

## Case reports

# Anaphase lag as the most likely mechanism for monosomy X in direct cytotrophoblasts but not in mesenchymal core cells from the same villi

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### Abstract

A 36 year old white female was referred for chorionic villus sampling for advanced maternal age. Direct (cytotrophoblast) preparations of chorionic villi were 45,X, but cultured mesenchymal core cells from the same villi were 46,XX. Study of embryonic and extraembryonic tissues showed the aneuploidy to be limited to cytotrophoblasts from specific placental sites. In aggregate, the cytogenetic findings can best be explained by anaphase lag during development of the cytotrophoblast, suggesting that this cytological mechanism and not non-disjunction is responsible for the common occurrence of monosomy X in villi.

Monosomy X (45,X) is observed relatively commonly in first trimester chorionic villi. The frequency is 1% in villi compared to 0.08% at amniocentesis. Several possible explanations for these differences have been proposed.<sup>1,2</sup> The difference could merely reflect the well known natural selection against 45,X fetuses as pregnancy advances. However, there could be an intrinsic tendency for 45,X cells to arise in extra-embryonic tissues like chorionic villi, compared to amniotic fluid cells which are shed from derivatives of the inner cell mass. We have encountered an instructive case in which monosomy X was limited to cytotrophoblasts from specific placental sites, but not mesenchymal core cells.

### Clinical findings

A 36 year old white female was referred for chorionic villus sampling (CVS) for advanced maternal age.

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With her first husband she had three healthy, liveborn children and two elective first trimester pregnancy terminations. With her second husband she had one healthy, liveborn child and one elective pregnancy termination. No family members had a birth defect or a history of multiple spontaneous abortions.

Ultrasound before CVS showed a single intra-uterine gestation with no demonstrable abnormalities. Consistent with menstrual dates, the crown-rump length was 42 mm, representing the mean for a gestational age of 11.1 weeks. Transcervical CVS yielded approximately 30 mg chorionic villi. Using the technique described elsewhere,<sup>3</sup> trypsin-EDTA was used to dissociate cytotrophoblasts from mesenchymal core. The dissociated cytotrophoblasts were used for direct analysis, whereas mesenchymal core cells from the same villi were used to initiate cultures.

Direct preparations available 24 hours later showed a 45,X chromosome complement in all 15 metaphase spreads available for analysis. The patient was informed of this result and counselled to await results of mesenchymal core cells before making definite decisions concerning pregnancy management. However, she elected to terminate the pregnancy before the results of cultured villi were available.

### Cytogenetic findings

All 15 metaphases examined from direct preparation of villi obtained at the time of CVS showed a 45,X chromosome complement. However, 15 cells derived from mesenchymal core cultures of the *same villus specimen* were all 46,XX. That all direct cells were 45,X and all cultured cells were 46,XX cannot reasonably be explained by random sampling of a mosaic complement, the probability of observing such results being extremely low. Thus, the abortus and many sections of the placenta were subjected to further cytogenetic studies. Villi were obtained from three placental sites selected to maximise the distance between the sites. Villi from all three sites were independently processed, using both direct and culture techniques on the same villi. Cultures were also initiated from fetal skin and amnion.

## Results of cytogenetic examination.

Tissue	No of cells with karyotype	
	46,XX	45,X
Initial CVS		
Direct cytotrophoblast cells	0	15
Cultured mesenchymal cells	15	0
After termination		
Villi placental site No 1		
Direct	0	20
Culture	22	0
Villi placental site No 2		
Direct	0	6
Culture	15	0
Villi placental site No 3		
Direct	18	0
Culture	30	0
Amnion culture	30	0
Fetal skin culture	50	0

Results of cytogenetic analysis from these various preparations are shown in the table. All villus cultures were 46,XX, as were fetal skin and amnion. By contrast, direct preparations from CVS and two other placental sites showed 45,X. Direct preparations from the third placental site showed a normal chromosome complement (46,XX).

## Discussion

In this case, 45,X was confined to cytotrophoblasts (direct preparations) from certain areas of the placenta; cytotrophoblasts from another area of the placenta as well as cultures of mesenchymal core cells, fetal skin, and amnion were 46,XX.

The frequency of cytogenetic discrepancies between chorionic villi and fetal tissue is 0.7 to 2%.<sup>2 4-7</sup> Discrepancies between direct and culture CVS analyses have been calculated to be 0.54%.<sup>2</sup> In the US collaborative study, 3884 cases were studied by the direct method; only 23 cases showed an abnormal 'mosaic' chromosome complement.<sup>2</sup> Of these 23, analysis of fetal tissue was available in 10 cases, with none corroborating the mosaicism. Other discrepancies involving 45,X complement have been reported.<sup>2 8 9</sup> In most reported discrepancies, CVS interpretation was based on either direct cytotrophoblast preparations only or on cultured mesenchymal core cells only, or both. In the latter, villi were divided into two groups, direct analysis being performed on some villi and cell cultures initiated on other villi. By contrast, our methodology of performing direct and culture analyses on the same villi is different, allowing us to derive information not available by other methods. We first observed that direct preparations of cytotrophoblasts obtained from one site of the placenta differed in chromosome complement from cytotrophoblasts obtained from other sites. However, no mosaicism was observed. That we used the same villus for both direct cytotrophoblast and cultured mesenchymal core cells and failed to find mosaicism may be relevant to frequent

claims of 45,X/46,XX 'mosaicism'. If cases similar to the present case had been studied by the aforementioned methods,<sup>2 8 9</sup> spurious diagnosis of aneuploid (45,X) or mosaic (45,X/46,XX) complements could have been obtained. Thus, analysing cytotrophoblast and mesenchymal core cells from the same villi is more informative than using either cell type alone or analysing separated batches of villi.

An extension of the frequently cited model of Kalousek<sup>4</sup> could be used to explain our findings. Assume the zygote was 46,XX. Anaphase lag in one of the progenitor cells of the primary villi (trophoblast) would result in a 45,X complement. Progeny of that 45,X cell could form cytotrophoblasts, specifically in those areas from which direct preparations showed the 45,X complement. Cytotrophoblasts from other areas of the placenta, mesenchymal core cells, and fetus itself would then be expected to show a normal 46,XX complement. Mitotic non-disjunction seems a far less likely explanation for 45,X because such an event would also result in formation of an accompanying 47,XXX line. No cells with a 47,XXX complement were detected in the villi or in other tissues examined from this fetus.

We emphasise that couples should be counselled that sex chromosome aneuploidy in direct preparations from cytotrophoblasts may well not be representative of the fetal sex chromosome complement. In these situations, patients should be counselled to await results of mesenchymal core cell cultures before making decisions concerning pregnancy management.

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