Clinical and Chromosome Studies in Fanconi’s Aplastic Anaemia

JOHN PERKINS, JOHN TIMSON, and ALAN E. H. EMERY

From Royal Albert Edward Infirmary, Wigan; University Department, Medical Genetics, Manchester; and University Department, Human Genetics, Edinburgh

A pernicious anaemia-like picture of the peripheral blood associated with congenital abnormalities was first described by Fanconi (1927) in three brothers. Since then a similar syndrome has been reported from many parts of the world under the title of Fanconi’s aplastic anaemia. Gmyrek and Syllm-Rapoport (1964) have reviewed 152 cases in the literature in considerable detail. Bloom et al. (1966) described gross chromosome abnormalities which appear to be present in all patients with this peculiar anaemia so far tested.

We present clinical and chromosome studies in a family (Fig. 1) in which two brothers suffer from Fanconi’s aplastic anaemia and their sister has typical congenital abnormalities and chromosome changes without the anaemia.

Materials and Methods

Twenty ml. of freshly drawn venous blood from each patient was added to 2 ml. of anticoagulant (heparin in dextran) in a sterile container and gently mixed. The erythrocytes were allowed to settle at 37°C, and the supernatant plasma and leucocytes were drawn off. The number of leucocytes per cu. mm. was determined, and for each patient cultures were set up in triplicate making the volume of each culture up to 10 ml. with TC 199 (Glaxo) so that the final concentration of leucocytes was about 10³ per ml.

Two drops of phytohaemagglutinin P (PHA, Difco) were added to each culture which was then incubated at 37°C for 72 hours. At this stage 0·2 ml. of demecolcine at a concentration of 1 mg./100 ml. of TC 199 was added to each culture; these were then incubated at 37°C for a further 2 hours. Each culture was then spun down at 2000 r.p.m. for 10 minutes and the supernatant discarded. The cells were resuspended in 3 ml. hypotonic saline, 0·25% (w/v), for 15 minutes at 37°C, fixed in acetic alcohol, resuspended in sufficient 45% acetic acid to give an opalescent suspension, spread on to cold slides, and air-dried. The preparations were then stained with 10%, Giemsa at pH 6·4.

Clinical Findings

Case 1. (III. 8, date of birth December 24, 1950.) This boy was first seen in the Paediatric clinic in April 1962 at the age of 11 years, because a full blood count, requested by the family doctor, had revealed an unusual anaemia. The initial blood count in February 1962 showed Hb 74%, but two months later it was 57%. The cells were macrocytic and there was a neutropenia: total white cell count (WCC) was 3000 per cu. mm., of which 40% were neutrophils and 60% were lymphocytes. The platelet count was only 80,000 per cu. mm. All his life his appetite was poor and in the last two years he bruised easily.

On examination he was found to be undersized (Fig. 2). Café-au-lait spots were prominent on the abdomen and there was a generalized pigmentation of the skin.

Further haematological investigations at this time revealed only pancytopenia, with a normoblastic marrow. Because of repeated bruising, splenectomy was carried out in October 1962. The spleen was a normal size (45 g.) but the platelet count after an initial rise fell to low levels again. He was treated with prednisolone 5 mg. t.d.s. but unfortunately with no effect.

In 1963 after an attack of haematuria he was investigated further, and it was found that his blood contained 10% of foetal haemoglobin and that the half-life of chromium-tagged red cells was reduced to 14 days (normal 24–32 days). Serum levels of iron, vitamin B12, and folic acid were all normal. There was no evidence of hypopituitarism, in that the kochesteroid excretion was normal and there was a normal water diuresis. The radiological bone age was normal. In March 1965 one of our colleagues, Dr. F. Hillman, suggested the correct diagnosis, and as a result the rest of the family were investigated.

The patient is maintained with transfusions of 3 or 4 pints of whole blood every three weeks, with the result that his Hb level varies between 33% and 66%. He does not complain of dyspnoea or tiredness and seems well adapted to a permanently low Hb level. He has been troubled very little by bruising or epistaxis since splenectomy, but haemolysis seems to be a major factor in his transfusion requirements.

Case 2. (III. 5, date of birth May 12, 1947.) This patient was first seen in February 1966 at the age of 18

Received March 28, 1968.
years, and his Hb was 41%. He denied all symptoms except breathlessness whenever he developed purulent bronchitis. He shaved only once a month. He was at that time still working as a light engineer. His only previous illness had been Perthe's disease of both hips. On examination he was only 158 cm. tall (Fig. 2) and weighed only 37.2 kg. He too had a generalized pigmentation and café-au-lait spots on the trunk and limbs. There was a feminine distribution of hair, and the testes were small.

His blood examination revealed neutropenia, with a WCC of 3200 per cu. mm. (24% neutrophils, 70% lymphocytes, 6% monocytes), and the platelet count was 128,000 per cu. mm. The red cells were macrocytic.

Radiological bone age was normal. His chest x-ray showed changes at the left lung base suggestive of bronchiectasis. He has been treated by blood transfusion of 3 pints of whole blood every three or four months and his Hb level is usually 50–55%. There seems to be no haemorrhagic or haemolytic tendency.

**Case 3.** (III. 10, date of birth November 12, 1954.) This patient is the only sister of the previous two cases and she has no symptoms. Physical examination revealed a pigmented skin and café-au-lait spots. She is undersized, for in April 1965 when 10 years 5 months old she was 117 cm. tall and in September 1967 she was 129 cm. tall and weighed only 25 kg. She has had an extra nail removed from the left thumb. There is no evidence of pancytopenia so far. In March 1966 her Hb was 84%, WCC 7800 per cu. mm. (63% neutrophils). A recent count (September 1967) showed Hb 80%, WCC of 4100 per cu. mm. (60% neutrophils), and a platelet count of 174,000 per cu. mm.

**Family Investigation**

No further cases of this condition have been noted among the individuals shown in the pedigree (Fig. 1), nor are any known in other relatives. The parents are not known to be related by blood.

None of the remaining four brothers has shown any congenital or haematological abnormality except III. 11 who had a mild iron-deficiency anaemia when 9 years old. The remainder have had no abnormality of the white cells or platelets.

Fig. 2 shows the relation between height and age in the seven children, and they fall into two groups lying on the lines AB and CD. All the three children whose height/age relation falls on line CD are pigmented, and none of the children represented on line AB are pigmented or anaemic, yet the rate of growth between the ages of 10 years and 18 years is the same in the two groups.

**Chromosome Findings**

The results obtained from karyotype analysis of the patients, their parents, and sibs are given in the Table.

**Family Pedigree**

![Figure 1: Pedigree of the family.](image)

**Fig. 2.** The chart shows the relation between height and age in the seven sibs. III. 5–11. The results for the three children with abnormal chromosomes are found on the line CD, those for the normal children are on the line AB.
Perkins, Timson, and Emery

TABLE

CHROMOSOME RESULTS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Karyotype of Normal Cells</th>
<th>Age (yr.)</th>
<th>Percentage of Cells</th>
<th>Breaks and Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>II.5</td>
<td>46,XY</td>
<td>44</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>II.8</td>
<td>46,XX</td>
<td>38</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>III.5</td>
<td>46,XY</td>
<td>19</td>
<td>88</td>
<td>12</td>
</tr>
<tr>
<td>III.6</td>
<td>46,XY</td>
<td>18</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>III.7</td>
<td>46,XY</td>
<td>17</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>III.8</td>
<td>46,XY</td>
<td>16</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>III.9</td>
<td>46,XY</td>
<td>13</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>III.10</td>
<td>46,XX</td>
<td>12</td>
<td>89</td>
<td>11</td>
</tr>
<tr>
<td>III.11</td>
<td>46,XY</td>
<td>10</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

Note—For each subject, 100 mitoses were examined. “Normal” in the above Table refers to those cells that do not show breaks, dicentric formation, or endoreduplication.

The chromosome abnormalities found were divided into two groups: endoreduplications (Fig. 3) and breaks and fragments. The second group included all mitoses in which breakage of the chromosome material was observed and ranged from mitoses in which a single chromatid break was found (Fig. 4) to cells in which complete fragmentation of the chromosomes had occurred. In patient III.8 one cell was found in which 151 chromatid fragments could be counted. In some cells stickiness of the chromosomes was seen. This may be a stage in the formation of dicentrics (Fig. 5) to be followed by fragmentation. The second group of abnormalities, therefore, includes cells which if found in isolation might be regarded as within the normal variation, i.e. single chromatid breaks, and cells which would be regarded as grossly abnormal in any circumstances.

The effect of a patient’s (III.8) plasma on normal chromosomes and of normal plasma on the patient’s chromosomes was investigated by separating off the leucocytes and washing them three times in sterile...
normal saline. The cells were then added to the appropriate plasma and cultured in the usual manner.

The results showed that the patient's white cells still showed the chromosome abnormalities previously found when cultured in his own plasma or in control plasma. The control white cells when cultured in his own plasma or in the patient's plasma had normal chromosomes. These results suggest that the chromosome abnormalities found in Fanconi's anaemia are unlikely to be caused by the presence of some agent circulating in the plasma, but are probably due to an abnormality at the precursor stage.

**Discussion**

Though this condition has been recognized since 1927, only about 160 cases have so far been described. The mean age of onset is about 8 years, but ranges from the first year of life to as late as the third decade. The ratio of affected males to females is roughly 3:2 (Gmyrek and Syllm-Rapoport, 1964). There is a wide range of clinical manifestation. Estren and Dameshek (1947) described pancytopenia and pigmentation without other congenital abnormalities. Varela and Sternberg (1967) have recently described a 7-month-old infant who had dislocation of hips, bilateral absence of thumbs, and growth retardation, and who had the typical chromosome abnormalities found in Fanconi's anaemia but without any haematological abnormality. Case 3 of this report had congenital and chromosome abnormalities without anaemia and may represent either a forme fruste or pre-anaemic state. The illness may be fulminating and result in death from septicemia or acute leukaemia. Some patients have marked haemolytic anaemia and this we found in Case 1. The anaemia may not incapacitate the patient in any way; certainly in our own study we found that Case 2 was working without symptoms though his Hb was only 40%. This adaptation to a low Hb level has been noted by other authors (Nelson, Lewis, and Robertson, 1964).
Fig. 5. Chromosomes of patient III. 8 showing dicentric formation.

The prognosis is usually poor. The commonest causes of death are acute leukaemia, septicaemia, and cerebral or intestinal haemorrhage. Mean survival is around two years, but after splenectomy survival times of 12½ and 10 years have been reported by Nelson et al. (1964) and Francis, Moir, and Swift (1955), respectively. Androgen and steroid therapy combined, as suggested by Shahidi and Diamond (1959), has also produced temporary improvement. In view of the wide range of clinical severity, it is possible that these good results are coincidental; certainly splenectomy has at times been of no use and post-operative death is a real possibility. The indications for splenectomy appear to be intractable thrombocytopenic haemorrhage, possibly if there is haemolytic anaemia and where there are high reticulocyte counts in the peripheral blood (Estren, Suess, and Dameshek, 1947), for in this latter situation splenectomy invariably improves the patient. The cornerstone of treatment

Peripheral blood picture shows macrocytosis but lacks the white cell changes seen in pernicious anaemia and other megaloblastic anaemias. Sternal marrow specimens may be difficult to obtain, but when obtained show normoblastic maturation.

The congenital abnormalities most frequently found in this anaemia are dwarfism and skin pigmentation. The latter consists of a fine generalized hypermelanosis (Baumann, 1951), and in addition numerous café-au-lait patches can be found. Skeletal deformities are particularly common in the upper limbs, and frequently the thumbs are missing or duplicated in part. Hypogenitalism is common in the males. Strabismus and cardiac anomalies may be coincidental but are frequently referred to in the literature. Gmyrek and Syllm-Rapoport (1964) point out that practically all the congenital abnormalities affect tissues derived from the mesoderm which differentiates between the 26th and 35th days of embryonic life.
is repeated blood transfusion together with antibiotic therapy for even minor infections.

There have been a number of reports of families with multiple affected sibs (Gmyrek and Syllm-Rapport, 1964). However, affected individuals do not usually live long enough to have children, and affected males are often hypogonadal. No parents have been reported to be abnormal, and certainly in the family reported here there was no clinical, haematological, or chromosome abnormality detected in the parents. The available family data, therefore, suggest that Fanconi’s aplastic anaemia is inherited as an autosomal recessive trait, and the present study shows that the heterozygote cannot be detected by present techniques.

Chromosome abnormalities in patients with Fanconi’s anaemia have been recorded by a number of authors. Schroeder, Anschütz, and Knopp (1964) reported two brothers with this disorder in which about 25% of the cells examined showed chromosome abnormalities, about 10% of these being endoreduplications. The anomalies were not found in the chromosomes of the parents or an unaffected younger brother. Schmid et al. (1965) described three cases, two of them brothers, in which some 50% of the cells examined had chromosome abnormalities. The parents and three sibs of the brothers were cytogenetically normal, though a few breaks were found in the mother’s chromosomes.

Hoefnagel et al. (1966) described two cousins with Fanconi’s anaemia, who showed a high frequency of endoreduplications and structural chromosome changes. Eighteen other members of the family were studied and all had normal chromosomes. One chromatic interchange was found in 47 metaphase plates in the paternal grandfather of one of the patients who was the uncle of the other patient, but the significance of this finding is questionable.

In the present study the parents and the apparently unaffected sibs had normal karyotypes, with no cells showing endoreduplication or breaks. This suggests that it is not possible to detect the carrier state cytogenetically.

Swift and Hirschhorn (1966) report two sisters with this disorder in which a high rate of chromosomal damage was found in the lymphocytes, fibrocytes, and bone-marrow cells. The similarity of the chromosomal anomalies found to those seen in viral infections suggested to these authors that abnormal susceptibility to virus-induced chromosomal breakage may be the cause of the cytogenetic abnormalities found in Fanconi’s anaemia.

In the present study culturing the leucocytes from two normal individuals in the plasma of patient II.8 did not produce any chromosome abnormalities. However, this does not exclude the possibility of a viral or other exogenous agent being the cause of the chromosome damage found in patients with Fanconi’s anaemia, but does suggest that if such an agent is responsible it acts on the precursors rather than on the peripheral cells.

Summary

A family is described in which two of seven sibs have Fanconi’s aplastic anaemia. Chromosome abnormalities, including endoreduplication, were found in the peripheral blood of the two patients, as well as in a sister who was not anaemic but had several of the congenital abnormalities usually associated with this syndrome. The remaining sibs and both parents are free of clinical, haematological, and chromosomal abnormalities.

The authors are grateful to Dr. R. M. Forrester, Royal Albert Edward Infirmary, Wigan, for permission to use the clinical notes concerning the propositus, and to Dr. F. Hillman of the same hospital who first suggested the correct diagnosis. The Department of Medical Photography, Manchester Royal Infirmary, kindly provided Fig. 1, 3, 4, and 5, and we are grateful to Mr. F. Seddon and Mr. J. Molyneux for the preparation of Fig. 2.

REFERENCES


Clinical and chromosome studies in Fanconi's aplastic anaemia.
J Perkins, J Timson and A E Emery

doi: 10.1136/jmg.6.1.28

Updated information and services can be found at:
http://jmg.bmj.com/content/6/1/28.citation

These include:

**Email alerting service**
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/