Trisomy 8 restricted to cultured fibroblasts*

RAINER NISS† and EBERHARD PASSARGE

From the Institute für Humangenetik, Universität Hamburg, Hamburg, Germany

Summary. In the course of re-examining cultured fibroblasts stored in liquid nitrogen from a patient with developmental retardation, solitary left kidney, and Wilms tumour, a cell line trisomic for chromosome 8 was found. Trisomy 8 was restricted to fibroblasts in the first 22 subcultures and was absent in later passages as well as in lymphocytes. A familial pericentric inversion of chromosome 2 was observed in three generations including the propositus but was thought to be unrelated to the clinical problem. Multiple spontaneous chromosomal rearrangements were seen in several late subcultures.

This study reports the association of a Wilms tumour in the solitary kidney of a patient with trisomy 8. In addition, it describes unusual diagnostic difficulties encountered in correlating karyotype and phenotype owing to the restriction of the trisomic cell line to cultured fibroblasts in their first 22 subcultures, and the presence of an apparently unrelated familial pericentric inversion of chromosome 2, along with multiple chromosomal structural rearrangements presumably arisen in vitro.

Methods

This family came to light in 1969 when the propositus was referred at the age of 6 years for evaluation of developmental retardation of undetermined origin. A familial pericentric inversion of chromosome 2 had previously been diagnosed elsewhere (Dr. R. A. Pfeiffer, Münster), which was confirmed. The patient was subsequently seen on a few occasions before his death in 1973 at the age of 10 years.

Metaphase chromosome preparations from cultured lymphocytes were obtained in 1969 and 1972. Fibroblast cultures were established from a skin biopsy taken in 1972 and frozen in liquid nitrogen after their fourth subculture, as described elsewhere (Böhmer et al., 1973). Fibroblast-like cells were grown in Dulbecco's modified Eagle's medium (Grand Island Biological Co., New York), supplemented with 16.6% fetal calf serum (Gibco), 20 mmol HEPES as buffer, penicillin (100 units/ml), streptomycin (100 μg/ml), and neomycin (100 μg/ml). The cultures have not been tested for mycoplasma.

After mitotic arrest with Colcemid (0.1–0.3 μg/ml) for 2 hours and exposure to hypo-osmotic conditions (KCl 0.075 mol/l for 5 to 10 minutes in lymphocytes and NaCl 0.3%, for 10 minutes in fibroblasts), chromosomal Giemsa (G) bands were produced according to Seabright (1972) with the following modifications: 0.16% trypsin for 10 to 20 s (0.23% trypsin for 50 to 70 s in fibroblasts), rinse in ethanol 70% and 96%, Giemsa stain at pH 6.8 in Sorensen-buffer. In addition, preparations with standard Giemsa stain were available. Metaphases selected according to the quality of their banding pattern were studied directly by microscopy and from microphotographs.

Results

Clinical data. The propositus was born at 40 weeks gestation with a birthweight of 3500 g and length of 50 cm to a 23-year-old mother and 26-year-old father.

His subsequent clinical course revealed severe failure of both mental and physical development as summarized in Table I. When first seen by us at the age of 6½ years his height was 104 cm (−12 cm), weight 16 kg (−5), and head circumference 50.5 cm. He had an antimongoloid slant of his eyelids, a high arched round palate especially in its anterior part. A 2×2 cm occipital area showed nearly unpigmented hair. His mental development was clearly impaired and corresponded to that of a child of approximately 2 years of age. He was unable to speak. Sight and hearing appeared normal. Clinically and on x-ray his pelvis appeared narrow.

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**Pathological data.** Data, made available through the courtesy of Professor J. Oehme of Braunschweig Children's Hospital, revealed a large and invasive tumour conglomerate in the retroperitoneal area. The diagnostic impression was that this was an undifferentiated metastasizing malignant tumour of the retroperitoneal area whose points of origin could not be determined macroscopically. The histological picture suggested that it originated from an undifferentiated nephroblastoma (Wilms tumour), with the somewhat unusual feature of an even and diffuse infiltration of the solitary left kidney. A neuroblastoma as well as a reticulosarcoma could be excluded.

The right kidney was absent and the right renal artery was extremely hypoplastic overlaid with an abortive renal artery ostium. A hydroureter was present on the right. The foramen ovale was anatomically open but functionally closed. The right testis was in the abdominal cavity, the left in the inguinal canal. There was internal and external hydrocephalus.

**Family and genetic marker data.** The pedigree is shown in Fig. 1. Reproductive and developmental history in this kindred was unremarkable and physical examination of the proband's two sibs as well as the parents and grandparents did not reveal any evidence of a developmental anomaly. Eighteen autosomal loci were tested as genetic markers without evidence of irregular inheritance. The full data are available on request from the authors. The activities of 14 red blood cell enzymes were normal.
Cytogenetics. The cytogenetic results are summarized in Table II. In both lymphocytes and fibroblasts one chromosome 2 was replaced by a submetacentric chromosome, with a reduced centromere index, in all metaphases of the propositus and 4 relatives (Figs. 2 and 3). This was interpreted as a pericentric inversion of chromosome 2 (inv(2)p21q11). No loss of chromosomal material could be detected in standard as well as special stains. The banding pattern of the propositus and the other affected family members looked alike (Fig. 3). A detailed analysis of the G banding pattern in 12 metaphases indicated that the break points were located in region 2 band 1 of the short arm (p21) and region 1 band 1 of the long arm (q11).

Presence of vanishing cell line with trisomy 8 in cultured fibroblasts. During the course of studying the inversion with special staining procedures, metaphases of cultured fibroblasts from the propositus were found to contain 47 chromosomes with an additional chromosome of the C+X group. The G-banding pattern clearly showed that the additional chromosome was chromosome 8 (Fig. 4). Fibroblasts from the propositus stored in liquid nitrogen were recovered twice and analysed for the presence of an additional chromosome 8. In the first series chromosomal preparations were done after the 7th subculture after thawing.

In 20 metaphases analysed, an additional C-group chromosome was present, which could be identified as chromosome 8 in 14 cells owing to a clear banding pattern of this group.

Analysis of the second series confirmed the presence of an additional chromosome 8 in chromosomal preparations from early subcultures. As the number of subcultures increased, the number of cells with trisomy 8 decreased (Table II). The fibroblasts did not grow very well after the 17th

TABLE II
SUMMARY OF CYTOGENETIC FINDINGS IN PROPOSITUS

<table>
<thead>
<tr>
<th>Tissue Examined</th>
<th>No. of Chromosomes</th>
<th>Additional C Group</th>
<th>Total No. of Metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 46 47</td>
<td>Chromosome Present</td>
<td>Examined</td>
</tr>
<tr>
<td>Lymphocytes 1969 (age 6)</td>
<td>3 98</td>
<td>0</td>
<td>101</td>
</tr>
<tr>
<td>1972 (age 8)</td>
<td>2 101 1</td>
<td>0</td>
<td>104</td>
</tr>
<tr>
<td>Skin fibroblasts 1972</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thaw 1: passage 11</td>
<td>1 3 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thaw 2*: passage 2</td>
<td>7 (5)†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17 (33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22 (47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 (76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 (78)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All cells with less than 46 chromosomes (result of technical loss) discarded.
† Number of days from thawing to subculture. Chromosomal preparations of thaw 2 were done 2 days after subculture, except passage 23 when it was possible only after 7 days.

Pericentric inversion of chromosome 2 (inv(2)p21q11) present in all cells.
subculture and only 3 metaphases were available from chromosome preparations prepared from subculture 22 (47 days from thawing). Two of these 3 cells showed trisomy C (Table II). It appears, therefore, that the trisomic cells disappeared between the harvest of the 22nd and the 23rd subculture within 9 days, i.e. 51 days after the fibroblasts had been thawed out. All chromosomal preparations of thaw 2 were done two days after subculture, with the exception of subculture 23 which was the first without trisomic metaphases and took about a week to grow a sufficient number of cells. No trisomic cells were found in lymphocyte cultures (Table II).

**Structural rearrangements presumably arisen in vitro.** While analysing the metaphases from cultured fibroblasts of consecutive subculture (thaw 2, Table II), we found 9 different cells containing structurally abnormal chromosomes other than the pericentric inversion of chromosome 2, summarized in Table III. An additional screening of about 200 G-banded metaphases from passage 30 revealed 10 cells with structural alterations similar to those above (t(13p;Yq) in 2; 3q+ in 8).

**Discussion**

In this report the trisomic cells were restricted to cultured fibroblasts in their first 22 subculture generations, while none was found in lymphocytes. This restriction of trisomy 8 to cultured fibroblasts has not been recorded previously and raises obvious diagnostic difficulties. Furthermore, they disappeared rapidly from cultured fibroblasts and were absent after the 22nd subculture. One report (Neu, Bargman, and Gardner, 1969) has shown the disappearance of a cell line trisomic for a C group chromosome from blood in contrast to bone-marrow (fibroblasts not studied), while in another the proportion of trisomic cells increased in late fibroblast cultures (Chang, Niewczas-Late, and Uchida, 1969).

In other observations, trisomy 8 mixoploidy was present in both blood and skin, either without different distribution of the two cell lines or preponderance in blood (Bijlsma, Wijffels, and Tegelaers, 1972; Caspersson et al., 1972; Crandall et al., 1974; Grouchy, Turleau, and Leonard, 1971; Jacobsen, Mikkelsen, and Rosleff, 1974; Laurent et al., 1971) or with a relatively higher proportion in fibroblasts (Atkins, Holmes, and Riccardi, 1974; Crandall et al., 1974; Schaumann, Červenka, and Gorlin, 1974; Walnavens et al., 1974). Our observations indicate that lymphocyte cultures may fail to indicate the state of mixoploidy, contrary to evidence in trisomy 21 (Taysi, Kohn, and Mellman, 1970). Non-disjunction occurring in vitro (Martin, Sprague, and Bryant, 1967) could not be formally excluded.

Presumably the familial pericentric inversion of chromosome 2 seen in our case is unrelated to the

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**TABLE III**

**TYPES OF CHROMOSOMAL STRUCTURAL REARRANGEMENTS PRESUAMABLY ARISEN IN CULTURE (THAW 2 OF TABLE III)**

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Passage No.</th>
<th>Type of Alteration</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>Abnormal chromosome</td>
<td>Inversion?</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>14q+</td>
<td>Trisomy 8</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>14q+</td>
<td>Fig. 5b</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>3q+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>3dic(3p+)</td>
<td>Fig. 5a, cell with 60 chromosomes</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>3q+; chromatid break in other 3p; + ace</td>
<td>Fig. 5c and 6b</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>3q+; t(13p;22q)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>t(13p;Yq)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>t(13p;22q); 13q+</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 5. Abnormal chromosomes 3 (see Table III).**
clinical problem and cannot be related to the phenotype. In other instances, pericentric inversion of chromosome 2 has been observed as a coincidental finding in phenotypically normal individuals (Jacobs et al., 1974; Weitkamp et al., 1969). Crossing over within the inverted segment, however, may lead to chromosomal imbalance, as has been observed in pericentric inversion of chromosome 13 (Parrington and Edwards, 1971; Taysi et al., 1973) (and our own unpublished observations). This apparently has not happened in the family described here, but should be expected in offspring of carriers of the inversion. Earlier, the concept of aneusomy by recombination has been invoked to explain phenotypic abnormalities in carriers of a cytologically inconspicuous inversion (Grouchy et al., 1966). The unspecific nature of the phenotypic finding in some of the observations (Grouchy et al., 1966; Therkelsen et al., 1973) makes one reluctant to accept here a concise karyotype-phenotype correlation. Our observation shows that it is possible to miss the actual anomaly if the important chromosomal aberration occurs as mosaic in only one of the tissues examined and if it is under selective pressure from the normal cells. The discovery of trisomic cells in our patient was a coincidental finding when re-examining fibroblasts in search for detectable chromosomal deficiencies or duplications in the pericentric inversion of chromosome 2 present in our patient. Up to this time we had considered the possibility that the abnormal phenotype in our patient could have been the result of aneusomy by recombination (Grouchy et al., 1966). Our study shows how precarious this interpretation would have been.

The observation of structural chromosomal rearrangements including translocations, presumably arisen in culture, deserves mention though it cannot yet be interpreted in a meaningful manner.

Spontaneous translocations occurring in cultured skin fibroblasts have not been reported often (Ellis, 1963; Ford, 1964; Kohn, Aronson, and Mellman, 1974). Kohn et al. (1974) related their finding to an infection with mycoplasma in culture. Mycoplasma infection has not been ruled out in our fibroblasts, but the rather ubiquitous event of mycoplasma infection and the presumably rare occurrence of spontaneous chromosomal rearrangements in cultured cells suggest that other mechanisms could be responsible. Structural chromosomal alterations of clonal origin have been described in cultured fibroblasts from patients with xeroderma pigmentosum (German, 1972), porokeratosis of Mibelli (Taylor, Harnden, and Fairburn, 1973), and in normal fibroblast cultures (A. M. R. Taylor, 1974, personal communication). We saw cells with seemingly identical structural anomalies as shown in Table III. The three trans-
locations involving chromosome 13 (Fig. 6) could have been viewed as of clonal origin from a common ancestral cell, had the special staining method not been applied and disclosed their difference. Furthermore, the identical appearing chromosomes 3 (3q +) were observed in cells with and without the 13p22q translocation, and one of the 13p22q translocations was seen in a cell with two normal chromosomes 3 (cell 9). Therefore, either the two D/G translocations must have arisen independently or the abnormal chromosomes 3 are not of clonal origin. Possibly all these observations are related to senescence of the cells.

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References


