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# Placental Mosaicism and Intrauterine Survival of Trisomies 13 and 18

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## Summary

**Cytogenetic analysis of 14 placentas from live newborn infants or from terminated pregnancies with trisomies 13 and 18 revealed that all were mosaic. The mosaicism was confined to the cytotrophoblast and not detected in villous stroma, chorionic plate, or amnion. The percentage of cells with a normal karyotype varied from 12% to 100%, the average being 70%. No such confined mosaicism could be detected in 12 placentas of trisomy 21 fetuses. These findings suggest that a postzygotic loss of a trisomic chromosome in a progenitor cell of trophectoderm facilitates the intrauterine survival of trisomy-13 and -18 conceptuses. They also imply that it is placental function which determines the intrauterine survival and that the mother plays no active role in rejection of trisomic conceptions. The combination of both a pre- and postzygotic cell division defect in viable trisomy-13 and -18 conceptions points to the possibility of a genetic predisposition to such events. The detection of only a diploid cell line in the cytotrophoblast of some pregnancies with trisomies 13 and 18 also suggests that direct preparation is unreliable for prenatal diagnosis of these trisomies on chorionic villi sampling and that long-term villous culture should be used.**

## Introduction

At least 25% of all conceptuses are lost prior to implantation (Kline and Stein 1985). This high rate of loss continues in the early postimplantation period before pregnancy becomes clinically recognized (Edmonds et al. 1982), and a further 15% are lost between the sixth and 28th weeks of pregnancy (Stein 1981).

The reasons for embryonic loss before recognition of pregnancy are not yet known. However, as the incidence of chromosomal errors in sperm from normal healthy males is 8%–9% (Brandiff et al. 1985; Martin et al. 1987) and as that for human ova is estimated to be even higher (Wramsby et al. 1987), cytogenetic abnormalities derived from meiotic errors, together with defects arising at fertilization (triploidy) and due to abnormal cleavage (tetraploidy), are the most likely reason for the majority of both preimplantation and early postimplantation losses. Among pregnancies which become clinically recognized, the spontaneous losses of chromosomally defective conceptions are well recorded and are highest in the first trimester. About 50% of these are trisomies, 20% are monosomies, and 25% are polyploidy (Jacobs and Hassold 1987).

Trisomy for every chromosome except chromosome 1 has been described in spontaneously aborted conceptions. Some trisomies, such as trisomies 15, 16, 22, occur more commonly, while others, such as trisomies 5, 17, 19, are rare. In spite of the variation in frequency of their detection, chromosomal trisomies have one feature in common: they usually act as a lethal mutation for embryonic and fetal development. Only a few infants with nonmosaic trisomies 8, 9, and 22 have survived to term (de Grouchy and Turleau 1982). Of conceptions with trisomy 13 and 18, less than 5% are live born. For trisomy 21 the figure is 20%–35% (Bond and Chadley 1983, pp. 15–22).

What makes these few trisomic conceptions different from all the others and allows for their intrauterine survival? Studies of developmental defects of spontaneously aborted embryos and fetuses with trisomies 13, 18, and 21 show that in many cases their phenotypes consist of the same range of developmental defects as

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seen in live-born infants. For example, a spontaneously aborted embryo with trisomy 13 may show abnormal eye development, facial clefting, postaxial polydactyly, and major heart defects involving greater vessels (Kalousek and Poland 1984). The identical phenotype is characteristic of a live-born infant with trisomy 13. As there is no obvious difference in embryonic and fetal development of either aborted or viable trisomic conceptions, we decided to examine the placenta to explain the differences in intrauterine survival of chromosomal trisomies. Differences in placental function of trisomic and normal gestations have been described elsewhere (MacAfee et al. 1970). As the trophoblast layer represents the most active component in placental transport and hormonal production, we decided to study its chromosomal complement in detail and to compare it to that of villous stroma, amnion, and chorion and to the trisomic fetus.

## Material and Methods

We karyotyped direct preparations of cytotrophoblast and long-term cultures from villous stroma, chorion, and amnion from placentas of viable trisomic fetuses/newborns having gestational ages of 20–42 wk. In addition, cord blood and bone marrow specimens were processed for cytogenetic analysis. The fetuses/newborns were identified cytogenetically either by prenatal diagnosis, as trisomy 18 or 21, or were clinically recognized at term, as trisomy-13, -18, or -21 syndrome.

Cultures of peripheral blood lymphocytes were established from cord blood and were harvested according to standard cytogenetic techniques. Several explants of the chorionic villi, the chorion, and amnion from central and peripheral areas of each placenta were seeded on plastic petri dishes containing nutrient media and incubated at 37°C. After 2–3 wk cells were harvested in situ (Cox et al. 1974). A modified direct preparation from chorionic villi obtained from several sites was prepared using a 24–48-h incubation (Vekemans and Perry 1986). All chromosome preparations were G-banded.

At least 15 cells were analyzed for each tissue. A preparation was considered to exhibit true mosaicism if three or more normal diploid cells were identified. As half of the newborns and fetuses were males, sex chromosomes could be used to rule out maternal contamination as a source of diploid cells.

## Results

Fourteen viable trisomy-13 and -18 fetuses/newborns and their placentas, age 20–42 gestational weeks, were studied. All revealed placental mosaicism, involving a normal diploid chromosomal complement, confined to cytotrophoblast. There was no mosaicism in cells from villous stroma, amnion, chorion, or the fetus/newborn (table 1). In some cases of trisomies 13 and 18, no trisomic cells were detected in the cytotrophoblast. No evidence of any mosaicism was found in trisomy 21 in any of the studied tissues. The detailed analysis of cytotrophoblast cells is presented in table 2, and similar data for placental long-term cultures and amniotic fluids are presented in table 3. Table 4 summarizes results from short-term blood/bone marrow cultures from seven live borns having the typical phenotype of trisomies 13, 18, and 21.

## Discussion

These findings demonstrate the presence of chromosomal mosaicism confined to the cytotrophoblast in viable gestations, of age 20 wks and older, with trisomies 13 and 18. As the mosaicism was found in tissues

**Table 1**

**Gestational Ages and Details of Chromosomal Analysis of 26 Placentas and Viable Fetuses/Newborns with Trisomies 13, 18, and 21**

| Trisomy (*N*) | Gestational Age (wk) | Cytotrophoblast (% of Trisomic Cells) | Chorion/Amnion, Villous Stroma, Fetal Tissue, Cord Blood, and Bone Marrow (% of Trisomic Cells) |
|---|---|---|---|
| 13 (3) ..... | 37–40 | 0–67 | 100 |
| 18 (11) ..... | 20–40 | 0–88 | 100 |
| 21 (12) ..... | 20–40 | 100 | 100 |

**Table 2**

**Results of Cytogenetic Analysis of Cytotrophoblast from 26 Placentas Supporting Viable Fetuses/Newborns with Trisomies 13, 18, and 21**

| Gestational Age (wks) | Sex | No. of Analyzed Metaphases | | | | |
|---|---|---|---|---|---|---|
| | | Hypodiploidy | Pseudodiploidy | Diploidy | Trisomy | Total |
| Trisomy 13: | | | | | | |
| 40 | F | ... | ... | 15 | ... | 15 |
| 37 | F | ... | ... | 14 | 6 | 20 |
| 38 | M | 1 | ... | 7 | 18 | 26 |
| Trisomy 18: | | | | | | |
| 32 | F | 1 | ... | 14 | ... | 15 |
| 20 | M | 1 | ... | 2 | 23 | 26 |
| 20 | M | ... | 1 | 9 | 19 | 29 |
| 37 | F | ... | ... | 10 | 19 | 29 |
| 40 | M | 2 | ... | 3 | 9 | 14 |
| 20 | F | ... | 2 | 10 | 11 | 23 |
| 20 | M | ... | 1 | 7 | 20 | 28 |
| 20 | M | 1 | 1 | 4 | 17 | 23 |
| 22 | M | 1 | ... | 6 | 19 | 26 |
| 37 | M | 1 | ... | 6 | 5 | 12 |
| 38 | M | ... | ... | 9 | 10 | 19 |
| Trisomy 21: | | | | | | |
| 20–40[a] | 7M 14F | 9 | 9 | 1 | 221 | 240 |

[a] 11 cases of pregnancy termination because of prenatal detection of trisomy 21 and 1 case full-term newborn.

processed for modified direct preparation, it cannot be due to an artifact of tissue culture.

This is the first study of term placenta chromosomes that uses a modified direct method for harvesting cytotrophoblast. Although in the third trimester cytotrophoblast is described as inactive and sparse, it contains enough mitoses for meaningful analyses and interpretation.

Evidence collected from mice suggests that intrauterine survival of murine aneuploid conceptions is influenced by genetics of maternal environment (Vekemans and Trasler 1987). There is no evidence for the concept of maternal selection and rejection of aneuploids in humans (Warburton et al. 1983). The results presented in the present report suggest that in trisomies 13 and 18 fetuses a fine symbiosis between the development of embryo proper and placenta plays the most significant role in their intrauterine survival. It appears that in 5% of trisomy-13 and -18 conceptions mosaic placenta may facilitate complete embryonic and fetal development through functional compensation provided by diploid cells in cytotrophoblast, while the re-

**Table 3**

**Results of Cytogenetic Analysis of Cultured Amniotic Fluids and of Long-Term Cultures of Amnion, Chorion, and Villous Stroma from 26 Placentas Supporting Viable Fetuses/Newborns with Trisomies 13, 18, and 21**

| | No. of Analyzed Metaphases | | | | |
|---|---|---|---|---|---|
| | Hypodiploidy | Pseudodiploidy | Diploidy | Trisomy | Total |
| Trisomy 13 ($N$ = 3) | ... | ... | ... | 90 | 90 |
| Trisomy 18 ($N$ = 11) | ... | 1 | 1 | 523 | 525 |
| Trisomy 21 ($N$ = 12) | ... | 1 | ... | 364 | 365 |

**Table 4**

**Results of Cytogenetic Analysis of Short-Term Blood/Bone Marrow Cultures from Seven Liveborns with Typical Phenotype of Trisomy 13, 18, and 21**

| | No. of Analyzed Metaphases | | | | |
|---|---|---|---|---|---|
| | Hypodiploidy | Pseudodiploidy | Diploidy | Trisomy | Total |
| Trisomy 13 ($N$ = 3) .... | ... | ... | ... | 29 | 29 |
| Trisomy 18 ($N$ = 3) .... | ... | ... | ... | 26 | 26 |
| Trisomy 21 ($N$ = 1) .... | ... | ... | ... | 11 | 11 |

maining 95%, nonmosaic conceptions are spontaneously aborted prior to fetal viability. Although this statement cannot be supported by specific data, as there is no study comparing placental and embryo/fetal mosaicism in aborted trisomies 13 and 18, available data on direct analysis of cytotrophoblast from 150 first-trimester abortions does not indicate the existence of cytotrophoblast-confined mosaicism among any spontaneously aborted conceptions (Eiben et al. 1987; Yu et al. 1987; Gärtner et al. 1988).

A diploid cell line seen in the trophoblast in viable trisomies 13 and 18 is produced by postzygotic nondisjunction or anaphase lag. It is not clear whether this mutation represents a genetically influenced event in a conceptus which already carries a meiotic nondisjunctional error, or whether it is the result of an environmental insult to which the developing trisomic blastomeres were exposed in the fallopian tube prior to implantation. If it is assumed that all 5% of viable trisomy-13 and -18 conceptions have confined placental mosaicism, the incidence of nondisjunction/anaphase lag in their cytotrophoblast progenitors would be significantly higher compared with the frequency of cytotrophoblast-confined mosaicism seen at chorionic villi sampling (CVS) in chromosomally normal conceptions or in cytotrophoblast of viable fetuses with trisomy 21, a result pointing to a specific not yet understood mechanism.

A complete lack of mosaicism in cytotrophoblast of viable gestations having trisomy 21 is an interesting finding. It indicates that random chromosome loss through anaphase lag or nondisjunction is not a fairly common event in trophoblast and is governed by not yet discovered rules. It has been shown that tissue cultures from spontaneously aborted trisomies involving nonacrocentric chromosome are more likely to have a normal cell line than are those from spontaneously ahorted trisomies involving acrocentric chromosome (Stene and Warburton 1981). This finding cannot be applied to mosaicism in cytotrophoblast, as both mosaic and nonmosaic cases involve acrocentric chromosomes 13 and 21. A different mechanism for trisomy-21 intrauterine survival must exist, such as a less deleterious effect of trisomy 21 on placental function.

From a prenatal diagnosis point of view, the practical implications of these observations are simple. First, when only a direct preparation on CVS is used, prenatal diagnosis is unreliable for the detection of viable trisomies 13 and 18. This already has been shown in isolated reports of false-negative findings on CVS (Eichenbaum et al. 1986; Linton and Lilford 1986; Martin et al. 1986; Simoni et al. 1987; Wirtz et al. 1988). Second, any mosaicism detected by CVS can represent confined placental mosaicism and therefore requires confirmation by either amniocentesis or fetal blood sampling.

Discrepancies between cytogenetic findings in cytotrophoblast, villous stroma, and fetus have been reported in about 2% of pregnancies studied by CVS at 10–11 wk gestation (Simoni et al. 1985; Mikkelsen and Aymé 1987). These discrepancies can assume three different forms: type I—mosaic or nonmosaic aneuploidy in the cytotrophoblast associated with chromosomally normal placental stroma and fetus; type II—diploid cytotrophoblast and fetus but mosaic placental stroma; and type III—mosaic or nonmosaic diploidy in the cytotrophoblast, associated with nonmosaic aneuploidy in placental stroma and the fetus. Although in the current literature such findings are labeled as CVS discrepancies or pseudomosaicism, they exemplify constitutional mosaicism confined to placenta.

By definition, constitutional chromosomal mosaicism means the presence of two or more cell lines, with different chromosomal complements, in one individual. It originates in early embryonic development through nondisjunction, anaphase lag, or structural rearrangement. The resultant mosaic pattern in the conceptus depends

on many factors: the number of blastomeres at the time of mutational event, the cell lineage affected by the mutational event, and cell selection based on viability of mutant cells. Therefore, constitutional chromosomal mosaicism can be present in a generalized or lineage-confined form. In its generalized form, where every tissue in a conceptus is affected, it is a well-recognized entity and most likely originates from a viable mutational event in the first or second postzygotic division.

The existence of confined forms of constitutional mosaicism is less well known. Mosaicism can be confined to either placenta or embryo/fetus. Confined placental mosaicism (CPM) most likely results from viable mutations occurring in trophoblast or extraembryonic progenitor cells during the morula or blastocyst stage, while confined embryonic mosaicism originates after early implantation and initiation of development of the embryo proper from a designated small number of embryoblasts (Gardner 1978; Markert and Petters 1978; Kalousek 1985).

The precise role of CPM in intrauterine development cannot be determined yet. In type I an aneuploid clone in placenta will affect implantation and morphogenesis of placenta and, through placental function, the growth and development of the embryo/fetus. However, there are reports of pregnancies with type I CPM producing a normal newborn (Mikkelsen and Ayme 1987), as well as reports of the same condition being associated with intrauterine death or intrauterine growth retardation (Johnson et al. 1988; Kalousek 1988). Similarly, type II CPM has been described in perfectly normal pregnancies and in pregnancies with marked intrauterine growth retardation (Kalousek and Dill 1983; Kalousek et al. 1987). Type III as documented in the present report appears to provide the necessary biological protection for trisomy-13 and -18 conceptuses and to facilitate their intrauterine survival. From a research point of view, these findings raise tantalizing questions about both the cause of CPM and the significance of chromosomal mosaicism in placental morphogenesis and function. It is obvious that a large body of information must be collected regarding placental function in specific aneuploidies, to develop an understanding of how different types of CPM influence the obstetrical outcome of affected pregnancies.

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