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Fanconi Anemia and its Diagnosis

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Abstract

Fanconi anemia (FA) is a genetically and phenotypically heterogeneous recessive disorder characterized by diverse congenital malformations, progressive pancytopenia, and predisposition to both hematologic malignancies and solid tumors. Congenital anomalies vary from patient to patient and may affect skeletal morphogenesis as well as any of the major organ systems. Although this highly variable phenotype makes accurate diagnosis on the basis of clinical manifestations difficult in some patients, laboratory study of chromosomal breakage induced by diepoxybutane (DEB) or other crosslinking agents provides a unique cellular marker for the diagnosis of the disorder either prenatally or postnatally. Diagnosis based on abnormal response to DNA crosslinking agents can be used to identify the pre-anemia patient as well as patients with aplastic anemia or leukemia who may or may not have the physical stigmata associated with the syndrome. This overview will present our present knowledge regarding the varied phenotypic manifestations of FA and procedures for diagnosis based upon abnormal DNA damage responses.

Keywords

Fanconi anemia diagnosis; congenital anomalies; hematologic abnormalities; cancer predisposition; DNA crosslink sensitivity; somatic mosaicism

Introduction

In 1927, Fanconi described a family in which three male children between the ages of 5 and 7 had pancytopenia and birth defects [1]. Based on his observations in this family and others, Fanconi's chief criteria for the diagnosis of Fanconi anemia (FA) included pancytopenia, hyperpigmentation, skeletal malformations, small stature, urogenital abnormalities and familial occurrence. Fanconi's observations formed the basis for the diagnosis of FA for many years. Consideration of FA in the differential diagnosis of a patient manifesting clinical features of the syndrome depends on the clinician's concept of the FA phenotype. In a study comparing the frequencies of congenital anomalies among probands, i.e. the first affected member of families seeking medical attention for a genetic disorder, and their affected siblings, Glanz and Fraser observed that there were significantly fewer congenital anomalies among the affected siblings compared to probands [2]. Affected siblings with milder phenotypic features were only diagnosed following the diagnosis of FA in another affected family member and not because of their phenotypic presentation. In fact, patients with "Fanconi-like" bone marrow failure who completely lacked congenital malformations, previously described as having the

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Estren-Dameshek syndrome [3], were found in the same sibships as "classical FA". The delay in diagnosis in the majority of FA patients, even those with congenital malformations, indicates the need for an increased awareness among physicians of the clinical features associated with the syndrome [4].

Since hypersensitivity to the clastogenic effect of DNA crosslinking agents provides a unique marker for the FA genotype, this cellular characteristic can be used to identify pre-anemic cases as well as patients with aplastic anemia or leukemia who do not have characteristic physical findings [5,6]. It is useful for prenatal as well as postnatal diagnosis of FA. The availability of such testing has revealed increasing numbers of individuals affected with FA who by clinical criteria appear to have idiopathic aplastic anemia and appear phenotypically normal. The issues of misdiagnosis and therefore mismanagement have thus become more pressing.

Case Reports in the Literature

FA is found in all races and ethnic groups, and has been widely reported to have a carrier frequency of 1 in 300. This estimate was based on the incidence of affected individuals before the full spectrum of the FA phenotype was recognized. The true gene frequency is likely to be considerably higher than this; a low estimate would result from an incomplete ascertainment of cases before the widespread application of chromosomal breakage tests for FA diagnosis. Up to 0.5 percent of the general population may be heterozygous at an FA locus. It should be noted that cells from carriers do not have increased sensitivity to DNA crosslinking agents.

It is now recognized that congenital malformations in FA patients may range from none [7] to many, and may involve any of the major organ systems. Abnormalities involving central nervous system, gastrointestinal system and skeletal system, in addition to radial-ray defects (i.e. radius and thumb abnormalities) have been added to the original FA phenotype [4]. FA patients may present with Vertebral anomalies, Anal atresia, Cardiac abnormalities, Tracheo-Esophageal fistula, Renal anomalies, and radial Limb (VACTERL), and may also include hydrocephalus. Several cases of VACTERL with hydrocephalus and Baller-Gerold syndrome have been reported in the literature, and then changed to a diagnosis of FA when the patients developed bone marrow failure and had a positive chromosomal breakage test with a crosslinking agent [8]. FA patients may also present with a phenotype characteristic of Seckel syndrome, Nijmegen breakage syndrome, Dubowitz syndrome, Holt-Oram syndrome, thrombocytopenia absent radius (TAR) syndrome, Townes-Brocks syndrome, Saethre-Chotzen syndrome (TWIST1 mutation), velocardiofacial syndrome, Diamond-Blackfan anemia, and dyskeratosis congenita. Thus, the clinician must recognize the considerable overlap of FA phenotype with these other syndromes and not be misled by preexisting 'diagnostic labels'. Between 1927 and 2001 1,300 cases of FA were reported in varying detail in the literature [9]. These cases, particularly those reported before the last decade, were made when aplastic anemia or leukemia developed in individuals with characteristic physical abnormalities; thus, reviews in the literature are biased toward the most severe clinical cases, as well as those with interesting outcomes.

International Fanconi Anemia Registry (IFAR) Overview

In order to study a large number of patients with a rare genetic disorder, and find the full spectrum of diverse features of the syndrome, the International Fanconi Anemia Registry (IFAR) was established at The Rockefeller University in 1982. The registry serves as a centralized repository for clinical and genetic information on patients with FA, as well as biological samples from patients. Patients with one or more clinical features associated with FA are referred to the IFAR by their physicians. Patients in the IFAR have had the diagnosis confirmed by chromosomal breakage studies, mostly using the DNA crosslinking agent

diepoxybutane (DEB) [5]. Between May 1982 and August 2008, 1075 patients from North America were registered in the IFAR. Complementation groups (see J. de Winter – Introduction of FA proteins and FA pathway, pp. xxx-xxx) are known for 681 IFAR patients, and are distributed as follows: FA-A=411; FA-B=10 (X-linked; affected are all males); FA-C=108; FA-D1=20; FA-D2=16; FA-E=9; FA-F=16; FA-G=67; FA-I=6; FA-J=13; FA-L=2; FA-M=0; FA-N=3. Thus the approximate percentage (rounded to nearest 0.5%) of IFAR patients with known complementation group is distributed as follows: FA-A=60.5%; FA-C=16%; FA-G=10%; other genes in the “nuclear core complex” (FA-B, FA-E, FA-F, FA-L)=5.5%; ubiquitinated gene complex (FA-D2/FA-I)=3%; “downstream genes” (FA-D1; FA-J; FA-N)=5%. Approximately 85% of all IFAR patients with known complementation group are defective in one of the three most common disease-causing genes *FANCA*, *FANCC*, *FANCG*. *FANCA* mutation analysis in IFAR patients reveals a high degree of heterogeneity with a large number of private mutations, as well as various large genomic deletions. There are two founder mutations in *FANCA*, c.1115_1118delTTGG in exon 13, and c.3788_3790delTCT in exon 38. However, these account for only 2% and 5% of all *FANCA* mutations in IFAR patients, indicating that founder mutations do not account for the high percentage of patients this complementation group. These results suggest that *FANCA* may be hypermutable due to the presence of sequence-specific mutation “hot spots”. This is distinct from the role of a founder effect on the mutation spectrum seen in *FANCC* and *FANCG*. Approximately 50% of FA-C patients in the IFAR are Ashkenazi Jews, who display the mutation c.456+4A>T, also known as IVS4 +4 A>T. Additional founder mutations in different ethnic populations from which the IFAR population is drawn have been demonstrated in *FANCC* as well as *FANCG*. Detailed information on all FA mutations identified in IFAR patients, as well as all published mutations in the thirteen FA genes, can be found online in the Fanconi Anemia Mutation Database at <http://www.rockefeller.edu/fanconi/mutate/>.

Congenital Abnormalities in IFAR Patients

Major Congenital Malformations

In a survey of the clinical findings obtained from the IFAR, a variety of congenital malformations associated with FA have been described [4]. Major congenital malformations were reported in approximately two-thirds of the patients, while a third were reported as not having congenital malformations [7]. A review of these data indicated that the FA phenotype is more variable than previously recognized. Gastrointestinal, central nervous system, and skeletal malformations in FA patients, not previously included as part of the FA phenotype, were observed [4]. Our analysis showed that most FA patients with congenital malformations are not diagnosed until after the onset of hematologic abnormalities; delayed diagnosis might be due to lack of physician awareness of the phenotypic spectrum of FA. Major congenital malformations reported in IFAR patients are summarized in Table 1. From a developmental standpoint, it is interesting that radial ray abnormalities in FA patients can be bilateral or unilateral [4]. Even patients with bilateral abnormalities usually exhibit asymmetry, with their limbs having different specific anomalies (Fig. 1). The presence unilateral congenital anomalies reveal a stochastic component to the disease. In other words, a defect in the FA pathway is not sufficient to cause a developmental defect, but it dramatically increases the odds that a defect will occur.

Minor congenital abnormalities in the IFAR

Notably, approximately one-third of FA patients do not manifest major congenital malformations [7]. In these patients the diagnosis of FA generally is made only after a patient presents with clinical symptoms of hematologic dysfunction; the mean age of diagnosis in this group is considerably older than that for FA patients with malformations. FA patients without congenital malformations frequently have alterations in growth parameters, with height,

weight, or head circumference below the fifth centile. Other very common findings are skin pigmentation abnormalities, hypoplastic thenar eminence, microcephaly and/or microphthalmia. Increased awareness of the facial anomalies as well as the complete spectrum of minor malformations seen in these patients should enable an earlier diagnosis to be made among patients without major congenital anomalies.

Ear Anomalies & Hearing Problems

A comprehensive study of otologic manifestations of FA has not been reported previously. Ear abnormalities have been reported in 232 of the 1075 patients (21.6%) currently enrolled in the IFAR. The frequency of abnormalities is the same for all three common complementation groups (FA-A, FA-C, FA-G). These abnormalities include morphologic anomalies affecting the ear structures and hearing loss. Hearing loss, reported in 126 of the patients with ear abnormalities, is usually conductive hearing loss, but sensorineural hearing loss has also been observed. Since many FA patients have not had formal hearing tests at the time of diagnosis and entrance into the IFAR, the incidence of hearing loss is likely to be higher than reported. The more common morphologic anomalies reported in patients with or without hearing loss include abnormal or absent pinna, prominent ears, abnormally positioned ears (low set or posteriorly rotated), small or absent ear canals, absent tympanic membrane, microtia, and fused ossicles.

Variable Expressivity

An analysis of congenital malformations among siblings in the IFAR revealed that there is intrafamilial phenotypic variation in the specific types of congenital malformations among affected siblings [10]. Fifty-three sibships composed of 120 siblings were studied. Even two sets of monozygotic (MZ) twins were phenotypically discordant. MZ twin pair 1 was comprised of two affected fetuses examined after pregnancy termination. Prenatal diagnosis was performed because of the presence of two prior affected siblings in the pedigree. Twin A had a bifid thumb, whereas twin B had no physical stigmata of FA. The proband in this sibship had duodenal atresia, whereas a second affected sib had no congenital abnormalities. In MZ twin pair 2, 15-year-old girls with FA, twin A had unilateral absence of the radius, bilateral absent thumbs, and an absent right clavicle. Twin B had a bifid right thumb, a hypoplastic left thumb and an absent left clavicle (Fig. 1). However, the analysis showed that the occurrence of malformations among FA siblings is nonrandom; siblings usually were concordant for the presence or absence of multiple congenital malformations.

Growth and Endocrine Abnormalities

Short stature is a well-recognized feature of FA and is often secondary to hormonal deficiencies, which include pituitary hypofunction with hypogonadism, growth hormone deficiency, thyroid dysfunction and insulin deficiency or resistance with glucose intolerance. The syndrome is usually associated with abnormal growth parameters both prenatally and postnatally. The mean height, weight and head circumference of FA patients in the IFAR is near the 5th centile. A prospective study by Wajnrajch *et al.* of 54 IFAR patients with FA, 30 males and 24 females from 47 unrelated families, showed that endocrinopathies are a common feature of FA, primarily manifesting as glucose/insulin abnormalities, growth hormone insufficiency and hypothyroidism [11]. Although short stature is a feature of FA, it is notable that in this study 23 patients (43%) were within two standard deviations of the 50th centile, and five (9%) were above the mean height for the general population. As expected, patients with endocrine dysfunction are more likely to have short stature. These data indicate that short stature is an integral feature of FA, but that the addition of endocrinopathies magnifies the growth failure in a significant proportion of patients. The finding of abnormal endogenous growth hormone secretion may demonstrate an underlying hypothalamic-pituitary dysfunction

that results in poor growth. Since correction of growth hormone or thyroid hormone deficiency may improve final height outcome and quality of life, endocrine evaluations are recommended for all FA children at an early age well before use of androgens and HCT if possible.

Results of this IFAR study were recently confirmed among 23 patients intensively evaluated at the National Institutes of Health, in whom anthropometric measurements, GH, IGF-I, IGF binding protein-3, thyroid, gonadal hormone, lipid levels, glucose homeostasis, brain imaging, and bone mineral density were obtained [12]. Endocrine abnormalities were present in 73%, including short stature and/or GH deficiency, hypothyroidism, midline brain abnormalities (these patients had very short stature and 60% were GH-deficient); abnormal glucose/insulin metabolism; obesity; dyslipidemia; and metabolic syndrome. Patients with any endocrine abnormality were shorter than those without; only GH deficiency correlated significantly with short stature ($P = 0.01$). In addition, 65% of peripubertal or postpubertal patients had gonadal dysfunction. Ninety-two percent of the patients 18 yr or older had osteopenia or osteoporosis. These authors conclude that endocrine dysfunction is widespread in children and adults with FA and expand the FA phenotype to include early onset osteopenia/osteoporosis and lipid abnormalities.

Further evidence of the importance of endocrine abnormalities in FA was obtained in a study of 44 patients with FA referred to Cincinnati Children's Hospital Medical Center (CCHMC) between 1975 and 2005 [13]. Of these, 33 had neuroimaging studies, including 11 who had cranial magnetic resonance imaging (MRIs). When compared to the age-gender matched on-site control sample, the mean pituitary height of FA patients was significantly smaller ($P < 0.0001$). 50% of patients with small pituitary gland were short. In another recent study from CCHMC it was found that among 63 children with FA, 63% had borderline thyroid function tests [14]. Those with borderline thyroid hormone results, compared to those with completely normal thyroid function, were shorter. A separate report describes thirty-nine children with FA referred to Cincinnati Children's Hospital Medical Center (CCHMC) who underwent 2-hr oral glucose tolerance test (OGTT). This study confirmed that abnormalities in glucose metabolism are frequent in young FA patients without prior diagnosis of diabetes, and are associated with marked defects in insulin secretion [15].

Infertility

Infertility is also common in FA; approximately half of all female patients with FA are infertile. Menopause usually starts during the 4th decade. However, 15% of females cited in the literature or reported to the IFAR who reached at least 16 years of age and were not receiving androgen therapy had at least one pregnancy [16]. While pregnancy is possible in some females, it is often associated with significant complications, such as rapid and marked progression of marrow failure, preeclampsia and premature labor. In contrast, males are very rarely fertile. Genital malformations and hypoplastic gonads are common findings in males with FA. There are extremely few reported cases of affected males having offspring [17]. Results of semen analyses typically reveal very low or absent sperm counts as well as evidence of abnormal spermatogenesis.

Hematologic Manifestations

Hematologic abnormalities occur in virtually all patients with FA at a median age of 7 years (range: birth to 41 years) [18,19]. Based on clinical data in the IFAR ($n=754$ patients), the cumulative incidence of bone marrow failure by age 40 years was 90%. Initial hematologic findings were diverse. Thrombocytopenia was often associated with elevated levels of fetal hemoglobin and macrocytosis and usually preceded onset of anemia or neutropenia. Notably, some patients presented with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) without prior diagnosis of aplastic anemia (AA). Of the 754 IFAR FA patients, 120

(16%) patients experienced MDS and/or AML. The cumulative incidence of MDS or AML by age 40 years was 33% [19]. Based on a survey of FA patients performed by Rosenberg *et al.* [20], the median age of onset of leukemia was 11.3 years.

Presence of bone marrow clonal cytogenetic abnormalities becomes increasingly common with age. The actuarial risk of identifying clonal cytogenetic abnormalities at the time of marrow failure is 67% by 30 years of age [18]. Among the most frequent clonal abnormalities observed are duplications and triplications of the long arm of chromosome 1, gains of portions of the long arm of chromosome 3, and monosomy 7 or loss of material from the long arm of chromosome 7. Deletions of 5q, 11q, rearrangements of 6p, and gains of chromosomes 8 and 21 have also been noted by different groups [21]. In addition, AML in FA patients rarely involves chromosomal rearrangements observed in non-FA patients with AML (e.g. t(15;17), t(8;21), and inv(16) or t(16;16)). Frequently, abnormal karyotypes in FA AML are complex. Recent advances in single and multicolor fluorescence *in situ* hybridization (FISH) and other molecular/cytogenetic techniques now permit more definitive characterization of clones.

Cancer Predisposition

In addition to the extraordinarily high frequency of AML in FA patients (actuarial risk of 52 percent for the development of MDS and/or AML by 40 years of age) [18], the high incidence of nonhematologic malignancy in FA patients is especially striking because of the predicted early death from hematologic causes associated with the syndrome (median estimated survival is 23 years; actuarial risk of death from hematologic causes is 81 percent by 40 years of age). Thus patients are unusually young when they develop cancer, and the incidence of malignancy probably would be considerably higher if patients had a longer life expectancy [22]. Most of the nonhematologic tumors in FA patients are squamous cell carcinomas (SCC) especially of the head and neck and anogenital regions [19,20]. Fanconi anemia patients have a 500- to 700-fold higher incidence of head and neck SCC than the general population and a 14% cumulative incidence of head and neck SCC by the age of 40 years [19,23]. A recent analysis of clinical findings in patients in the German Fanconi Anemia (GEFA) Registry validates previous epidemiological studies [24]. A study by Kutler *et al.* suggests that Fanconi anemia is associated with increased susceptibility to HPV-induced carcinogenesis and that SCC in Fanconi anemia patients is probably associated with the inactivation of p53 by HPV-associated oncoproteins rather than by direct mutagenesis [25]. It is now clear that Fanconi anemia is both a major bone marrow failure syndrome, and also a highly penetrant cancer susceptibility syndrome. Epidemiologic analyses strongly suggest that solid tumors will become the predominant clinical problem of patients with Fanconi anemia, as hematopoietic transplant becomes available for more patients because of an increased pool of alternative donor options (i.e. one and two antigen-mismatched cord blood transplant), and new transplant protocols result in an improved probability of survival to an age when the incidence of solid tumors begins to increase.

Diagnostic Tests

Schroeder *et al.* [26] first suggested the use of spontaneous chromosomal breakage as a cellular marker for FA, but longitudinal studies of chromosome instability in FA patients showed this finding to be inconsistent. In contrast, hypersensitivity of FA cells to the clastogenic (chromosome-breaking) effect of cross-linking agents provides a reliable cellular marker for the diagnosis of this disorder (Fig. 2). DEB and mitomycin C (MMC) are the agents most widely used for FA diagnosis. Extensive experience with MMC and DEB testing has demonstrated the sensitivity, specificity and reproducibility of the results [5,6]. It is recommended that all patients exhibiting any congenital malformation known to be associated with FA or AA at any age, or any patient with MDS with complex cytogenetic abnormalities,

have a peripheral blood sample tested for cross-linker hypersensitivity [4]. Because of the lack of concordance of FA phenotype among affected siblings, all full siblings of an FA patient should also be screened.

Data from DEB testing indicate that there is great variability in the degree of hypersensitivity in FA patients [5]. Although a few cells with chromosomal breaks may be seen in non-FA individuals, there is little overlap with FA patients in either the number of breaks per cell, or number of breaks per aberrant cell (Fig. 3A and B). Patients with somatic mosaicism (see below) have a population of resistant cells, but usually exhibit multiple breaks per aberrant cell. Therefore only a small number of affected individuals cannot be discriminated on the basis of mean breaks/cell and the presence of a pathogenic mutation is necessary for diagnosis.

Importantly, the clinician should know that the chromosomal breakage test could also be applied to the study of fetal cells obtained by chorionic villus sampling (CVS), amniocentesis, or percutaneous umbilical blood sampling [26,27,28]. The availability of prenatal diagnosis of Fanconi anemia played a major role in the use of this syndrome as a model for the development of umbilical cord blood transplantation as an alternative to bone marrow transplantation in the treatment of hematologic disorders [29]. The first human cord blood transplant was in a Fanconi patient in 1988 [30]; the patient is still alive and well after 20 years. The successful use of cord blood as a source of hematopoietic progenitor cells for matched sibling transplantation was a stimulus for the application of preimplantation genetic diagnosis (PGD) for selection of embryos produced by in vitro fertilization (IVF) that are unaffected by FA and HLA-identical to the FA proband [31,32]. The creation of such “designer babies” has led to discussion of the ethical, legal, and policy issues that must be addressed because of the expanding use of this technology.

Alternative diagnostic methods for FA, such as cell-cycle analysis using flow cytometric methods on lymphocytes exposed to cross-linking agents, are sometimes used to discriminate between FA and non-FA individuals [33]. While it is clear that duration of the G2/M phase is increased in FA cells as compared to unaffected cells, clinical diagnostic laboratories do not often use this method. If the specific complementation group and mutations are known within the family, testing for the presence of the mutation on limited quantities of uncultured cells can be used for diagnosis.

Somatic Mosaicism

Somatic mosaicism, defined as the presence of genetically distinct populations of somatic cells in a given organism, is relatively common in FA and needs to be considered when performing diagnostic tests for the syndrome. Somatic mosaicism in FA patients has been shown to be caused by new DNA mutations or the spontaneous reversion of inherited mutations [34,35]. This can lead to cells with a selective advantage due to loss of the characteristic DNA crosslink sensitivity. A population of cells which has lost crosslink sensitivity may appear early in embryogenesis or after birth, in a distinct tissue such as bone marrow stem cells or lymphocyte progenitor cells.

Somatic mosaicism is usually first detected in FA patients when they have a diagnostic test with a DNA crosslinking agent on a peripheral blood sample. In our laboratory DEB testing will reveal two populations of phytohemagglutinin A (PHA)-stimulated peripheral blood lymphocytes; one demonstrating an FA phenotype with chromatid breaks and exchanges and the other a normal one [36]. While there is currently no generally accepted level of aberrant cells at which an individual is considered mosaic, about 10 percent of FA patients have 50 percent or fewer aberrant cells and approximately 25 percent have 75 percent aberrant cells (Fig. 4).

The clinical significance of this type of mosaicism is unclear and is under investigation. Thus far, no correlation has been discerned between the degree of cross-linker hypersensitivity and the severity of the phenotype in FA patients or individual patient sensitivity to chemoradiotherapy [37]. However, data exist suggesting that genes not in the FA pathway can modify the crosslinker hypersensitivity [38].

Conclusion

The availability of laboratory diagnosis for FA, based on procedures which test for abnormal cellular response to DNA damage, has revealed increasing numbers of affected individuals who by clinical criteria appear to have idiopathic aplastic anemia and appear phenotypically normal. Likewise, clinicians must recognize the considerable overlap of the FA phenotype with other malformation syndromes, and the need for applying a specific laboratory diagnostic test. The clinician needs to be aware of the need for timely diagnosis in order for the family to receive genetic counseling regarding the availability of prenatal diagnosis and preimplantation diagnosis (PGD), including the possibility of embryo selection to provide a healthy, HLA-identical sibling for hematopoietic stem cell transplant. Early diagnosis will enable timely testing of siblings for FA and HLA-type, and if a matched sibling already exists, hematopoietic cell transplant should be pursued before the onset of severe pancytopenia, MDS or leukemia. The issues of misdiagnosis and therefore mismanagement are pressing, as accurate and timely diagnosis are essential to implement appropriate therapy for patients and to enable parents to make informed reproductive decisions.

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References

1. Fanconi G. Familiäre infantile perniziösaartige Anämie (pernizioses Blutbild und Konstitution). *Jahrb Kinderh* 1927;117:257–280.
2. Glanz A, Fraser FC. Spectrum of anomalies in Fanconi anaemia. *J Med Genet* 1982;19:412–416. [PubMed: 7154038]
3. Estren S, Dameshek W. Familial hypoplastic anemia of childhood. Report of eight cases in two families with beneficial effect of splenectomy in one case. *Am J Dis Child* 1947;73:671–687.
4. Giampietro PF, Adler-Brecher B, Verlander PC, Pavlakis SG, Davis JG, Auerbach AD. The need for more accurate and timely diagnosis in Fanconi anemia: a report from the International Fanconi Anemia Registry. *Pediatrics* 1993;91:1116–20. [PubMed: 8502512]
5. Auerbach AD, Rogatko A, Schroeder-Kurth TM. International Fanconi Anemia Registry. Relation of clinical symptoms to diepoxybutane sensitivity. *Blood* 1989;73:391–396. [PubMed: 2917181]
6. Auerbach AD. Fanconi anemia diagnosis and the diepoxybutane (DEB) test. *Exp Hematol* 1993;21:731–733. [PubMed: 8500573]
7. Giampietro PF, Verlander PC, Davis JG, Auerbach AD. Diagnosis of Fanconi anemia in patients without congenital malformations: an International Fanconi Anemia Registry study. *Am J Med Genet* 1997;68:58–61. [PubMed: 8986277]
8. Rossbach HC, Sutcliffe MJ, Haag MM, Grana NH, Rossi AR, Barbosa JL. Fanconi anemia in brothers initially diagnosed with VACTERL association with hydrocephalus, and subsequently with Baller-Gerold syndrome. *Am J Med Genet* 1996;61:65–67. [PubMed: 8741921]
9. Alter BP. Cancer in Fanconi's anemia, 1927–2001. *Cancer* 2003;97:425–440. [PubMed: 12518367]
10. Giampietro PF, Verlander PC, Maschan A, Davis JG, Auerbach AD. Fanconi anemia: A model for somatic gene mutation during development. *Am J Med Genet* 1994;52:36–37.

11. Wajnrajch MP, Gertner JM, Huma Z, Popovic J, Lin K, Verlander PC, Batish SD, Giampietro PF, Davis JG, New MI, Auerbach AD. Evaluation of growth and hormonal status in patients referred to the International Fanconi Anemia Registry. *Pediatrics* 2001;107:744–754. [PubMed: 11335753]
12. Giri N, Batista DL, Alter BP, Stratakis CA. Endocrine abnormalities in patients with Fanconi anemia. *J Clin Endocrinol & Metabolism* 2007;92:2624–2631.
13. Sherafat-Kazemzadeh R, Mehta SN, Care MM, Kim MO, Williams DA, Rose SR. Small pituitary size in children with Fanconi anemia. *Pediatr Blood Cancer* 2007;49:166–170. [PubMed: 17274008]
14. Eyal O, Blum S, Mueller R, Smith FO, Rose SR. Improved growth velocity during thyroid hormone therapy in children with Fanconi anemia and borderline thyroid function. *Pediatr Blood Cancer* 2008;51:652–656. [PubMed: 18623197]
15. Elder DA, D'Alessio DA, Eyal O, Mueller R, Smith FO, Kansra AR, Rose SR. Abnormalities in glucose tolerance are common in children with Fanconi anemia and associated with impaired insulin secretion. *Pediatr Blood Cancer* 2008;51:256–260. [PubMed: 18454466]
16. Alter BP, Frissora CL, Halperin DS, Freedman MH, Chitkara U, Alvarez E, Lynch L, Adler-Brecher B, Auerbach AD. Fanconi's anaemia and pregnancy. *Br J Haematol* 1991;77:410–418. [PubMed: 2012768]
17. Liu JM, Auerbach AD, Young NS. Fanconi anemia presenting unexpectedly in an adult kindred with no dysmorphic features. *Am J Med* 1991;91:555–557. [PubMed: 1951421]
18. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio A, Auerbach AD. Hematologic abnormalities in Fanconi anemia. An International Fanconi Anemia Registry study. *Blood* 1994;84:1650–1655. [PubMed: 8068955]
19. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Auerbach AD. A 20-year perspective of The International Fanconi Anemia Registry (IFAR). *Blood* 2003;101:1249–1256. [PubMed: 12393516]
20. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood* 2003;101:822–826. [PubMed: 12393424]
21. Neitzel, H.; Kühl, J.; Gerlach, A.; Ebell, W.; Tönnies, H. Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: Results and implications. In: Schindler, D.; Hoehn, H., editors. *Monogr Hum Genet 15: Fanconi Anemia. A Paradigmatic Disease for the Understanding of Cancer and Aging*. Karger; Basel: 2007. p. 79-94.
22. Satagopan JM, Ben-Porat L, Berwick M, Robson M, Kutler D, Auerbach AD. A Note on Competing Risks in Survival Data Analysis. *Brit J Cancer* 2004;91:1229–1235. [PubMed: 15305188]
23. Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, Goberdhan A, Shah JP, Singh B. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. *Arch Otolaryngol Head Neck Surg* 2003;129:106–112. [PubMed: 12525204]
24. Rosenberg PS, Alter BP, Ebell W. Cancer risks in Fanconi anemia: findings from the German Fanconi Anemia Registry. *Haematologica* 2008;93:511–517. [PubMed: 18322251]
25. Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, Huvos AG, Giampietro P, Levran O, Pujara K, Diotti R, Carlson D, Huryn LA, Auerbach AD, Singh B. Higher levels of human papilloma virus integration and lack of p53 mutations in squamous cell carcinoma from patients with Fanconi anemia. *J Natl Can Inst* 2003;95:1718–1721.
25. Schroeder TM, Anschultz F, Knoff A. Spontane chromosomenaberrationen bei familiärer Panmyelopathie. *Humangenetik* 1964;19:194–196;1
26. Auerbach AD, Sagi M, Adler B. Fanconi anemia: Prenatal diagnosis in thirty fetuses at risk. *Pediatr* 1985;76:794–800.
27. Auerbach AD, Zhang M, Ghosh R, Pergament E, Verlinsky Y, Nicolas H, Boue J. Clastogen-induced chromosomal breakage as a marker for first trimester prenatal diagnosis of Fanconi anemia. *Hum Genet* 1986;73:86–88. [PubMed: 3458668]
28. Auerbach, AD. Diagnosis of Fanconi anemia by diepoxybutane analysis. In: Dracopoli, NC.; Haines, JL.; Korf, BR.; Moir, DR.; Morton, CC.; Seidman, CE.; Seidman, JG.; Smith, DR., editors. *Current Protocols Human Genetics*. Vol. 37. John Wiley & Sons, Inc; Hoboken, NJ: 2003. p. 8.7.1-8.7.15.
29. Auerbach AD, Liu Q, Ghosh R, Pollack MS, Douglas GW, Broxmeyer HE. Prenatal identification of potential donors for umbilical cord blood transplantation for Fanconi anemia. *Transfusion* 1990;30:682–687. [PubMed: 2219253]

30. Gluckman E, Broxmeyer HE, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P, Copper S, English D, Kurtzberg J, Bard J, Boyse EA. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 1989;321:1174–1178. [PubMed: 2571931]
31. Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. *JAMA* 2001;285:3130–3133. [PubMed: 11427142]
32. Grewal SS, Kahn JP, MacMillan ML, Ramsay NKC, Wagner JE. Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. *Blood* 2004;103:1147–1151. [PubMed: 14504102]
33. Seyschab H, Friedl R, Sun Y, Schindler D, Hoehn H, Hentze S, Schroeder-Kurth T. Comparative evaluation of diepoxybutane sensitivity and cell cycle blockage in the diagnosis of Fanconi anemia. *Blood* 1995;85:2233–2237. [PubMed: 7718895]
34. Lo Ten Foe JR, Kwee ML, Rooimans MA, Oostra AB, Veerman AJ, van 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 anaemia: molecular basis and clinical significance. *Eur J Hum Genet* 1997;5:137–148. [PubMed: 9272737]
35. Gregory JJ, Wagner JE, Verlander PC, Levran O, Batish SD, Eide C, Steffenhagen A, Hirsch B, Auerbach AD. Somatic mosaicism in Fanconi anemia: evidence of genotypic reversion in lymphohematopoietic stem cells. *Proc Natl Acad Sci (USA)* 2001;98:2532–2537. [PubMed: 11226273]
36. Auerbach AD, Flit Y, Batish SD, Morales J, Berwick M. A 25 year perspective of the DEB test for Fanconi anemia: correlation of mosaicism with clinical outcome. *Am J Hum Genet* 2004;(Suppl): 90.
37. Gillio AP, Verlander PC, Batish SD, Giampietro PF, Auerbach AD. Phenotypic consequences of mutations in the Fanconi-anemia *FAC* gene: an International Fanconi anemia Registry study. *Blood* 1997;90:105–110. [PubMed: 9207444]
38. Davies SM, Radloff GA, DeFor TE, Levran O, Batish SD, Hanenberg H, Auerbach AD. GST genotype may modify clinical phenotype in patients with Fanconi anemia. *Brit J Hematol* 2005;131:118–122.



Figure 1. Fifteen-year-old female monozygous twin pair homozygous for deletion of exons 30 and 31 of *FANCA*. In panel A, Twin A (on left) had a bifid right thumb (surgically repaired), hypoplastic left thumb and absent left clavicle. Twin B (on right) had unilateral absence of the radius, bilateral absent thumbs, and absent right clavicle. The hands of Twin A are seen in panel B; the hands of Twin B are seen in panel C.



Figure 2.

A, Part of a metaphase spread of a Fanconi anemia (FA) lymphocyte showing spontaneous chromatid aberrations. B, Part of a metaphase spread of an FA lymphocyte treated with 0.1 μ ml of diepoxybutane (DEB). Multiple complex chromatid exchange figures are seen. (From Auerbach *et al.* *Pediatrics* 67 (1981) 128–138, by permission).

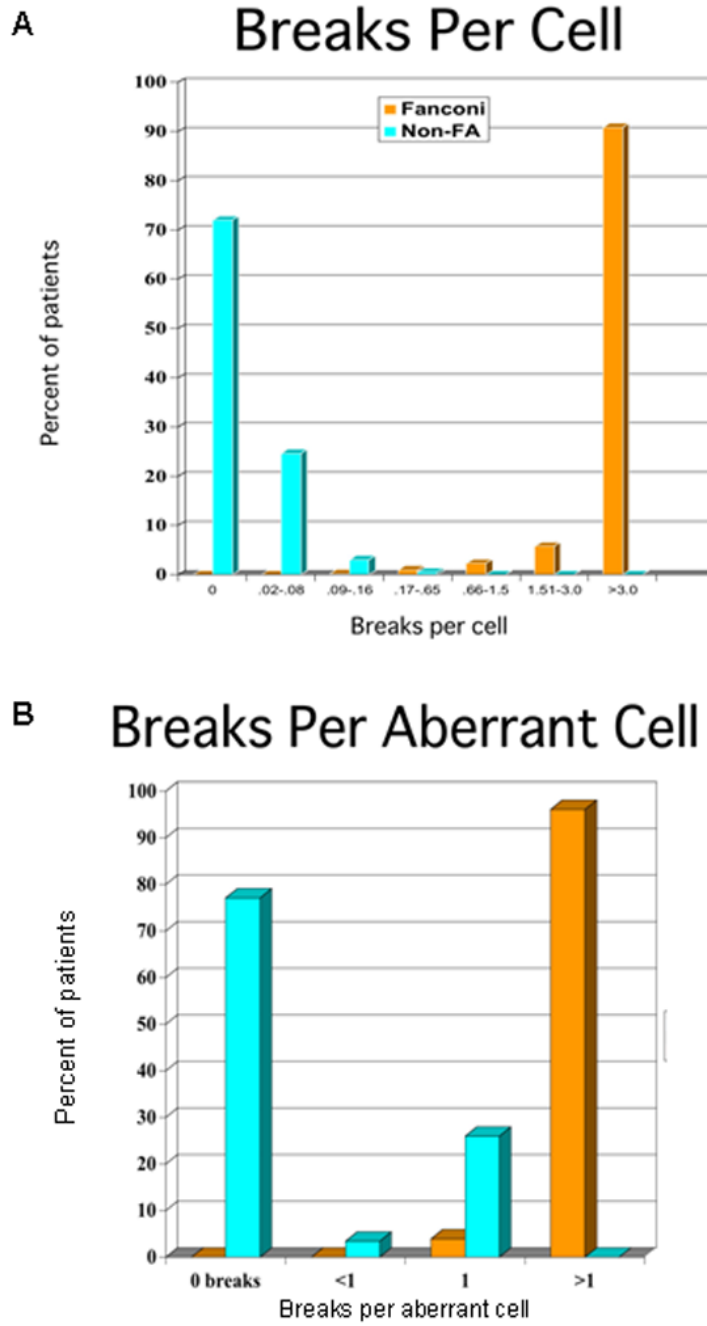


Figure 3. Comparison of FA and non-FA cells. Data is expressed as breaks/cell (A) and breaks/aberrant cell (B). 98.9% of patients diagnosed as FA and none of the patients diagnosed as non-FA had greater than 0.65 mean breaks/cell. 96.6% of non-FA subjects and 0% of FA patients had less than 0.09 mean breaks/cell. Only a small number of affected individuals could not be discriminated on the basis of mean breaks/cell.

Percent of Aberrant Cells

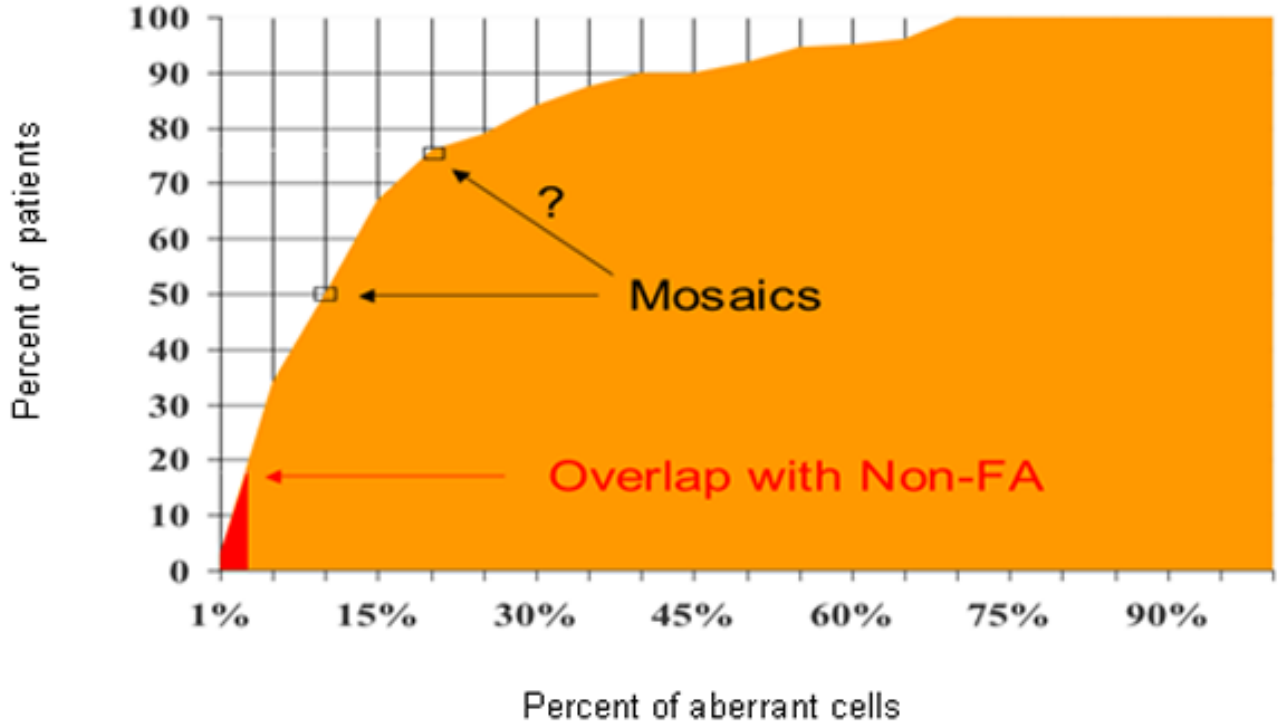


Figure 4. About 10% of FA patients have 50% or less of their cells exhibiting chromosomal abnormalities after treatment with 0.1 μ /ml of diepoxybutane (DEB). About 20% of FA patients have 75% or less aberrant cells with this treatment. Discrimination between FA subjects with very highly skewed mosaicism and non-FA subjects was uncertain in less than 2% of cases tested. It is recommended that retesting (or testing of another tissue such as skin fibroblasts) be performed in these cases.

Table 1

Congenital malformations in patients in the International Fanconi Anemia Registry (IFAR)

Skin	Café-au-lait spots, hyper- and hypopigmentation
Growth	Intrauterine growth retardation, short stature, endocrine abnormalities
Eyes	Microphthalmia, short or almond shaped palpebral fissures, ptosis, epicanthal folds, hyper- and hypotelorism, strabismus, cataracts
Thumb and radius	Thenar hypoplasia, absence or hypoplasia of radius and/or thumb, floating thumb, bifid thumb, digitalized thumb/abnormal thumb placement
Other skeletal	Dysplastic or absent ulna, micrognathia, frontal bossing, spina bifida, Klippel-Feil, vertebral anomalies, absent clavicles, Sprengel's deformity, Perthes disease, congenital hip dysplasia/dislocation, scoliosis, rib abnormalities, clubfoot, sacral agenesis (hypoplasia), leg length discrepancy, kyphosis, brachydactyly, arachnodactyly, humeral abnormality, craniosynostosis
Kidney and urinary tract	Ectopic, horseshoe, rotated, hypoplastic or absent, dysplastic, hydronephrosis, hydroureter, urethral stenosis, reflux
Ears	Deafness (usually conductive), abnormal or absent pinna, prominent ears, abnormally positioned ears (low set or posteriorly rotated), small or absent ear canals, absent tympanic membrane, microtia, fused ossicles
Genital	Males: micropenis, penile/scrotal fusion, undescended or atrophic or absent testes, hypospadias, chordee, phimosis, azospermia Females: bicornate uterus, aplasia or hypoplasia of vagina and uterus, atresia of vagina, hypoplastic uterus, hypoplastic/absent ovary, hypoplastic/fused labia
Cardio-pulmonary	Patent ductus arteriosus, ventricular septal defect, pulmonic or aortic stenosis, coarctation of the aorta, double aortic arch, cardiomyopathy, tetralogy of Fallot, pulmonary atresia
Gastrointestinal	Esophageal atresia, duodenal atresia, anal atresia, tracheoesophageal fistula, annular pancreas, intestinal malrotation, intestinal obstruction, duodenal web, biliary atresia, foregut duplication cyst
Central nervous system (CNS)	Microcephaly, hydrocephalus, Bell's palsy, CNS arterial malformations, abnormal pituitary, absent septum pellucidum/corpus callosum, hyperreflexia, neural tube defect, Arnold-Chiari malformation, Moyamoya, single ventricle