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Chromosomal Mosaicism in Amniotic Fluid Cell Cultures

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SUMMARY

Over the past 6 years, using in situ processing methods, we have identified 32 cases of mosaicism in amniotic fluid cell cultures prepared from 1,100 samples. Two of these (45,X/46,XX and 46,XX/47,XX,+21) were called true mosaics because multiple colonies demonstrated the same abnormal chromosome complement, and on subsequent evaluation of the newborn blood or fetal tissues, mosaicism was confirmed. Of the remaining cases, 29 were designated as pseudomosaics because only single or partial colonies exhibited an aberrant chromosome complement, 12 having a trisomy 2 line. In the final case, a double trisomy was demonstrated in only one of eight colonies in the first culture, but in the culture from a repeat sample an additional two colonies showed the same double trisomy. Since no abnormal cells were observed in infant blood, it was postulated that the mosaicism may only have been present in the extraembryonic tissues. It is our conviction that the use of these cloning methods should diminish the danger of misdiagnosis in genetic amniocentesis.

INTRODUCTION

A major problem facing laboratories involved in prenatal diagnosis is the identification of chromosomal mosaicism [1–16]. In only a few cases has the mosaicism diagnosed in utero been confirmed in the fetus or newborn infant [17–21]. In situ processing of cultured amniotic fluid cells enhances the possibility of distinguishing between true and

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pseudomosaicism, since mitoses can be analyzed from multiple colonies derived from multiple culture vessels. In this study, true mosaicism is defined as mosaicism which is present in the cells of the fetus and/or the extraembryonic tissues, whereas pseudomosaicism is probably the result of in vitro change. In pseudomosaicism, the cells with an abnormal karyotype are not representative of the chromosome constitution of the fetus.

MATERIALS AND METHODS

Three plastic centrifuge tubes (Corning 25310) of amniotic fluid are obtained from each patient. The first tube has approximately 2.0 ml of initial aspirate which may contain some blood. This sample is used to prepare slides for Y-chromatin screening [22]. The fluid in the remaining two tubes, usually 10–20 ml, is centrifuged at 25 *g* for 10 min, the supernatant removed, and the cells resuspended in 4.0 ml of medium (McCoy's 5A modified, supplemented with 5% fetal calf serum and 13% human cord serum). Then 0.5 ml of thoroughly mixed suspension is carefully deposited on a 22 sq mm coverslip in each of eight 35 mm plastic tissue culture dishes (Falcon 3001), the drop being confined to the coverslip. The dishes are placed in a 37°C (5%) CO_2 incubator and 1.0 ml of medium is added to each dish after 48 hr incubation. The dishes are checked microscopically every 2 to 3 days when the medium is changed. When small colonies of actively dividing cells are observed, usually by 6 to 14 days, the processing procedure is initiated by adding colcemide (final concentration 0.0025 μg/ml) and continuing the incubation for 3 hr. Keeping the dish level, the medium is carefully removed from the edge of the dish with a pasteur pipette. Two ml of 0.8% sodium citrate is added slowly down the side of the dish and allowed to stand at room temperature for 20 min. An equal volume of freshly prepared fixative (1:3 acetic acid:methanol) is slowly added to the hypotonic solution, and after 2 min the total liquid volume is carefully removed. Fixative (2.0 ml) is added to the dish and removed after 20 min, and a second fixation is carried out for a similar interval. Following removal of the fixative, the coverslip is blown on directly three or four times, and the dish inverted over the lid to allow excess fluid to drain off. Finally, the coverslip is stained by a modification of the Seabright trypsin technique for G-banding [23].

Initially, up to 15 mitoses are analyzed from multiple colonies derived from at least three coverslips. If a mitosis with an abnormal chromosome complement is found, all analyzable mitoses in that colony are checked, and if all demonstrate the same abnormality, mitoses from as many other colonies as possible are evaluated.

RESULTS

In a series of 1,100 samples processed by the above in situ methods, 32 cultures (3%) demonstrated some degree of mosaicism. In this laboratory the criterion used for defining true mosaicism has been the presence of two or more colonies from different culture vessels with the same unique chromosome complement. Where the aberrant complement was confined to a single colony, or part of a colony, the interpretation would be pseudomosaicism.

In two cases more than one colony was found to have the same aberrant chromosome complement. These cases were therefore interpreted as true mosaicism (table 1). The first case was referred because of a maternal age of 42 years. Following the analysis of the amniotic fluid culture, the couple was counseled with respect to what was known and what was not known about the prognosis for an individual with X/XX mosaicism, and they elected to continue the pregnancy. At term, a female infant was delivered demonstrating none of the physical findings associated with Turner syndrome. A blood sample was obtained by heel stick, and the mosaicism found in the amniotic fluid was

TABLE 1

CASES OF TRUE MOSAICISM

	Chromosome constitution	Amniotic fluid colonies	Mitoses examined
			Newborn Blood
Case 1	45,X	13	9
	46,XX	11	11
Total		24	20
			Placental Tissue
Case 2	46,XX	33	2
	47,XX,+21	3	38
Total		36	40

confirmed in the lymphocytes with roughly the same proportions of mitoses having the two karyotypes.

The second case, mosaic for trisomy 21, was also referred because of maternal age, being 41 years old. The couple elected to have the pregnancy interrupted following counseling about the amniotic fluid results. This was accomplished by hypertonic saline injection. A grossly normal fetus was delivered, and specimens were obtained for cell culture from a variety of tissues. The sample from the placenta was the only one that yielded any cell growth, and the mitoses examined demonstrated the same mosaicism found in the amniotic fluid culture. The relative proportions of the two cell types, however, were reversed. The two mitoses with a normal complement were each found in preparations from different initial culture dishes. This second case emphasizes the fact that the relative proportions of normal and abnormal cell lines found in the amniotic fluid in true mosaicism will not necessarily represent the proportions found in fetal or placental tissues, and therefore no valid statement can be made relative to the ultimate prognosis based on the proportions of chromosomally normal vs. abnormal cells in amniotic fluid.

In neither of these cases did a single colony contain both cell lines in the amniotic fluid cultures, confirming our belief that the colonies were derived from single cells.

Twelve of the remaining 30 cases had mitoses with an extra no. 2 chromosome (table 2). In all cases, these mitoses were present in only one colony. In some instances where only a few mitoses could be analyzed, they were all trisomic for chromosome 2. In others at least one mitosis in the colony had a normal complement, and these are designated as having a partial colony with the trisomic complement.

An additional 17 cases demonstrated a variety of different mosaic constitutions (table 3). Again, only single or partial colonies demonstrated the abnormal complement.

All live-born infants with pseudomosaicism (tables 2 and 3) have been phenotypically normal. In the seven cases that we have thus far been able to karyotype postnatally, the analysis of 50 mitoses has shown no mosaicism.

The final case presented special problems. The mother was referred because she was 40 years old. On the initial amniotic fluid culture one mitosis demonstrated a double trisomy, having an extra chromosome 7 and an extra chromosome 14. No other mitoses

TABLE 2

CASES DESIGNATED AS PSEUDOMOSAICS DEMONSTRATING TRISOMY OF CHROMOSOME 2

Case No.	Mitoses	Colonies	Trisomy 2 status
3	10	4	1 Mitosis
4	20	8	Partial colony
5	10	5	1 Mitosis
6	20	10	2 Mitoses (1 colony)
7	15	12	3 Mitoses (1 colony)
8	15	14	Partial colony
9*	18	11	2 Mitoses (1 colony)
10	18	7	Partial colony
11*	13	4	Partial colony
12*	15	8	Partial colony
13	15	7	Partial colony
14*	15	6	1 Mitosis

* Postnatal karyotype normal.

in the colony were analyzable, and the other seven colonies examined revealed normal male chromosome complements. According to our criteria, this case would be designated as a tentative pseudomosaic. The alpha-fetoprotein level of this amniotic fluid was slightly elevated, and a repeat sample was requested for that reason. In view of the chromosome findings, repeat cultures were established from this second sample. Two of 12 colonies presented with the same double trisomy that was identified in the original culture (table 4). This result from the repeat sample changed the interpretation from pseudomosaicism to true mosaicism. The parents were notified of these results and of our concern about the double trisomy. They elected to continue the pregnancy, a

TABLE 3

CHROMOSOMAL CONSTITUTION OF AMNIOTIC FLUID PSEUDOMOSAICS EXCLUDING TRISOMY OF CHROMOSOME 2

Chromosome constitution	Mitoses	Colonies	Aberration status
−G,+mar	15	12	2 Mitoses (1 colony)
i(Dq)	18	11	2 Mitoses (1 colony)
+20	13	5	2 Mitoses (1 colony)
t(X;15)	16	9	Partial colony
+3	15	11	Partial colony
+11	20	18	2 Mitoses (1 colony)
+6	30	16	Partial colony
t(9;14)*	15	9	2 Mitoses (1 colony)
t(1;13),+t(1;13)	13	9	2 Mitoses (1 colony)
+4	15	12	3 Mitoses (1 colony)
+16*	15	13	Partial colony
+16	15	12	Partial colony
t(5;5)*	15	6	Partial colony
+7	15	6	Partial colony
+19	19	6	Partial colony
+1	17	11	Partial colony
+7	15	6	Partial colony

* Postnatal karyotype normal.

TABLE 4

KARYOTYPIC FINDINGS ON AMNIOTIC FLUID OBTAINED FROM TWO SEPARATE TAPS REVEALING CONSISTENT MOSAICISM FOR THE SAME DOUBLE TRISOMY

CHROMOSOME CONSTITUTION	AMNIOTIC FLUID COLONIES		MITOSES EXAMINED
	Tap 1	Tap 2	Infant blood
46,XY	7	10	100
48,XY,+7,+14	1	2	0
Total	8	12	100

decision in which we supported them. The baby was delivered at 42 weeks gestation, was meconium stained, and weighed 5 lbs. 4 oz. Apart from being small for gestational age, he appeared phenotypically normal and has developed normally for the first 4 months. The baby's current karyotype, in an evaluation of 100 lymphocyte mitoses, was found to be normal. It is possible, therefore, that the mosaicism was confined to the extraembryonic tissues. In screening 40,000 newborns for X-chromosome aneuploidy using amnionic membrane, we observed similar instances where the newborn blood did not reveal the anticipated mosaicism indicated by the membrane evaluation ([24] and A. Robinson, unpublished results). Extraembryonic chromosome mosaicism has also been reported in spontaneous abortion material [25].

DISCUSSION

Chromosomal mosaicism in amniotic fluid cell cultures has been described by several authors, but only in relatively few cases has the mosaicism been confirmed in the fetus or newborn. Cox et al. [5] were the first to point out the usefulness of in situ processing as an aid in differentiating true from pseudomosaicism, and in situ methods have been described and/or advocated by a number of other authors [7, 18, 26–30]. The occurrence of trisomy 2 mosaicism in 12 of 29 cases in this series suggests that this may be relatively common in amniotic fluid cell cultures. One of the pseudomosaics identified by Cox et al. [5] also involved a trisomy 2 cell line. To our knowledge, trisomy 2 has been found only in spontaneous abortions (Warburton et al. [25] found the incidence of trisomy 2 second only to that of trisomy 16 in a series of karyotyped spontaneous abortions), and neither a complete trisomy 2 nor a mosaic trisomy 2 has been found in a live-born infant. It is of interest, in view of our finding of preferential malsegregation of chromosome no. 2 and the incidence of trisomy 2 and trisomy 16 in abortion material [25], that both these chromosomes have been demonstrated to have a fragile site in the long arm with resultant selective endoreduplication or malsegregation of the terminal segment [31–33].

The interpretation of mosaic findings should always be carried out with caution, but the results of this study can be utilized to develop some useful guidelines if in situ processing is used. 1. A diagnosis of true mosaicism may be made when two or more colonies from different culture vessels demonstrate the same chromosome abnormality. 2. A diagnosis of pseudomosaicism may be made when the aberrant chromosome

complement is found in a single colony together with normal mitoses. This was the case in over half of the analyses in this study where isolated colonies were involved. 3. A more tentative diagnosis of pseudomosaicism should be made when a single colony with a chromosome anomaly is identified. One can feel more secure, however, if many more colonies can be checked.

This method also aids in ruling out maternal cell contamination, since the more colonies that are checked, when a 46,XX complement is identified, the greater the possibility that at least some colonies are derived from fetal cells. The maintenance of colony integrity also eliminates the overgrowth of tetraploid cells which has been a frequent occurrence. Since the cultures are usually processed within 6–14 days of initiation, in vitro changes can be kept to a minimum.

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