

Hatice ILGIN
Ayşe Nurten AKARSU
Fatma Işık BÖKESÖY

Cytogenetic and Phenotypic Findings in Turkish Patients With Fanconi's Anemia

Received: April 21, 1998

Medical Biology Department Faculty of
Medicine, Ankara University, Sıhhiye, 06100
Ankara-Turkey

Abstract: Fanconi's anemia is an autosomal recessive disorder with manifestation of chromosomal instability inducible by alkylating agents. Since 1989 we studied 85 patients with suspected Fanconi's anemia (FA) to confirm diagnosis. Cytogenetic analyses were performed on spontaneous and induced cultures of peripheral blood samples. Induction was carried with mitomycin-C (MMC) or diepoxybutane (DEB). The normal range of spontaneous, MMC and DEB-induced breaks were 0.00–0.10, 0.00–0.16 and 0.00–0.16 per cell respectively. Fanconi's anemia was

confirmed when breaks were above the normal threshold. According to this criterion 59 patients were confirmed as having FA. Some of the siblings were also investigated to exclude disease especially when donors for bone marrow transplantation are sought. Among 28 studied siblings, we found 6 affected. Therefore, 65 cases were diagnosed as FA according to cytogenetics criteria. In 13 out of the 65 patients (20%) instability was detected only after induction.

Key Words: *Fanconi's Anemia, Mitomycin-C, Diepoxybutane, Chromosome breakage.*

Introduction

Fanconi's Anemia (FA) is the most common genetic form of aplastic anemia which is an autosomal recessive disorder characterized by progressive pancytopenia, diverse congenital abnormalities and predisposition to malignancy (1–13). It was first described in 1927 in three brothers who had aplastic anemia and congenital physical abnormalities (1–3, 5, 8, 11). The features seen in FA are growth retardation, skin hypo–hyperpigmentation, combined radial ray and thumb deformities, hypogenitalism, microcephaly, renal malformation, mental retardation, ear malformation and microphthalmia (1–13). The physical findings range from extremely abnormal to normal, covering one or more systems. The haematological manifestations also show a broad spectrum; ranging from severe aplastic anemia to normal, depending on the time of diagnosis. Leukemia, carcinoma or liver tumor are the most frequent malignancies seen in the course of the disease (2, 4, 5, 7). FA occurs in all racial and ethnic groups (8, 10). The disease frequency varies among ethnic groups, with an estimated heterozygote frequency of 1/200–1/300 (5, 10). FA has a prevalence of about 1 in 350.000 in North America (11). Although the exact figure for FA is not

known in Turkey, observations indicate a high incidence (12).

The observation of increased chromosomal breakage in FA patients was made in the early 1960's (5). Since then chromosomal breakage studies have been used to confirm clinical diagnosis. Sensitivity of cells to DNA crosslinker agents such as MMC, nitrogen mustard and DEB has been noted and application of these has been carried out for pre–and postnatal diagnosis (6, 10). In this respect patients were classified as FA or non–FA based on the sensitivity of cultured peripheral blood lymphocytes to DNA crosslinking agents.

We report our experience with patients and their sibs to confirm diagnosis of FA since 1989.

Materials and Method

Peripheral blood samples or bone marrow aspirates were derived from 85 referred patients and 28 sibs of 59 patients after cytogenetic confirmation. Typical congenital malformations or haematologic involvement alone were the criteria for referral to cytogenetic analysis. Spontaneous and MMC and/or DEB–induction were performed on each sample. MMC was applied to 44

samples, MMC and DEB to 5 samples and only DEB to 64 samples along with untreated cultures. MMC (0.01 µgr/ml) or DEB (0.01 µgr/ml) was added at the last 24 hours and cells were harvested at 48 hours. Slide preparations were made according to standard procedures (14). Chromosomal breaks were counted on 50 Giemsa stained metaphases from each untreated and treated culture. Each cell was scored for chromosomal breaks. Achromatic areas less than a chromatid width were considered as gaps and was not scored, whereas exchange configurations, translocations, dicentrics, and ring chromosomes were grouped as rearrangements. Rearrangements were scored as two breaks. In our laboratory the normal range of spontaneous, MMC and DEB-induced breaks in cells were 0 to 0.10, 0 to 0.16 and 0 to 0.16 respectively.

Pedigree analysis and physical findings were also evaluated.

Results

Fifty-nine out of the 85 referred patients (69.4%) and 6 out of the 28 sibs (21.4%) were diagnosed as

having FA on the basis of chromosomal breakage studies (Table 1). Thirteen of these cases (20%) had a normal score for spontaneous breaks but high frequency of MMC or DEB-induced breaks. Consanguinity in FA families was observed with a frequency of 79.6%, whereas, the non-FA group had a frequency of 42.3%. The difference was significant with the χ^2 test (P<0.001). On the other hand, figures from both groups were higher than the mean consanguinity in Turkey (22.6%) (15) (Z test-P<0.001).

Ninety-four percent of the patients with FA were under 16 years of age at the time of diagnosis. Fifteen percent of non-FA patients were 16 years of age or older at the time of diagnosis.

In our groups (FA and non-FA) males were predominant with a sex ratio of 2.09 (FA) and 2.25 (non-FA), respectively.

Table 2 shows the various physical abnormalities seen in our patients with FA and non-FA. Prominent abnormalities which deviate significantly (P<0.001) in patients with FA from non-FA were skin hypo-hyperpigmentation, café au lait spots and upper

| | FA (n=65) | non-FA (n=48) |
|---|---------------|---------------|
| The range of spontaneous chromosome breaks/cell | 0.02-0.80 | 0.00-0.10 |
| The range of MMC-induced chromosome breaks/cell | 0.20-multiple | 0.06-0.16 |
| The range of DEB-induced chromosome breaks/cell | 0.20-multiple | 0.02-0.16 |

Table 1. The cytogenetic findings in FA and non-FA.

| | FA (%) (n=60*) | non-FA (%) (n=48) |
|------------------------------|-------------------|-------------------|
| Growth retardation | 56.7 | 38.5 |
| Skin hypo-hyper pigmentation | 58.3 | 3.8 ^Φ |
| Skin café au lait spots | 56.7 | 3.8 ^Φ |
| Upper limb | 50 | 3.8 ^Φ |
| Hypogenitalism | 20 | 3.8 |
| Microcephaly | 15 | 3.8 |
| Eye | 18.3 | 0 [∅] |
| Renal | 16.7 | 0 [∅] |
| Lower limb | 11.7 | 0 |
| Ear | 5 | 0 |
| Haematologic involvement | 90.8 ^τ | 92.3 |
| Cancer | 6.7 | 0 |

Table 2. Physical abnormalities and medical problems in FA and non-FA.

* 5 patients were not available for physical examination.

τ n=65

Φ P<0.001 using χ^2 test

∅ P<0.05 using FET

limb defects (combined radial ray defects, radial ray reduction deformities, malformed thumb, supernumerary thumb). Eye and renal malformation differences were less pronounced ($P < 0.05$). In the non-FA patients the most frequent finding (apart from the haematologic involvement) was growth retardation. Growth retardation and other findings showed no difference between the groups.

Discussion

Fanconi's Anemia is in the group of genetic disorders with autosomal recessive inheritance featuring chromosomal instability. The main consistent findings in FA are physical features, haematological findings and cytogenetic anomalies. However, the age of onset of the anemia is variable and it is possible that haematologic involvement may not be present at early ages in spite of physical signs or chromosomal breakage.

In our study 65 cases out of 113 were diagnosed as FA on the basis of chromosomal breakage studies. In thirteen of the FA cases (20%), spontaneous breaks were within the normal range while MMC or DEB induced breaks were high. Therefore if FA is to be ruled out we highly recommend induction with MMC or DEB, as is seen, normal spontaneous breakage count can give false negative results.

Earlier studies reported a distortion in the sex ratio, with more males than females being affected (1, 2, 8, 9). Our data is consistent with this, but higher than another study from Turkey (12).

Pedigree analysis revealed a high incidence of consanguinity in our patients, a proportion higher than Turkey's average. It was highest in the FA group. This is not surprising when we consider the role of recessive genes in the etiology of anemias.

In the studied patients with FA, the prominent physical findings were skin hypo-hyperpigmentation, café au lait spots, upper limb defects, eye and renal malformations which deviate among FA and non-FA groups. In our series non-FA patients did not exhibit physical findings apart from growth retardation and anemia. On the other hand, the absence of dysmorphic features does not rule out the diagnosis of FA because of the clinical heterogeneity. The question of whether the extensive phenotypic variation seen in this syndrome is related to genetic differences has been addressed through complementation groups. Complementation groups in FA are likely to represent distinct disease genes, eight of which (FA-A through FA-E) have been identified (16). In spite of genetic heterogeneity, chromosomal instability to crosslinking agents is common for all. Although the role of FA genes at the biochemical and molecular level are not known, DNA crosslinking hypersensitivity of FA cells can be used in diagnosis.

References

- Schroeder TM, Tilgen D, Krüger J, Vogel F. Formal Genetics of Fanconi's Anemia. *Hum Genet* 32: 257-288, 1976.
- Gordon-Smith EC, Rutherford TR. Fanconi Anemia: Constitutional Aplastic Anemia. *Seminars in Hematology* 28: 104-112, 1991.
- Jones KL. *Smith's Recognizable Patterns of Human Malformation*, WB Saunders Company. Philadelphia 1988, pp: 274-275.
- Berger R, Le Coniat M, Gendron MC. Fanconi Anemia Chromosome Breakage and Cell Cycle Studies. *Cancer Genet Cytogenet* 69: 13-16, 1993.
- Alter BP. Annotation: Fanconi's Anemia and its variability. *British J of Haemato* 85: 9-14, 1993.
- Auerbach AD, Rogatko A, Schroeder TM. International Fanconi Anemia Registry: Relation of Clinical Symptoms to Diepoxybutane Sensitivity. *Blood* 73: 391-396, 1989.
- Cavenagh JD, Richardson S, Gibson RA, Mathew CG, Newland AC. Fanconi's anemia presenting as acute myeloid leukaemia in adulthood. *British J of Haemat* 94: 126-128, 1996.
- Juhn HJ, Wesenberg RL, Gwinn JL. Roentgenographic Findings in Fanconi's Anemia. *Radiology* 89: 646-653, 1967.
- Glanz A, Fraser FC. Spectrum of anomalies in Fanconi anemia. *J of Med Genet* 19: 412-416, 1982.
- Auerbach AD, Sagi M, Alder B. Fanconi Anemia: Prenatal Diagnosis in 30 Fetuses at Risk. *Pediatrics* 76: 794-800, 1985.
- Santos CC, Gavish H, Buchwald M. Fanconi Anemia Revisited: Old Ideas and New Advances. *Stem Cells* 12: 142-153, 1994.
- Altay C, Alikasıfoğlu M, Kara A, Tunçbilek E, Ozbek N, Schroeder TM. Analysis of 65 Turkish patients with congenital aplastic anemia (Fanconi anemia and non-Fanconi anemia): Hacettepe experience. *Clin Genet* 51: 296-302, 1997.

13. Lo Ten Foe JR, Kwee ML, Rooimans MA, Oostra AB, Veerman AJP, Weel M, Pauli RM, Shahidi NT, Dokal I, Roberts I, Altay C, Gluckman E, Gibson RA, Mathew CG, Arwert F, Joenje H. Somatic Mosaicism in Fanconi Anemia: Molecular Basis and Clinical Significance. *Eur J Hum Genet* 5: 137-148, 1997.
14. Rooney DE, Czepulkowsky BH. *Human cytogenetics: a practical approach*. IRL Press. Oxford, Washington DC 1996. pp: 47-50.
15. Tunçbilek E. Genetic Services in Turkey. *Eur J Hum Genet* 5 (suppl 2): 178-182, 1997.
16. Joenje H, Oostra AB, Wijker M, Summa FM, Berkel CGM, Rooimans MA, Ebell W, Weel M, Pronk JC, Buchwald M, Arwert F. Evidence for at Least Eight Fanconi Anemia Genes. *Am J Hum Genet* 61: 940-944, 1997.