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Aneuploid Correction and Confined Placental Mosaicism

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INTRODUCTION

Uniparental disomy (UPD) can be caused by various genetic mechanisms such as gamete complementation, chromosome duplication in a monosomic zygote or postzygotic aneuploid correction. This latter mechanism has been recently well documented in human reproduction and seems to be strictly related to placental mosaicism. We have therefore studied some aspects of confined placental mosaicism (CPM) which are useful to clarify one of the most common sources of UPD in humans.

Abnormal distribution of chromosomes in postzygotic mitotic cell divisions may result in a mosaic condition with two or more cell lines showing different chromosome constitutions. The effects on fetal phenotype and pregnancy development depend on the chromosomes involved, the distribution of the abnormal cells among tissues and on the precise stage at which chromosome mutation occurs.

As shown in Fig. 1, when the mutational event occurs in the blastocyst, prior to the differentiation of embryonic and chorionic compartments, the mosaicism is found in both the placental and fetal tissues. In contrast, when the chromosome mutation occurs at a later stage, after embryonic and chorionic compartment separation, the abnormal cells may be confined to the placenta or to the embryo, and are not necessarily found in both.

The possibility that mosaicism might be confined to extraembryonic tissue was first suggested by Ford [2] in 1969 who described an aneuploid cell line in the fetal membrane of the mouse. In 1978, Warburton et al. [3] found an unexpectedly high frequency of mosaic autosomal trisomies in cultures from spontaneous abortions. They suggested that these findings, as well as the relatively high rate of mosaicism in amniotic fluid cell cultures, which are usually not confirmed in the newborn, could be related to cells of extra-fetal origin. In fact, as reported by Priest et al. [4] in 1977,

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there is evidence that the most common cell type found in amniocyte cultures is derived from the fetal membranes. Early diagnostic application of chorionic villus sampling (CVS) for first trimester fetal karyotyping revealed discrepancies between chorionic villi cells and the embryo proper [5]. The general experience, using chorionic villi, is that abnormal cell lines are encountered more frequently in placental tissues than in the fetus itself.

Various hypotheses have been advanced by Gosden [6] to explain how this may occur, such as the ability of embryonic tissues to limit the proliferation of chromosomally abnormal cells or the possibility that specific chromosome abnormalities could give a positive advantage to trophoblast cells at different stages of placental development. Another explanation, based on the demonstration by Markert and Petters [7] that in mammals the embryo proper develops from only 3 or 4 cells of the 64-celled blastocyst, is that mitotic non-disjunction or chromosome structural rearrangements could occur much more frequently in chorionic cells than in the embryo. This hypothesis is strongly supported by the higher incidence of false-positive than false-negative findings from CVS. Numerous studies report a frequency of false-positive results ranging from 1 to 2%. The incidence of false-positive with the direct method of analysis, using spontaneous metaphases of cytotrophoblast cells, is twice that found in long-term culture, in

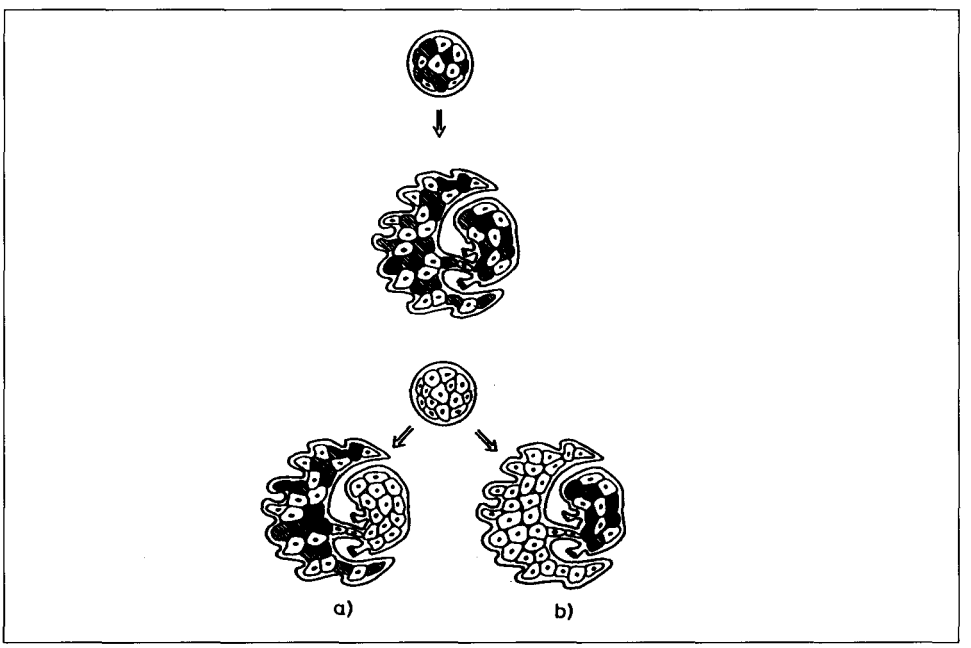

Fig. 1 - Diagrammatic representation of mosaicism involving both fetus and placental tissues a confined to the placenta *a*) or to the embryo proper *b*). The distribution of the mosaic is dependent on the time of chromosome mutation. (Modified of the mosaic is dependent on the time of chromosome mutation. Modified from Kalousek [1].

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which the metaphases scored are those of the mesenchymal cells (1.2 vs. 0.6% for a total of 11,855 analyses) [8]. False-negative results are found only exceptionally and have also been preferentially observed using the direct method of analysis. An estimate of their frequency compiled from 12,017 CVS derived from five series [9-13] can be given as 0.04%.

Effects of the presence of chromosomally abnormal cells in the placenta

Chromosome abnormality in chorionic villi, which is not confirmed in amniotic fluid cell cultures, is generally disregarded. However, despite a normal fetal karyotype, the presence of a chromosomally abnormal placenta could play a negative role in fetal development and/or pregnancy outcome.

The possibility of a positive association between chromosomal abnormalities in extra-fetal cells and growth retardation of the fetus was first suggested by Kalousek and Dill [14] in 1983, when they found two instances, among 46 pregnancies, of impairment of fetal development associated with a trisomic cell line in the placenta.

Recently, several cases have been reported in the literature suggesting a positive correlation between abnormal cells in the placenta and delay of intrauterine growth, fetal death, or poor perinatal outcome [15-19].

In an attempt to associate different types of CPM and intrauterine growth retardation, Kalousek [20] proposed in 1991 that CPM could be classified into three different types (Table 1). Type 1 is the most common. The aneuploid cells are detected in the cytotrophoblast (by the direct method) and the condition does not seem to have any influence on intrauterine growth. In type 2 CPM, the abnormal karyotype is confined to mesenchymal cells and it is revealed by the long-term culture method. This type of CPM is less common and has been associated with some cases of fetal growth retardation. Type 3 CPM occurs very rarely, when aneuploid cells are present in both cytotrophoblast and mesenchymal cells. Pregnancies with this type of placental mosaicism are frequently complicated by intrauterine growth retardation and fetal death.

Table 1 - Types of CPM in pregnancies with a chromosomally normal fetus

Tissue	CPM		
	type 1	type II	type III
Cytotrophoblast (direct or short-term method)	mosaic or nonmosaic aneuploidy	normal	mosaic or nonmosaic aneuploidy
Mesenchyme (long-term culture)	normal	mosaic or non-mosaic aneuploidy	mosaic or non-mosaic aneuploldy
Fetal tissue	normal	normal	normal

Modified from Kalousek [20].

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UPD in fetoplacental discrepancy

From a genetic point of view, the dichotomy between the karyotypes of fetal and placental cells may be explained by two main mechanisms. If the zygote is chromosomally normal (Fig. 2) an anaphase lag event may generate trisomic cells with one paternal or one maternal supernumerary chromosome. This may lead to differing effects on fetal development, depending upon the parental origin of the trisomic chromosome. When there is a trisomic zygote (Fig. 3), which has originated by paternal or maternal nondisjunction at the 1st or 2nd meiotic cell division, mosaicism will result from chromosome loss and the restoration of a 'normalized' diploid fetal karyotype.

A consequence of the latter mechanism could be the presence of uniparental disomy in the fetus. In such cases, we can assume that the effect on intrauterine growth could be related to both the parental origin of the disomy, and to the type of UPD involved, which can be either hetero or isodisomic [uniparental homologous pair or two copies of the same chromosome, respectively; ref. 21].

We performed a molecular study of eight cases of type 1 CPM where the presence of a trisomic cell line was revealed by direct chromosome preparations in cytotrophoblast metaphases only (Table 2). The analysis of short tandem repeat polymorphyisms in the fetus and both parents failed to reveal UPD in these cases; however, in the only case in which the trisomic cell line was available for this study, the presence of 3 different alleles demonstrated the original trisomic condition of the zygote (Table 3).

In 1993, Kalousek et al. [22] demonstrated the presence of maternal UPD in normal fetal cells of four cases with intrauterine growth retardation and trisomy 16 CPM of type III. A comparison of our findings with these seems to indicate that UPD could be related only to cases in which both cytotrophoblast and mesenchymal cells are homogeneously abnormal.

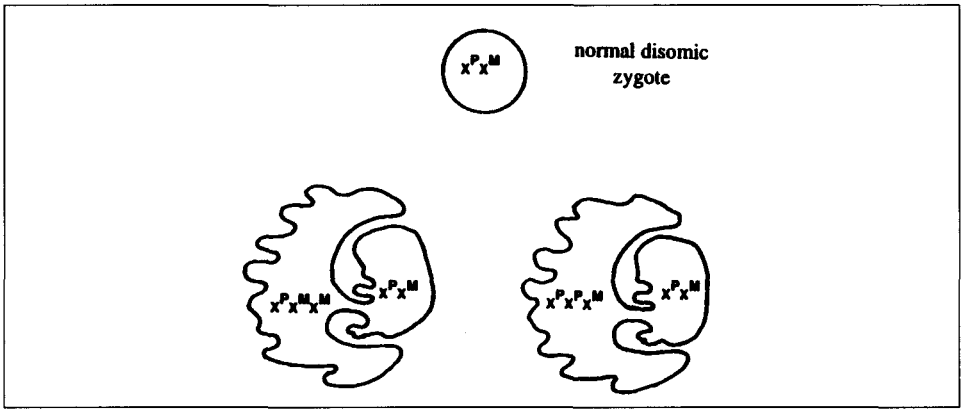

Fig. 2 - Possible mechanism by which a CPM could originate from a normal zygote. Chromosomal anaphase lag occurs at a late gestational stage, after fetal and placental compartments have separated. We can assume a different imprinting effect according to the maternal (M) or paternal (P) origin of the supernumerary chromosome.

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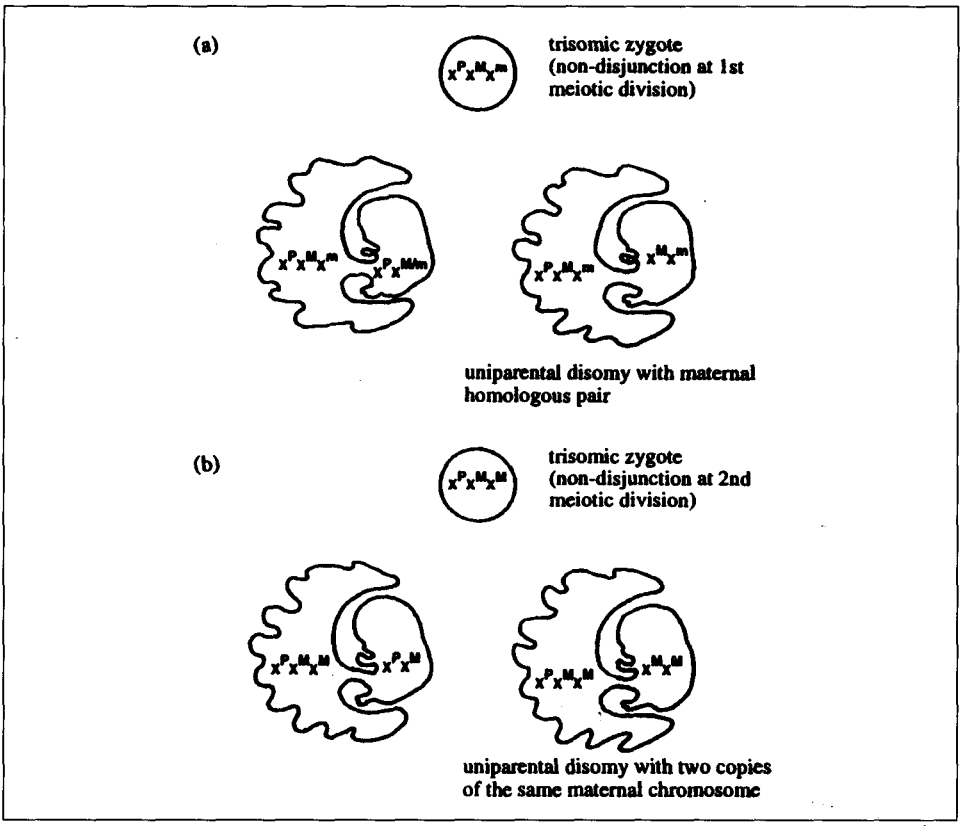

Fig. 3 - Possible mechanism by which CPM could originate from a trisomic zygote. The fetal karyotype could be "normalized" by a chromosome loss. We can assume different events would lead to uniparental heterodisomy *a*) or isodisomy *b*). M/m = Maternal; P = paternal.

Table 2 - Placental mosaics confined to the cytotrophoblast and revealed by the direct method of analysis

Case	
Case 1	mos 46,XX/47,XX,+8
Case 2	mos 46,XX/47,XX,+7
Case 3	mos 46,XX/47,XX,+8
Case 4	mos 46,XY/47,XY,+2
Case 5	mos 46,XX/47,XX,+13
Case 6	trisomy 47,XY,+20
Case 7	trisomy 47,XX,+16
Case 8	trisomy 47,XX,+15

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Table 3 - Molecular results from the eight cases of CPM

Case	System	Mother	Father	Zygote	Placenta
1	D8S85	1-3	2-2	1-2	1-2
	D8S88	1-3	2-4	1-2	1-2
2	D7S440	1-2	2-2	2-2	2-2
	D7S460	1-2	2-2	1-2	1-2
3	D7S461	1-1	2-2	1-2	1-2
3	D8S85	2-2	/-/	1-2	1-2
	D8S88	1-2	/-/	1-2	1-2
4	APO-B	3-3	1-2	1-3	1-3
	D2S102	2-2	1-3	2-3	2-3
	D2S103	2-2	1-1	1-2	1-2
5	D13S128	1-3	2-4	1-4	/-/
	D13S129	1-2	1-1	1-2	/-/
6	D20S66	1-2	2-2	1-2	1-2
	D20S75	1-2	2-3	1-3	1-2-3
7	D16S265	1-2	2-2	1-2	/-/
	D16S312	1-3	2-3	3-3	/-/
	D16S587				/-/
8	D15S102	2-4	1-3	1-2	/-/
	D15S104	1-3	1-2	1-3	/-/
	D15S87	1-2	1-3	1-2	/-/

/ = Not available.

Table 4 - True fetal mosaics revealed in amniotic fluid cell cultures

Case	
Case 9	mos 46,XX/47,XX,+14 (90%/10%)
Case 10	mos 46,XX/47,XX+21 (94%/6%)

In 1992, Cassidy et al. [23] suggested that aneuploid correction of a trisomic zygote involving chromosome 15 could be a cause of Prader-Willi syndrome in the fetus, both maternal chromosomes 15 being preserved in the "normalized" fetal karyotype. A similar mechanism could be responsible for other imprinted syndromes.

We studied two cases of true fetal mosaicism (as shown in Table 4) and were able to separate by in situ trypsinization pure trisomic and normal cell populations. In the euploid cell line of a fetus with mosaic 46/47,+14 karyotype, we found a chromosome 14

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Table 5 - Molecular results from the two cases of true fetal mosaicism

Case	System	Mother	Father	Diploid cells	Aneuploid cells
9	D14S43	2-3	1-3	2-2	2-2-3
	D14S49	2-2	3-1	2-2	2-2-1
	D14S50	1-2	2-2	1-2	1-2-2
	D14S51	1-2	2-1	1-1	1-1-1
	D14S59	1-2	2-2	1-2	1-2-2
10	D21S222	2-3	1-4	3-4	/-/
	D21S267	1-3	1-2	2-3	/-/

/ = Not available.

matemal disomy, demonstrating that UPD may occur not only in fetoplacental discrepancy, but also in the true mosaic condition (Table 5).

There are many unanswered questions regarding the possibility of UPD in chromosomal mosaicism. How many mosaics originate by chromosome loss from a trisomic zygote? Of these, how many with an apparently normal cell line hide UPD? Is the trisomic component of the mosaic the only cause of the phenotypic manifestations or is there a combined effect of the trisomic cells and the UPD? Understanding the role of UPD in mosaicism requires further analysis of polymorphic alleles in mosaics at prenatal and postnatal stages.

A new hypothesis: the meiotic origin of trisomic neoplasms

A final interesting point to consider in the hypothesis recently advanced by Haas and Seyger [24]. They suggest that specific trisomies found in tumors as the sole chromosome abnormalities, may represent tissue-confined residual cell populations of meiotic origin. The mechanism could be the same as that proposed to explain CPM. This hypothesis is in contrast with the current opinion that trisomies in tumors result from mitotic nondisjunction. Thus the trisomic cells in tumors may represent the original trisomic karyotype, whereas the apparently normal disomic karyotype may be acquired, corrected one.

To test this hypothesis, we investigated, by polymorphisms analysis, seven endometrial tumors where chromosome 10 trisomy has been found as the sole abnormality. In fact, molecular genetic analysis of trisomic neoplasms, originating from errors at the 1st meiotic division, could reveal polymorphisms not present in the constitutional karyotype. The results of this study are summarized in Table 6. In five cases, there was correspondence among the alleles of polymorphic loci of chromosome 10 comparing tumor and normal cells. In two cases, three or more alleles were found in tumor cells; however, a similar finding was also observed for several other chromosomes we studied by the same methodology. Our results could suggest a high variability of tandem repeat DNA sequencies in tumors rather than a meiotic origin of trisomic cells.

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In conclusion, we feel that detailed studies of some of the cytogenetic events occurring in the complex interaction of the fetoplacental system may be of great value for the study of some of the aspects of the origins and consequences of genetic imprinting.

Table 6 - Summary of molecular results obtained by DNA polymorphism analyses from seven endometrial tumors with trisomy 10 as the sole chromosal abnormality

	Case													
	2		5		6		7		18		19		20	
Probe	S	T	S	T	S	T	S	T	S	T	S	T	S	T
D10S173	2	2	2	4	2	2	2	2	1	1	2	2	2	3
D10S183	2	2	2	4	2	2	2	2	2	2	2	2	1	3
D10S89	2	2	2	3	1	1	1	1	1	1	2	2	2	4
D10S109	1	1	2	3?	2	2	2	2	2	2	1	1	1	2
D2S102	2	2	1	2	2	2	2	2	1	1	2	2	–	–
D2S103	2	2	2	2	1	1	–	–	2	2	1	1		
D7S460	–	–	1	1	–	–	–	–	–	–	–	–	1	1
D7S461	1	1	2	3	2	2	1	1	2	2	2	2	2	3
D7S440	–	–	1	2	–	–	–	–	–	–	–	–	1	2
MBC	–	–	1	1	–	–	–	–	–	–	–	–	–	–
YNC.64	–	–	1	2	–	–	–	–	–	–	–	–	–	–
LNS	–	–	1	1	–	–	–	–	–	–	–	–	2	2
D14S50	–	–	2	3	–	–	–	–	–	–	–	–	1	2
D14S51	2	2	2	3	2	2	2	2	1	1	2	2	1	2
D14S49	–	–	1	2	–	–	–	–	–	–	–	–	2	2
D14S59	–	–	1	2	–	–	–	–	–	–	–	–	2	3
D15S102	–	–	1	2	–	–	–	–	–	–	–	–	1	2
D15S104	2	2	2	3	1	1	2	2	1	1	1	1	2	3
D15S87	–	–	1	2	–	–	–	–	–	–	–	–	2	3
D16S265	2	2	2	3	1	1	2	2	2	2	1	1	–	–
D20S66	2	2	1	2	2	2	1	1	2	2	2	2	–	–
D20S75	2	2	2	2	2	2	2	2	1	1	2	2	-	–

Cases 5 and 20 show high microsatellite instability.

S = somatic cells.

T = tumor cells.

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