Identification of C trisomies in human abortuses*

JOËLLE BOUÉ, ANDRÉ BOUÉ, CHRISTIANE DELUCHAT, NICOLE PERRAUDIN,
and FRANCINE YVERT

Centres d'Etudes de Biologie Prénatale, INSERM Groupe, U.73, Château de Longchamp 75016—Paris, France

Summary. Fifteen cell lines initiated from embryonic tissues of abortuses with C trisomy were frozen and stored. They were thawed and karyotyped again with banding techniques. Trisomies 7, 8, 9, 10, and 12 were identified. Some characteristics of these abortuses are discussed in connection with the chromosome identifications.

The high frequency of chromosome anomalies in early spontaneous abortions had been clearly demonstrated. Recent studies (Boué and Boué, 1973a; Kajii *et al*, 1973; Therkelsen *et al*, 1973) have found about 60% of the products of these abortions to exhibit abnormal karyotypes. Many years of work were required to collect a sufficient number of specimens to permit epidemiological studies and statistical evaluation of these anomalies (Boué *et al*, 1974b). Thus most of the chromosomal analyses were carried out before the banding techniques for the identification of human chromosomes were developed.

From 1966 until 1972, 1498 abortuses were karyotyped in our laboratory and 921 chromosome anomalies were identified using the standard Giemsa staining technique for chromosome analysis. As far as technically possible, cell lines were initiated from primary cultures of embryonic cells and then stored frozen in liquid nitrogen. This material permitted retrospective identification of the precise chromosomes involved in the anomalies using banding techniques.

In this paper the identification of some C trisomies are reported.

Materials and methods

Cell lines. Fifteen cell lines with C trisomy which were initiated from embryonic tissues between 1966 and 1973 were stored frozen in liquid nitrogen. Details on the initiation and the storage of cell lines have been published elsewhere (Boué *et al*, 1968).

Tissue culture and chromosome preparation. After thawing, cells were cultivated first in Petri dishes in a CO₂ incubator then trypsinized and grown in glass prescription bottles. The medium used was BME Eagle with 10% calf serum. Chromosome preparations were made by conventional methods. Chromosome analysis was made on each cell line using three techniques. (1) The conventional Giemsa staining technique; (2) the Q-banding technique using quinacrine mustard staining; and (3) Giemsa staining after trypsin treatment. The G-banding technique routinely used in the laboratory (Boué and Boué, 1973b) was derived from Seabright's method (1971).

Results and discussion

The results of the analysis of the 15 cell lines with C trisomy are shown in Table I. In twelve C autosomal trisomies, Trisomies 7, 8, 9, 10 and 12 were identified among 12 cases of autosomal trisomy. In one case (523) a pericentric inversion of chromosome 9 is associated with trisomy 8. The pericentric inversion of chromosome 9 was transmitted by the father who is homozygous for this pericentric inversion and whose parents were unrelated. Fig. 1 shows the details of the C chromosomes in some of these autosomal trisomies after trypsin treatment. Other series were recently reported with the identification of 15 (Kajii *et al*, 1973; T. Kajii, personal communication, 1974), 10 (Therkelsen *et al*, 1973), and five (D. H. Carr, personal communication, 1974) abortuses with autosomal C trisomy. Two observations of trisomy 7 were also reported (Kuliev *et al*, 1973; McCreanor *et al*, 1973). These results are summarized in Table II. Thus all the autosomal C trisomies have been observed in abortuses. The most

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TABLE I

Karyotype	Case No.	Maternal Age (yr)	Previous Term	Pregnancies Aborted	Length of Pregnancy (wk)	Developmental Age of Embryo
47,XX,7	247	26	0	0	9	28 dy
47,XX,7	1184	30	0	3	13	35 dy
47,XY,8	523	38	2	4	9	25 dy
47,XX,8	1541	34	1	1	10	3 mm
47,XX,9	214	43	0	1	12	40 dy
47,XX,9	516	21	1	0	9	35 dy
47,XY,9	1154	33	1	0	11	30 dy
47,XX,9	1453	25	1	1	16	28 dy
47,XX,9	2875	25	1	0	9	38 dy
47,XY,10	1233	46	0	0	12	42 dy
47,XX,10	1595	31	1	0		42 dy
47,XY,12	1631	42	0	1	10	2.5 mm
47,XXY	1089	32	0	1	16	1.5 mm
47,XXY	1554	30	0	0	13	42 dy
47,XXY	3011	38	8	0		25 dy

FIG. 1. Partial karyotypes of C-group trisomies 7, 8, 9, 10, and 12 observed in abortuses. Giemsa staining after trypsin treatment.

frequent being apparently trisomies 7, 8, 9, and 10; but it is not possible to try to evaluate the relative frequency of each trisomy C in abortuses on the basis of these few observations.

In three of our cell lines the extra C chromosome was identified as an X chromosome. Before the Q-banding technique became available, the 47,XXY karyotype was difficult to identify with certainty in abortion specimens. A karyotype with 47 chromosomes, including 16 C chromosomes, five G

chromosomes, and a positive sex chromatin with one Barr body could be classified either as 47,XXY or as 47,XX,G+ in the absence of male phenotypic characteristics. Fluorescent staining allows the retrospective identification of these cell lines.

As precise identification of the extra chromosome in trisomies detected in human abortuses was not possible until the development of the banding techniques the analysis of different morphological and epidemiological features of these abortions was

TABLE II
AUTOSOMAL C TRISOMIES IN ABORTUSES

Extra Chromosome	Present Study	Kajii	D. H. Carr, personal communication, 1974	Therkelsen <i>et al</i> (1973)	Kuliev <i>et al</i> (1973)	McCreanor <i>et al</i> (1973)
6		1				
7	2	4	2	2	1	1
8	2	3	2	3		
9	5	1		3		
10	2	3	1	2		
11		2				
12	1	1				

difficult. With the classification of these anomalies in chromosome groups, trisomies involving different extra chromosomes were grouped together. This was particularly confusing in the C group which involved the X chromosome as well as seven autosomal pairs.

In the present study it must be emphasized that the C trisomies identified represent only a selected fraction of the C trisomies which were observed during a systematic study of early spontaneous abortions. In 921 abortuses with chromosomal aberrations, 86 C trisomies were observed; in 73 of these, macroscopic and microscopic pathological examination was possible. Thirty-eight were blighted ova with no detectable embryonic formation, 20 had embryos of less than 25 days' development, and in 16 the embryonic development was more than 25 days (Boué and Boué, 1973a).

From these results it is clear that autosomal C trisomies lead to early developmental arrest before any embryonic formation becomes evident in more than 50% of the specimens collected. Since initiation of cell lines from primary cultures of amniotic or chorionic cells is not possible, the retrospective identification of the extra chromosome on frozen cell lines selected the specimens in which there was an embryonic formation and in which the initiation of a cell line was successful. During these studies we found that the lifespan of cell cultures was related to the developmental age of the embryo (Boué *et al*, 1975) and consequently long-term cell cultures were more successful when the developmental age was longer.

In most of these cases a microscopic examination of the embryo was not possible, these embryos are so small that a choice between tissue culture or histological examination had to be made.

Nevertheless some differences seem to emerge; developmental age was shorter in trisomies 7, 8, and 12 and longer (28 to 42 days) in trisomies 9 and 10

(see Table I). The embryo with trisomy 7 studied by Kuliev *et al* (1973) was only 6 mm long.

Microscopic examination of the placentas was carried out on these specimens by Philippe (1974). The main characteristics were rare villi of small size mostly without blood vessels, underdeveloped trophoblast, and Breus' mole. In some cases large cytotrophoblastic cells were seen in the mesenchymal core of the villi. When the developmental arrest of the placenta could be determined it was found to occur before the developmental arrest of the embryo itself (case 1541: 19 days versus 25 days for the embryo; cases 1554 and 1555: 28 days versus 42 days for the embryos). A study of the growth characteristics of the cell lines with this trisomy has shown a mean doubling time of 50 hours (Cure *et al*, 1974); this decreased rate of cell growth may explain the decreased growth of the placenta.

In the epidemiological study on abortions the mean maternal age in the group of 441 trisomies was 31.3 (± 0.6) years, but variations were observed between the different groups of trisomies, trisomies D and G having a higher maternal age (32.5 and 33.2, respectively). The mean maternal age for 72 C trisomies was 30.9 (± 1.6) (Boué *et al*, 1974). In these 15 observations which belong to the whole group of 72 C trisomies the mean age was 32.9; the mean maternal age of the five trisomies 9 was 29.4, but the number of observations is too small to make comparisons.

Pearson *et al* (1973) have observed two chromosome 9 bodies in about 2.5% of human spermatozoa reflecting the high rate of meiotic non-disjunction of chromosome 9 in male gametogenesis. In our abortion material the mean maternal age for abortuses with a normal karyotype (509 specimens) is 27.5 ± 0.4 . This age is the same as the mean age of mothers giving birth to a live-born infant in France.

The previous obstetrical events of the patients in this group are similar to those observed in the epidemiological study. Eight of 15 had one or more deliveries (49% in the whole study); seven of 15 had abortions (39% in the whole study). Six of these patients were followed after their abortion with C trisomy. Three had no subsequent pregnancies (cases 1233, 1541, and 1555), one had one child (case 523) and one had two children (case 1154); all these offspring were normal. One case (2130) had another spontaneous abortion, a blighted ovum which was karyotyped as 47,XX,G+ (sex chromatin positive).

In living subjects, autosomal C trisomies have been observed mainly in mosaics; recently some cases of apparently homogeneous trisomy 8 have been

reported (Casperson *et al*, 1972; Kakati *et al*, 1973; Jacobsen *et al*, 1974). Trisomy 8 like trisomies 13, 18, and 21 and monosomy X and 47,XXY may lead either to spontaneous abortions or to liveborn offspring. But some differences appear: in abortuses with trisomies 13, 18, and 21 and monosomy X and 47,XXY the arrest of development take place more frequently at 5–6 weeks, sometimes at 7 to 12 weeks or even during the second trimester of pregnancy showing different stages of development from early abortion to still birth and to liveborn subjects. In the abortuses with trisomy 8, however, the arrest of development occurred very early (fourth week) and in living subjects with apparently the same karyotype this anomaly seems much less deleterious than trisomies 13, 18, or 21.

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