial cushion defect. The cytogenetic analysis revealed trisomy 22 and the infant died in the neonatal period. The patient's previous two pregnancies included a hydatidiform male and a normal liveborn female at 37 weeks. A fourth pregnancy resulted in a spontaneous abortion at ten weeks. Chromosome analysis was not possible on these products of conception.

The patient then had difficulty conceiving and believed she may have had other early losses but none was documented. The pregnancy studied was thought to be conceived on day 19 of a cycle, and was complicated by spotting and cramping on the 45th day. Because of the previous liveborn with trisomy 22, the patient requested prenatal diagnosis and had chorionic villus sampling with ultrasound at 10 weeks. Following the procedure, the patient experienced spotting and cramping for two days. After learning of the cytogenetic results, the couple elected to terminate the pregnancy. Parental karyotypes were normal.

Case 5

This 37-year-old gravida 3 was seen for advanced maternal age in her third pregnancy. The first pregnancy ended in a spontaneous abortion at 4½ months with no further details known. The second pregnancy resulted in a term delivery of a 9½ lb female infant.

This pregnancy was uneventful and chorionic villus sampling was done at 11 weeks. Ultrasound confirmed appropriate early growth. The cytogenetic results indicated a mosaicism for a marker chromosome. Parental karyotypes were normal. The mother elected to continue the pregnancy. Ultrasound scan and maternal serum alpha fetoprotein at 16 weeks were within normal limits. An 8 lb female infant was delivered at term. The baby showed no dysmorphic features other than 2–3 syndactyly of toes which is also present in mother.

Cytogenetic methodology

From amniotic fluid obtained in a standard way by amniocentesis in cases 1 and 2 cultures were established in 60 mm plastic culture dishes in Ham's F10 supplemented with 25% FCS. Chromosomes were harvested in situ (Cox et al. 1974) after 10–14 days in culture and were G-banded. Colonies from six dishes were analysed. In those colonies where an anomaly was found, several metaphases were analysed.

A direct preparation from chorionic villi obtained by transcervical aspiration using a Portex catheter under ultrasound guidance was prepared using a modified methodology of Simoni et al. (1983) and 48 h incubation (Vekemans and Perry 1986). Part of the sample was also used to establish a long-term culture. Confirmatory cultures were established following term delivery from amnion, chorion and blood. After pregnancy termination (cases 1 and 4), in addition to culturing amnion, chorion and fetal blood, multiple fetal tissues such as skin, fascia, gonads, kidney, lung and tongue were cultured and analysed cytogenetically. In full-term placentas (cases 2, 3, 5) both amnion and chorion were sampled at multiple sites. The location of each site was carefully recorded and cultured as several explants in three different dishes.

Evaluation of fetuses and newborns

Detailed morphological studies including external and internal examination and X-rays were performed on fetuses after pregnancy termination (cases 1, 4). Careful neonatal examination was done in cases 2, 3, 5.

Results

Confined chorionic mosaicism detected by amniocentesis

All available colonies obtained after 2 weeks of culturing amniotic fluid in cases 1 and 2 were analysed. In both cases a significant proportion of colonies in several dishes were aneuploid. These results were interpreted as true mosaicism for trisomies 14 and 17 (Table 1) respectively.

Table 1. Cytogenetic results of confined chorionic mosaics diagnosed from amniotic fluid cell cultures

Cultured tissue	Number of analysed metaphases		Total cells
Case 1	46,XY	47,XY,+14	
Amniotic fluid cells	29 (24 colonies)	13 (6 colonies)	42
After termination			
Amnion	50	0	50
Fetal tissues (fibroblast)[a]	50	0	50
Cord blood	25	0	25
Chorion and chorionic villi	114	20	134
Case 2	46,XY	47,XY,+17	
Amniotic fluid cells	12 (12 colonies)	11 (7 colonies)	23
After term delivery			
Cord blood	100	0	100
Amnion	38	0	38
Chorion and chorionic villi[b]	59	79	138

[a] Cultures were established and analysed from skin, fascia, gonad and kidney
[b] Table 2 provides details of the distribution of the two cell lines in the chorion and chorionic villi

Table 2. Cytogenetic results from different sites of chorionic plate from full term placenta of case 2

Site	Number of cells counted	46,XY	Percent	Trisomy 17	Percent
Peripheral locations					
Chorion and villi	11	4	36.5	7	63.5
Chorionic plate	16	1	6.2	15	93.8
Chorion and villi	12	7	58.3	5	41.7
Chorionic plate	12	0	0.0	12	100.0
Villi only	23	15	65.2	8	34.8
Chorion and villi	14	6	42.9	8	57.1
Chorion and villi	10	3	30.0	7	70.0
Villi only	10	7	70.0	3	30.3
Near cord insertion					
Villi only	16	15	93.8	1	6.2
Chorionic plate	14	1	7.1	13	92.9
Total	138	59	42.7	79	57.3

In case 1 cultures were established from amnion, fetal tissues (skin, fascia, gonad and kidney), blood and chorion after pregnancy termination. The trisomic cell line was identified only in cultured chorion.

In case 2 the pregnancy continued to term. Cytogenetic studies of cultured cord blood and amnion failed to show any aneuploid cells. However, in chorion, a large proportion of cultured cells revealed trisomy 17. Table 2 shows an unequal distribution of trisomic and diploid cells in the chorion. The samples (sites 2, 4 and 10) consisting of predominantly trisomic cell populations were taken from true chorionic plate and were macroscopically free of villi. On the other hand, samples (sites 5, 8 and 9) with mainly diploid karyotypes consisted of chorionic villi. Sites 1, 3, 6 and 7 represented a mixture of villi, villous septae and chorionic plate.

Table 3. Cytogenetic results of confined chorionic mosaics diagnosed from chorionic villus sampling

Cultured tissue	Number of analysed metaphases		Total cells
Case 3	46,XX	47,XX,+13	
Chorionic villus cells			
– direct preparation	26	3	29
Amniocentesis	18	0	18
After term delivery			
Cord blood	50	0	50
Amnion	25	0	25
Chorion	105	0	105
Case 4	46,XY	47,XY,+12	
Chorionic villus cells			
– direct preparation	0	23	23
– long-term cultures	15	0	15
After termination			
Amnion	50	0	50
Fetal tissue (fibroblast)[a]	59	0	59
Chorion	48	2	50
Case 5	46,XX	47,XX,+mar	
Chorionic villus cells			
– direct preparation	0	15	15
– long-term culture	20	0	20
After term delivery			
Cord blood	25	0	25
Amnion	25	0	25
Chorion	25	0	25

[a] Cultures established and analysed from cartilage, tongue, brain and kidney

Confined chorionic mosaicism detected by CVS

The cytogenetic results of three completely studied cases are summarized in Table 3.

In case 3 mosaicism 46,XX/47,XX,+13 was detected on direct preparation of chorionic villi. Long-term culture failed to produce any dividing cells. Amniocentesis revealed a normal female karyotype in all analysed colonies. The pregnancy continued to term and cytogenetic studies of cultured cord blood, amnion and chorion failed to show cells with trisomy 13.

In case 4 all analysed and available metaphases in the CVS direct preparation showed trisomy 12, while long-term cultures revealed only a normal male karyotype. Cultures initiated from multiple fetal tissues (cartilage, tongue, brain and kidney) and amnion yielded only diploid karyotypes. However the presence of trisomy 12 was confirmed in 4% of analysed cells from primary cultures of chorionic villi.

In case 5 all analysed metaphases from the direct CVS preparation revealed an aneuploid female karyotype with a metacentric marker chromosome, slightly smaller than chro-

mosome 20, consisting of a larger C-band positive centromeric region and a small amount of euchromatin on both arms. Long-term CVS cultures showed only 46,XX cells. After term delivery analysis of cultured cord blood, amnion and chorion yielded normal diploid cells.

Detailed morphological examination of fetuses (cases 1 and 4) from pregnancy terminations showed no developmental defects. The examination of newborn infants (cases 2, 3 and 5) also revealed well-developed, non-malformed newborns.

Discussion

The existence of confined chorionic mosaicism with its expression restricted only to the cytotrophoblast, the extraembryonic mesoderm, or both of these lineages, with a complete absence of mosaicism in the embryo, reflects the complexity of pre-implantation and early post-implantation placental development. Placental development starts at day 6 post-fertilization when the implanting blastocyst invades the endometrium in the area of polar trophectoderm as two cell populations: cytotrophoblast and syncytiotrophoblast. These cells also form primary villi between days 6–9 with the syncytiotrophoblast externally located and the cytotrophoblast internally situated. Secondary villi develop between days 9 and 18 by migration of cells from both extra-embryonic mesoderm and primitive embryonic streak into the villous core. Tertiary villi are characterized by the appearance of primitive capillaries in the villous core around day 18. Chorionic villi at the time of CVS have a structure of tertiary villi and are, therefore, derived from three different cell lineages: polar trophectoderm, extra-embryonic mesoderm and primitive embryonic streak. Although both extra-embryonic mesoderm and the embryo proper originate from the inner cell mass (ICM), in the case of a mosaic blastocysts, it is possible that the cells of the ICM that give rise to extra-embryonic mesoderm may have a different karyotype from the cells migrating from the primitive streak of the embryo proper. The reason for this is that only a small number of cells (3–8) from ICM are selected as progenitors of the embryo proper (Markert and Petters 1978; Boyd and Hamilton 1970). This obviously was the situation in both cases of confined chorionic mosaicism detected on amniocentesis described in this report. The villus core cells with a diploid karyotype originated from embryo proper and aneuploid cells from extra-embryonic mesenchyme.

The study of confined chorionic mosaicism is not only complicated by the complex embryonic derivation of placenta from three different lineages but also by its further development during its maturation. For example, the cell type used for direct preparation for CVS, cytotrophoblast, is the predominant cell in primary placental villi and is an actively dividing cell in the secondary and young tertiary villi. However, by the fourth month of pregnancy the cells of cytotrophoblast become attenuated, lose their appearance as a continuous layer and become difficult to demonstrate histologically. Using special immunohistochemical techniques the existence of this cell type can be documented in term placentas, but the cells appear inactive, sparse and non functional (Boyd and Hamilton 1970). Confirmation of the presence of an abnormal cell line in the cytotrophoblast that was identified at 10–12 weeks of gestation becomes an impossible task at the end of the pregnancy when these trophoectoderm-derived cells lose their proliferative capacity. Therefore, it is not surprising that no trisomic cells were found in cultures established from term placenta in case 3 and 5. On the other hand, trisomy 12, also confined to the cytotrophoblast in case 4, could be confirmed after termination at only 14 weeks gestation in epithelial cells in dishes harvested in situ.

First trimester prenatal cytogenetic diagnosis, based on direct preparations or preparations using a short incubation period (24–72 h), uses actively dividing cells of the cytotrophoblast. Therefore, using these techniques, it is the trophectoderm lineage that is being studied. When long-term culture of minced villi is employed, generally the initial outgrowth morphologically resembles the epithelial cells of the cytotrophoblast, but this initial growth is soon replaced by fibroblasts which become the dominant cell population. After trypsinization and harvesting long-term cultures, the cells used for cytogenetic diagnosis are derived from the extra-embryonic and embryonic mesoderm. As these two different methodologies use three different cell lineages for cytogenetic analysis, it is not surprising that unexpectedly large numbers and unusual types of aneuploid cell lines are detected in mosaic chorionic villi.

It is likely that use of both direct method and long-term cultures of chorionic villi for prenatal cytogenetic analysis will uncover a spectrum of previously unsuspected cases of chorionic chromosomal mosaicism. In these cases some chromosomal abnormalities will be confined to the trophectoderm, others to extra-embryonic mesoderm, yet others may be expressed in all lineages contributing to the development of placenta but not be present in the embryo/fetus proper. For this reason it is mandatory that any mosaicism detected by either direct or cultured method on CVS be followed up by amniocentesis or by fetal blood sampling to rule out fetal involvement.

Although doing both direct preparations and long-term cultures for first trimester prenatal diagnosis is labour intensive and expensive, detailed cytogenetic studies in pregnancies with different types of confined mosaicism combined with careful obstetrical follow up will probably result in a better understanding of the placental role in second and third trimester intrauterine death of unknown cause, unexplained intrauterine growth retardation and possibly many other such conditions.

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