Immunodeficiency, centromeric heterochromatin instability of chromosomes 1, 9, and 16, and facial anomalies: the ICF syndrome

PAOLA MARASCHIO*, ORSETTA ZUFFARDI*, TIZIANA DALLA FIOR‡, AND LUCIANO TIEPOLO*

From *Biologia Generale e Genetica Medica, University of Pavia; ‡Cattedra di Biologia Generale, Facoltà di Medicina, University of Firenze; and ‡Ospedale S Chiara, Trento, Italy.

SUMMARY Instability of the heterochromatic centromeric regions of chromosomes 1, 9, and 16 associated with immunodeficiency was found in a four year old girl. Similar phenotypic and chromosomal abnormalities were described in a previous patient studied by us and in four other published cases. All these patients have facial anomalies in addition to combined immunodeficiency and chromosomal instability. Stretching of the heterochromatic centromeric regions of chromosomes 1, 16, and to a lesser extent, 9 and homologous and non-homologous associations of these regions were the most common cytogenetic findings in all the patients. Multi-branched configurations and whole arm deletions of chromosomes 1 or 16 or both were also found.

Comparing clinical and chromosomal data we conclude that immunodeficiency, centromeric heterochromatin instability, and facial anomalies form a new syndrome, for which we propose the acronym ICF. A mutation interfering with the normal process of condensation of part of the centromeric heterochromatin is postulated as the basic chromosome defect in this syndrome.

At the 1978 Symposium of the European Society of Human Genetics two independent cases of immunodeficiency associated with chromosome multibranching were reported by Hultén and by Tiepolo et al and one of them was published in detail. Two similar cases have since been described by Fryns et al and Howard et al, and another has been recently found by G Valkova (1986, personal communication).

In all these patients, combined immunodeficiency was associated with facial anomalies and instability of the centromeric regions of chromosomes 1, 9, and 16. In the case of Hultén, centromeric instability was reported at first only in chromosome 1, but further analysis revealed the involvement of chromosomes 9 and 16 as well (M Hultén, 1986, personal communication).

We describe in detail a patient with similar chromosomal and clinical features and re-examine the clinical and cytogenetic findings of our first patient.

Case reports

PRESENT CASE

The proband was born in August 1981 to non-consanguineous parents, the mother aged 37 years and the father 68. The latter has agenesis of the gallbladder and one of his brothers developed bronchiectasis when young and died in adulthood from lung cancer. The older sister of the proband, born in 1972, is healthy. The patient was born at term by normal delivery with a birth weight of 3050 g. Up to two years her growth and development were normal. She started to walk at 16 months and the other milestones were normal.

From two years of age she suffered recurrent pulmonary infections and diarrhoea in the summer time. At the age of four years three months she was admitted to hospital with pneumonia. Her height was 98 cm (below the 25th centile) and her weight 13 kg (below the 3rd centile). Her face was dysmorphic with hypertelorism, flat nasal bridge, low set ears, and protrusion of the tongue.

Psychomotor development was not evaluated but she appeared ‘slow’.

Received for publication 19 January 1987.
Accepted for publication 20 January 1987.
Chest x-ray showed opacity in the lower left segment and tomography led to a diagnosis of bronchiectasis. Skull and paranasal sinus x-rays were normal. Content of sweat chloride was 47 mEq/l (normal values up to 60 mEq/l) and a slight hypochromic anaemia was found (Hb 9.9 g/dl). Blood levels of d-xylose after oral administration revealed normal absorption. Tests for autoantibodies gave negative results.

Quantitative determination of serum proteins showed a decrease in IgG (681 mg/dl, normal values 800 to 1800), IgA (4.7 mg/dl, normal values 90 to 450), and IgM (9.4 mg/dl, normal values 60 to 250).

In March 1986 she was again admitted to hospital with pneumonia and at that time a chromosome analysis was requested because of her dysmorphic facies.

THE CASE OF TIEPOLO ET AL.3
This patient died in 1978 and we could only re-examine his medical records, which showed that facial dysmorphism was present in this patient also. He is reported to have had a flat facies (prominence of malar and maxillary bones), freckles without telangiectasia, epicanthus, and a low nasal bridge. As reported, he had severe psychomotor retardation with an ataxic gait, choreoathetoid tremors, and progressive neurological degeneration. From three months of age he had recurrent respiratory infections and IgA and IgE deficiency was diagnosed. A brother died at three years after recurrent respiratory infections and a sister died at one year for unknown reasons.

CYTOGENETIC INVESTIGATIONS
In a first 72 hour blood culture in Difco medium, multi-branched configurations and breakages at the centromeric region of chromosome 16 were observed but not quantified because of the poor standard of the preparations. A second blood sample was cultured for 48, 72, and 96 hours in Difco and RPMI media. In these cultures, analysed with Q, C, and DA–DAPI banding, fragility of the centromeric regions of chromosomes 1, 9, and 16 were found (table 1). The most frequent abnormalities were various degrees of stretching, up to breakage, of the centromeric heterochromatin of chromosome 16 (fig 1 a–c). The long arm of this chromosome was deleted in some cells and duplicated in others, forming a multi-branched configuration (figs 1 f–j and 2).

Homologous and non-homologous associations of the centromeric regions of chromosomes 1, 16, and, less frequently, 9 were also found. In some cells these associations formed a single block of cen-
Immunodeficiency, centromeric heterochromatin instability, and facial anomalies

FIG 1 Stretching of the centromeric heterochromatin (a–c), whole arm deletions (d, e), and long arm duplications (f–j) of chromosome 16. (a, h) Giemsa staining; (b, d, e, i, j) Q banding; (c, f, g) DA–DAPI banding.

FIG 2 Multi-branched configurations of chromosome 16. (a, b) DA–DAPI, (c) Q, and (d) C banding.

tromeric heterochromatin (fig 3). In a proportion of cells one or more whole chromosomes appeared less spiralled than the others (fig 4). This phenomenon was present in 40 out of 225 cells (17.7%) from 48 hour cultures; in 72 and 96 hour cultures the frequency was 1/100 and 4/100 respectively. Identification of the despiralised chromosomes was possible in only a few cells and mainly in those in which only some of the chromosomes were despiralised. In these cases despiralisation of one or both chromosomes 2 was the most frequent finding.

In some of the cells, among the despiralised chromosomes, 1, 16, and 9 were identified sporadically (fig 4c). In the light of these findings, we re-examined the slides of our first case: 17/52 (32.6%) and 12/50 (24%) cells with despiralised chromosomes in 48 and 72 hour cultures were found, but identification of these chromosomes was not possible. In a control culture from a normal female using Difco medium, one cell with despiralisation was observed out of 250 analysed. No abnormality was found in fibroblast cultures from our patient and in blood cultures from her parents and sister.

Mitomycin C (MMC) induces chromosomal aberrations which in part overlap with those found in patients with centromeric fragility and immunodeficiency. We treated blood cultures in RPMI medium from our patient and from a normal subject with MMC, at concentrations of 50 and 80 ng/ml for 96 hours (table 2). This table shows the frequencies of the specific anomalies of chromosomes 1, 9, and 16 and of those found in all other chromosomes.

The frequency of centromeric associations, stretching, and multi-branching in the patient's cultures treated with MMC was from four (MMC 80 ng) to more than six (MMC 50 ng) times higher than
in treated cultures from controls after correction for the number of spontaneously occurring anomalies in cultures from the patient.

In the patient’s cultures the frequency of induced anomalies was also increased for chromosomes other than 1, 9, and 16, if compared with that found in treated controls: 1–7 and 2–3 times higher in cultures with 80 and 50 ng of MMC respectively.

Discussion

Anomalies of the centromeric regions of chromosomes 1, 9, and 16 were found in all patients shown in table 3, with the exception of the case of Howard et al,5 in which chromosome 9 was not involved. This chromosome was also less frequently involved than chromosomes 1 and 16 in the different anomalies in the other cases and never showed arm deletions or multi-branching. Associations, stretching, arm losses, and multi-branching of chromosomes 1 and 16 were observed in all cases, except in our present one, where chromosome 1 was never found to form multi-branching configurations.

Occasional cells with stretching of the centromeric region of chromosome 2 were found by Fryns et al4 and by G Valkova (1986, personal communication). We did not find this abnormality, although this chromosome was entirely decondensed in some cells of our present case (fig 4a, b). As shown in table 1, the overall frequency of anomalies in the present case varied both among cultures in different media and among different culture times. In Difco medium the frequencies of abnormal cells were generally higher than in RPMI; the main difference between the two media was found in 48 hour cultures, in which only 7% of the cells were abnormal in RPMI, while in Difco the frequency was 74%. At 72 and 96 hours the differences in the frequencies of abnormalities were less striking.

An increased frequency of abnormal cells with increasing culture times, as observed in our first patient, was found in the present case only from 48 to 72 hours in RPMI medium.

In our first patient and in those of Hultén1 and Howard et al,5 multi-branched configurations and arm losses or duplications were not present in 48 hour cultures, but this was not observed in our present patient, in whom these rearrangements were found mainly in 48 hour Difco cultures.

FIG 3  Associations of the centromeric regions of chromosomes 1/1 (a, b), 1/16 (c, d), 1/1/16 (e), 1/9 (f), 1/16 (g), and 1/16p (h). (a, c, e, f, g, h) Q banding, (b, d) DA–DAPI. In the latter the two centromeric regions appear fused in a single heterochromatic block.
Immunodeficiency, centromeric heterochromatin instability, and facial anomalies

We do not know if these differences in the frequencies of the various types of anomalies, both among cultures of the same patient and among different patients, are due to different selectivity of the medium used or to the fact that different media may selectively enhance the specific chromosomal abnormalities. For instance, a medium with no or very low levels of folic acid may, like the cases of folate sensitive sites, induce a higher frequency of chromosomal abnormalities. In fact, had we used only cultures in RPMI, we would probably have made a diagnosis of 16qh+ (the predominant abnormality in these cultures) and the true chromosomal situation of the patient could have remained undetected.

No abnormality was found in fibroblast cultures from our cases and from those of Hultén and Fryns et al. Only Howard et al. reported centromeric instability of chromosome 1 in 45% of the fibroblasts analysed.

Comparing the clinical data there is a high degree of similarity among the phenotypes of the six patients (table 4). Various facial abnormalities, mainly epicanthus, hypertelorism, and macroglossia, were reported for all of them. Developmental delay and recurrent infections were also present in all the cases. The immunodeficiency is always of the combined type, with reduced IgA, IgG, and IgM, the only exception being our first patient who had IgA and IgE deficiency.

FIG 4 Undercondensation of whole chromosomes. One chromosome 2 is undercondensed in (a); two chromosomes 2 and one chromosome 13 in (b); chromosomes 1, 9, and 16 in (c); and most of the chromosomes in (d). In a, b, and c arrows point to the undercondensed chromosomes (Q banding).
Both our present case and that of G Valkova (1986, personal communication) support the suggestion of Howard et al. that fragility of chromosomes 1 and 16 (and to a lesser extent 9), facial abnormalities, and combined immunodeficiency characterise a new syndrome. We examined several cases with combined immunodeficiency but without facial abnormalities (one of them familial) without finding any centromeric instability.

In an attempt to clarify the basic defect causing the chromosomal abnormalities found in these patients, we must consider that the centromeric regions of chromosomes 1, 9, 16, 15, and Y represent a special class of heterochromatin, characterised by positive staining with DA–DAPI, by highly methylated DNA, and by undercondensation and association in metaphase after treatment with 5-azacytidine.

This characteristic appears of particular interest, causing chromosomal abnormalities similar, at least in part, to those found in our patients. This cytidine analogue, incorporated into DNA during duplication, would interfere with DNA methylation, a probable prerequisite for normal chromosome condensation during mitosis.

According to Schmid et al. the persistence of a decondensed state of heterochromatic regions after DNA duplication would preserve their interphase somatic pairing and would therefore cause their association in metaphase. A defect in the structure of the heterochromatic regions similar to that caused by 5-azacytidine could explain all the anomalies found in our patients: in fact, undercondensed centromeric regions, in addition to homologous or non-homologous association in metaphase, can be prone to a high frequency of breakage and interchange among the associated chromosomes, leading to whole arm loss, to the fusion in a single chromocentre of the centromeric heterochromatin of associated chromosomes, and to multi-branched configurations.

However, the spontaneous anomalies found in the patients with immunodeficiency and those caused by 5-azacytidine are not exactly overlapping, mainly because in the former chromosomes 15 and Y do not show any abnormality.

This discrepancy could indicate either differences in the composition of heterochromatin between chromosomes 1, 9, and 16 and 15 and Y or that mitotic condensation of these two sub-groups of heterochromatin are under separate control. If a difference in the composition of heterochromatin between 1, 9, and 16 and 15 and Y exists, this does not seem to be in the classes of satellite DNA present; in fact, in chromosomes 9, 15, and Y all...
Immunodeficiency, centromeric heterochromatin instability, and facial anomalies

TABLE 3 Involvement of chromosomes 1, 9, and 16 in the anomalies present in patients with immunodeficiency and centromeric heterochromatin instability.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Chromosomes involved in metaphase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Association</td>
</tr>
<tr>
<td>1</td>
<td>1, 9, 16</td>
</tr>
<tr>
<td>3</td>
<td>1, 9, 16</td>
</tr>
<tr>
<td>4</td>
<td>1, 16</td>
</tr>
<tr>
<td>5</td>
<td>1, 16</td>
</tr>
<tr>
<td>G Valkova (1986, personal communication)</td>
<td>1, 9, 16</td>
</tr>
<tr>
<td>Present casea</td>
<td>1, 9, 16</td>
</tr>
</tbody>
</table>

TABLE 4 Clinical and laboratory data of patients with immunodeficiency and centromeric heterochromatin instability of chromosomes 1, 9, and 16.

<table>
<thead>
<tr>
<th>Hulténb</th>
<th>Tiepoloet al</th>
<th>Fryns et al</th>
<th>Howard et al</th>
<th>G Valkova (1986, personal communication)</th>
<th>Present casec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Parental age: mother, father</td>
<td>?</td>
<td>36, 37</td>
<td>26, 26</td>
<td>?</td>
<td>37, 68</td>
</tr>
<tr>
<td>Age at examination</td>
<td>5 y</td>
<td>11 y</td>
<td>3 mth</td>
<td>4 y</td>
<td>4 mth</td>
</tr>
<tr>
<td>Delivery</td>
<td>?</td>
<td>Term</td>
<td>Term</td>
<td>?</td>
<td>Term</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>?</td>
<td>3400</td>
<td>2600</td>
<td>2600</td>
<td>3400</td>
</tr>
<tr>
<td>Facial abnormalities</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Recurrent infections</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Immunodeficiency</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IgA</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>IgG</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>R</td>
</tr>
<tr>
<td>IgM</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cell mediated immunity</td>
<td>R</td>
<td>R</td>
<td>N</td>
<td>R</td>
<td>?</td>
</tr>
</tbody>
</table>

N=normal, R=reduced.

In conclusion, combined immunodeficiency, instability of the heterochromatcentromeric regions of chromosomes 1, 9, and 16, and facial anomalies seem to characterise a new syndrome. The chromosomal anomalies found in this syndrome could be due to an autosomal recessive mutation interfering with the normal process of condensation of part of the centromeric heterochromatin. We propose the acronym ICF for this syndrome.

We are grateful to Professor M Fraccaro for critical reading of the manuscript.

Note added in proof

We cultured a further blood sample obtained from our patient in January 1987 for 96 hours in 199 medium, to verify the effect of a low concentration of folic acid on the frequency of abnormal cells. As controls, lymphocytes were cultured in RPMI and

four classes of satellite DNA are represented, while in 1 and 16 only sat II is found.14

In Drosophila melanogaster, Gatti et al15 demonstrated that mitotic condensation of euchromatin and heterochromatin are under separate genetic control, having identified a mutant responsible for decondensation of all the heterochromatic regions in metaphase. It can be postulated that in man different sub-groups of heterochromatin are under the control of different genes for their condensation. In man heterogeneity in the time of duplication of heterochromatin has already been demonstrated.16 Heterogeneity in the control of condensation of heterochromatin could be an obvious consequence of this heterogeneity in time of duplication.

In our first case, a brother of the proband died in infancy after recurrent bronchitis and pulmonary infections. Also in the case observed by G Valkova (1986, personal communication), a brother of the proband was probably affected.
in Difco media. The frequencies of mitoses with anomalies were 62/200 (31%) in 199, 51/100 in RPMI, and 83/139 (60%) in Difco. These results do not support the hypothesis that the frequency of chromosomal abnormalities is related to the concentration of folic acid in the medium, as postulated on page 177.

The case quoted in this paper as a personal communication by G Valkova has been published in Clin Genet 1987;31:119–24.

References


Correspondence and requests for reprints to Dr Paola Maraschio, Dipartimento di Patologia Umana ed Ereditaria, Sezione di Biologia Generale e Genetica Medica, CP 217, I–27100 Pavia, Italy.
Immunodeficiency, centromeric heterochromatin instability of chromosomes 1, 9, and 16, and facial anomalies: the ICF syndrome.

P Maraschio, O Zuffardi, T Dalla Fior and L Tiepolo

doi: 10.1136/jmg.25.3.173

Updated information and services can be found at:
http://jmg.bmj.com/content/25/3/173

**Email alerting service**

These include:

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/