ARTICLE

A placental diploid cell line is not essential for ongoing trisomy 13 or 18 pregnancies

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Viable trisomy 13 or 18 pregnancies may be supported by the presence of a diploid cell line, confined to the outer layer of the placenta (cytotrophoblast). To establish the presence of diploid cells we investigated five random biopsies from placentas of trisomy 13 (_n=8_) and trisomy 18 cases (_n=6_) of newborn infants and terminated pregnancies by means of fluorescence _in situ_ hybridisation on interphase nuclei (_n=100_). In 12 of these 14 placentas (including all five liveborns) 80% or more of the analysed nuclei showed three spots, suggestive of the presence of a full trisomy. In the other two placentas (both cases of trisomy 18) mosaicism was detected at most investigated sites. Thus, in contrast with earlier studies, these results show that a significant diploid cell line present in the placenta, confined to the trophoblast, is not a pre-requisite for intrauterine survival in the investigated cases._ European Journal of Human Genetics_ (2001) 9, 286–290.

Introduction

Next to Down syndrome, trisomy 18 (Edwards syndrome) and trisomy 13 (Patau syndrome) are the most frequently reported numerical aberrations. In cytogenetic surveys of spontaneous abortions or in early embryos trisomies for every chromosome have been described,[1] but many of these trisomies are lethal. Still, a number of them lead to liveborn deliveries. For trisomy 21, 20–35% survive to term, while less than 5% of the trisomy 18 cases and 2.5% of trisomy 13 cases are liveborn.[2] Kalousek _et al_[3] were the first to suggest that possibly only cases with mosaic or non-mosaic diploidy in the cytotrophoblast of these trisomy 13 or 18 conceptions might survive prenatally. Their hypothesis was based on the cytogenetic analysis of 14 placentas from terminated pregnancies or from live newborn infants between 20 and 42 weeks of pregnancy, having a trisomy 13 or 18. It was shown that all were mosaic, in contrast with 12 cases of trisomy 21, where no diploid cells were seen. The authors

speculated that the presence of diploid cells in the outer layer of the placenta might provide the necessary biological protection and significantly enhance intrauterine survival of foetuses with trisomy 13 or 18. The relative surplus of mosaic trisomy 13 and 18 cases (in comparison with trisomy 21) in first trimester chorionic villus sampling, as well as false-negative findings may also reflect a better survival in case of placental mosaicism.[4,5]

It has been shown that the chromosomal complement of the placenta may have an effect on foetal outcome: among pregnancies with a diploid foetus the presence of an abnormal cell line confined to the placenta showed correlation with an increased frequency of foetal intrauterine growth retardation.[6–9] Others, however, could not confirm this.[10] The majority (around 90%) of trisomy 13 and 18 cases are maternal-meiotic in origin.[11,12] When a diploid cell line is formed through trisomic rescue, there is a 1:3 chance that both chromosomes 13 or 18 are derived from the parent whose chromosomes led to the trisomy (usually maternal), the so-called uniparental disomy (UPD). The presence or absence of UPD might play a role in the phenotypic consequences of trisomy rescue.

The distribution of an aberrant cell line in a placenta may differ greatly,[13] so multiple biopsies should be analysed to establish whether a diploid cell line indeed is a determining factor in intrauterine survival of viable trisomy 13 or 18

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pregnancies. In case of diploidy we wanted to determine if the presence of a specific UPD would influence the viability of a pregnancy.

Materials and methods

Placental tissues were collected from eight pregnancies with trisomy 13 and six pregnancies with (mosaic) trisomy 18, detected at prenatal diagnosis or at birth. The clinical histories of these 14 pregnancies are summarised in Table 1. The mean maternal age in these cases was 34.7 years. When foetal tissue was investigated after birth or after termination of pregnancy the results are also given in Table 1. Cases 8 and 9 are twin pregnancies, in both cases the other child had a normal female (diploid) karyotype. The complete placenta and, if possible, blood of both parents was collected after informed consent. In case of a twin pregnancy both placentas were investigated. Five random biopsies of the placenta of 250–500 mg each were taken. Samples were washed thoroughly in Hank's balanced salt solution (Hank's BSS) containing heparin. One half of each biopsy was stored at $-20°C$ for DNA analysis and DNA was also extracted from blood of both parents.

From the other half, a suspension was made consisting mainly of cells derived from the cytotrophoblast.[13] In one case (trisomy 18, case 13) the trophoblast suspension was made from one half of the biopsy using the direct method, as in all other cases. The other half of the fresh biopsy was used to isolate mesenchymal cells. For this, trophoblast cells were removed by trypsin-EDTA treatment (0.05% in Hank's BSS for 20 min at $37°C$) and a mesenchymal cell suspension was made using collagenase-A (0.13% in HAM's F10, activity 0.715–0.78 U/mg).[14] From all suspensions, slides were made and FISH was performed according to standard procedures. In case of trisomy 13 pregnancies we used a probe obtained by Vysis (LSI 13 spectrum green, Vysis UK Ltd, Richmond, UK), which hybridises to the long arm of chromosome 13 (13q14). Placental biopsies of trisomy 18 pregnancies were hybridised with a centromere-specific probe (L1.84).[15] A chromosome 1 centromere-specific probe (pUC 1.77)[16] was used as a control on polyploidy. These probes were biotin-labelled and for detection avidin-FITC was used. Total human DNA was counterstained with propidium iodide. For each biopsy and each probe a minimum of 100 cells was scored and according to standard criteria, the proportion of nuclei with one, two, three and four or more hybridisation signals was determined. A site was considered to be fully trisomic when 80% or more of the nuclei displayed three signals (see Discussion).

Results

The results of the FISH analysis of the tested trisomy 13 and 18 placentas are summarised in Table 2. Only percentages of cells with two and three spots are given, the total of one, four and more spots never exceeded 9%, and usually was less than 5%; individual results are not shown. All trisomy 13 placentas, with the exception of one site in case 5, and four out of six trisomy 18 placentas were fully trisomic at all sites. In the other two trisomy 18 placentas the highest percentage of two spots was 45% (Table 2). For each biopsy a control

Table 1 Clinical histories of pregnancies with trisomy 13 (cases 1–8) or trisomy 18 (cases 9–14) detected in prenatal diagnosis

Case	Indication	Prenatal tissue	G.A. (weeks)	Prenatal diagnosis	Outcome (weeks, birth weight, percentile[a])	Karyotype tissue after birth/TOP	Remarks
1	US	AF	32	47,XY,+13[16]	LB (37; 2850 g, P25-50)	n.d.	† 1 day p.n., no obduction. Trisomy 13 features
2	36 years	AF	16.2	47,XY,+13[10]	TOP (20.5; 400 g)	n.d.	Trisomy 13 features
3	US	AF	32.4	47,XX,+13[16]	LB (36; 2020 g, P10-25)	n.d.	† 12 days p.n.
4	US	AF	24.4	47,XX,+13[10]	TOP (26; 510 g, P5-10)	47,XX,+13[8]	Trisomy 13 features
5	41 years, US	CV	14.2	47,XY,+13[10]	TOP (15; 65 g)	47,XY,+13[5]	Trisomy 13 features
6	US	AF	26.4	47,XX,+13[16]	LB (36.6; 2360 g, P25)	n.d.	† 3.5 h.p.n., no obduction. Trisomy 13 features
7	38 years, US	AF	16	47,XX,+13[16]	TOP (20.6; 255 g)	47,XX,+13[10]	Trisomy 13 features
8	US	–[b]	–	–	LB (33; 1215 g, P5)[c]	47,XX,+13[10]	† 7 days p.n. Twin pregnancy
9	40 years	AF	15.6	47,XX,+18[10]	LB (33; 1130 g, P2.3–5)[d]	47,XX,+18[10]	† 4 weeks p.n. Trisomy 18 features. Twin pregnancy
10	36 years, US	AF	21.2	47,XY,+18[10]	TOP (23.5; 300 g)	n.d.	Trisomy 18 features
11	TT	AF	17.1	47,XX,+18[21]/46,XX[6]	TOP (20; 240 g)	47,XX,+18[5]/46,XX[12]	† a few hours p.n. Minor trisomy 18 features
12	41 years	AF	15.1	47,XX,+18[9]/46,XX[14]	TOP (19.1; 187 g)	47,XX,+18[5]	Macerated foetus, no visible trisomy 18 features.
13	US	AF, UCB	23.4	47,XY,+18[2]	TOP (26.4; 725 g, P10)	n.d.	UCB: 47,XY,+18[10]
14	44 years	AF	15.4	47,XY,+18[10]	TOP (18.6; 165 g)	47,XY,+18[5]	No obduction

[a]Percentiles not given for pregnancies <25 weeks. [b]No invasive prenatal tests performed. [c]Birth weight normal child: 3380 g. [d]Birth weight normal child: 2000 g. AF: amniotic fluid; CV: chorionic villi; G.A.: gestational age; LB: liveborn; n.d.: not done; p.n.: postnatally; TOP: termination of pregnancy; TT: triple test indicated increased risk of aneuploidy; UCB: umbilical cord blood; US: ultrasound anomalies.

Table 2 FISH results of placental biopsies of trisomy 13 cases (cases 1–78, probe LSI 13) or trisomy 18 (cases 9–14, probe L1.84), sites with less than 80% of nuclei showing three spots are indicated in bold

| | Biopsy number | | | | | | | | | |
| | 1 | | 2 | | 3 | | 4 | | 5 | |
Case number	2 spots (%)	3 spots (%)	2 spots (%)	3 spots (%)	2 spots (%)	3 spots (%)	2 spots (%)	3 spots (%)	2 spots (%)	3 spots (%)
1	6	94	4	95	3	96	8	91	5	94
2	3	93	6	91	7	91	6	92	6	93
3	5	92	4	95	3	94	6	92	4	95
4	15	82	8	88	9	83	7	90	6	90
5	13	83	14	83	13	86	**20**	**76**	16	82
6	16	81	5	93	7	88	2	94	8	90
7	5	92	8	89	3	94	4	95	6	89
8[a]	10	86	8	89	11	89	9	86	11	86
9[b]	6	91	4	96	12	88	14	82	11	88
10	2	97	6	94	5	94	5	95	7	91
11	5	94	6	92	6	93	8	92	8	91
12	5	93	7	89	4	90	3	93	8	85
13[c]	**21**	**77**	–	–	**21**	**72**	17	80	**33**	**58**
14	**32**	**64**	**45**	**54**	**35**	**62**	**18**	**79**	12	88

[a]Normal twin showed two spots at all sites in 90–95% of the nuclei; [b]Normal twin showed two spots at all sites in 94–98% of the nuclei; [c]Mesenchymal cells showed three spots in 51–75% of the nuclei.

probe on chromosome 1 was used. For this probe the percentage of two spots ranged from 86 to 100%, and for three spots a range from 0 to 9% was seen. Separate data are not shown.

Discussion

In 12 out of 14 investigated placentas involving trisomy 13 or 18 in this study 80% or more of nuclei at the investigated sites displayed three signals, which can be regarded as indicative of non-mosaic trisomy (Table 2). In only one of these (case 5, trisomy 18) a slightly lower level was found at one site. In two pregnancies with trisomy 18, in case 13 (in cytotrophoblast cells and mesenchymal cells) and in case 14, a diploid cell line may have been present at most of the placental sites. These sites, with less than 80% of nuclei with three spots, are indicated in Table 2. The FISH results of the control probe did not indicate polyploidy in any case.

From other studies we know that when interphase FISH is performed the expected number of signals (two signals for autosomes in case of diploidy) is (most often) not present in 100% of the nuclei.[17,18] Results were considered abnormal when at least 60% of the cells were aneuploid,[19] others diagnosed aneuploidy or euploidy when at least 85% of the nuclei showed equal numbers of signals.[18] These figures highly depend on the type of tissue and probe. Ruangvutilert *et al*[20] attempted to assess probe efficiency regarding interphase detection of trisomies. In non-mosaic trisomic or triploid fibroblast cultures 80–89% of nuclei displayed the expected number of signals. Two signals were seen in most of the nuclei that did not produce three signals. FISH on trisomic interphase nuclei proved to be less accurate than on disomic nuclei, most probably due to an increase of signal overlap. Therefore, in the present study we regarded sites

with 80% or more nuclei with three spots as fully trisomic. In one of the mosaic cases (case 13) the diploid cell line does not seem to be confined to the outer layer of the placenta, for diploid cells were also observed in mesenchymal cells (Table 2). Unfortunately no mesenchymal cells were available for FISH in the other mosaic case (case 14).

Our findings are in contrast with earlier reports. Kalousek *et al*[3] investigated three placentas with full trisomy 13 (gestational ages between 37–40 weeks) and 11 placentas with full trisomy 18 (gestational ages between 20 and 40 weeks). Direct preparations of cytotrophoblast cells were karyotyped and all revealed mosaicism, involving a normal diploid cell line. The percentage of metaphases with trisomy 13 varied from 0 to 67% and for trisomy 18 from 0 to 88%. On average, in their study, in all investigated sites no less than 70% of the metaphases had a normal diploid karyotype. For the same patients full trisomy was seen in cultures from villous stroma, chorion and amnion. Since a diploid cell line was found in all cases the authors suggested that mosaicism confined to the trophoblast might facilitate intrauterine survival of such conceptuses. Data shown by Harrison *et al*[21] in another study performed in the same laboratory supported this idea. They studied 12 placentas from pregnancies with trisomy 18 (gestational ages between 14 and 40 weeks) using FISH. In five placentas only one site was investigated, in five others two samples were taken, and three and four sites, respectively, were investigated in the other two cases. Statistical analysis was used to determine the minimum level of mosaicism for chromosomal diploidy detectable by FISH. The upper 95% confidence interval was used to assign a cut-off value and a sample was considered to be mosaic when more than 23.4% of the nuclei revealed two spots. Using this threshold, seven out of 12 placentas were mosaic, including all five stillborn and liveborn cases of over 25 weeks of

gestational age, whereas no significant levels of mosaicism were detected in cytotrophoblasts from spontaneously lost pregnancies (n=2). In both studies[3,21] the authors suggested that viable trisomy 18 and 13 pregnancies required a diploid cell line in the cytotrophoblast. A summary of the results of both studies is shown in Table 3. Only the placental analysis of pregnancies of over 24 weeks of gestational age are given (respectively eight out of a total of 14 cases and five out of 12 cases), and these were all regarded to be mosaics. When comparing these data with our own results (Table 2) in cases over 24 weeks of gestational age (cases 1, 3, 4, 6, 8, 9 and 13), for which significant levels of diploidy could be expected, in only one of these seven cases the percentage of trisomic cells was less than 80%. The gestational ages in these cases are comparable and can not explain the discrepancy between our findings and earlier studies. Two cases of mosaic trisomy 18 seen at amniocentesis (cases 11 and 12), also showed full trisomy in the placental biopsies. Since no complete diploid sites were found, testing for uniparental disomy was not carried out.

Different methods were used in the present study and both other studies, but in our view, this fact can not explain the difference in outcome. Kalousek et al[3] used the direct method and analysed actively dividing cells, Harrison et al[21] enzymatically isolated trophoblast cells and used interphase FISH. We performed FISH on trophoblast nuclei, obtained by the direct method. From earlier studies on term placentas we know that with this method, cytotrophoblast cells can well be isolated.[13,22] In these cases where a chromosomal aberration was observed in first trimester trophoblast cells and a normal karyotype was seen at amniocentesis, we were able to confirm the abnormal cell line (often confined to one or few sites) in term placentas. In some biopsies up to 90% of the cells, analysed by FISH, showed the same abnormality as initially seen at first trimester, indicating that mainly cytotrophoblast cells had been analysed.

Our present findings are concordant with the results of a recent study by Moore et al.[23] They studied foetal tissues of different gestational ages (11–26 weeks) of four cases of trisomy 13, 10 pregnancies involving trisomy 18 (mean maternal age in case of trisomy 13 or 18 was 39.1 years) and seven cases of trisomy 21, using FISH. Slides were made from frozen tissues. In three trisomy 13 cases and nine trisomy 18 cases (gestational ages less than 24 weeks) a single placental biopsy was among the examined tissues. Most likely, because of the technique used, mesenchymal cells as well as trophoblast cells were scored together. In their study, however, confined placental mosaicism could not be ruled out. In only one out of 14 cases of trisomy 13 or 18 a diploid cell line was reported (in a placental biopsy of a trisomy 13 pregnancy).

On the basis of our data we conclude that a diploid cell line does not seem to be a crucial factor in intrauterine survival of trisomy 13 or 18 pregnancies. The term 'trisomic rescue' introduced for this phenomenon, and widely accepted as a possible explanation for survival in these cases does not seem to hold for the viable trisomy 13 or 18 cases presented in our study and 'survivors' are not mosaics. Moore et al,[23] too, concluded that tissue-specific mosaicism is not likely to be responsible for potential survival to birth. Considering our combined results, there still is a lack of understanding which mechanisms are involved in intrauterine survival of different trisomies, which may differ considerably. Each aneuploidy also shows a wide variability of major malformations so, other, as yet unknown factors may play a role in morphogenesis and may thus affect (intrauterine) survival.

Table 3 Summary of the results on placental tissue in viable trisomy 13 cases (cases 13-1, 13-2 and 13-3) or trisomy 18 cases (all other cases) of over 24 weeks of pregnancy in a study by Kalousek et al[13] (A) using conventional cytogenetics, and Harrison et al[21] (B) using FISH

	Case number	Gestational age (weeks)	% diploid	% trisomy
Study A	13-1	40	100	0
	13-2	37	70	30
	13-3	38	27	69
	18-1	32	100	0
	18-4	37	34	66
	18-5	40	21	64
	18-10	37	50	42
	18-11	38	47	53
Study B[a]	18-8	25	18.2–27.4	69.6–77.4
	18-9	38	27.7–41.6	53.1–69.6
	18-10	38	29.2–33.8	61.8–68.8
	18-11	Term	26.7–27.8	67.8–70.4
	18-12	Term	97.3–98.8	1.2–2.7

[a]Two or more placental sites were investigated: the lowest and highest scores are indicated.

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