

An update on Fanconi anemia: Clinical, cytogenetic and molecular approaches (Review)

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Abstract. Fanconi anemia is a genetic syndrome clinically characterized by congenital malformations that affect several human systems, leads to progressive bone marrow failure and predisposes an individual to cancer, particularly in the urogenital area as well as the head and neck. It is commonly caused by the biallelic compromise of one of 22 genes involved in the FA/BRCA repair pathway in most cases. The diagnosis is based on clinical suspicion and confirmation using genetic analysis, where the chromosomal breakage test is considered the gold standard. Other diagnostic methods used include western blotting, multiplex ligation-dependent probe amplification and next-generation sequencing. This genetic condition has variable expressiveness, which makes early diagnosis difficult in certain cases. Although early diagnosis does not currently allow for improved cure rates for this condition, it does enable healthcare professionals to perform a specific systematic follow-up and, if indicated, a bone marrow transplantation that improves the mobility and mortality of affected individuals. The present review article is a theoretical revision of the pathophysiology, clinical manifestations and diagnosis methods intended for different specialists and general practitioners to improve the diagnosis of this condition.

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1. Introduction

Fanconi anemia (FA) was first described in 1927 by Dr. Guido Fanconi, who observed a family of 3 siblings that presented with several physical abnormalities and pernicious anemia (Fig. 1) (1). FA is defined as a rare genetic disease of chromosomal instability that affects the proteins involved in DNA repair and the regulation of the cell cycle (2). In the majority of cases, patients with FA present with an autosomal recessive inheritance pattern, where the clinical effect is progressive depletion in bone marrow function, congenital malformations and a high risk of developing solid and hematological tumors at an earlier age than the general population (2). FA is not the same as Fanconi syndrome; the latter is a hereditary or acquired defect of the proximal tubule that leads to the malabsorption of multiple electrolytes and substances usually reabsorbed in this region (3).

FA has an incidence of 1 in 300,000 live births and a prevalence of 1-9 per million (4). The carrier frequency varies according to the populations based on the founding mutations; this is how the carrier prevalence reported in the Afrikaans population in South Africa is 1 in 83 (5), in Ashkenazi Jews is 1 in 100 (6) and in the Spanish gypsies is 1 in 64 to 1 in 70 (7), compared with the general population, where it is ~1 in 189 (8). In general, the male:female ratio of the presentation of the disease is 1.2:1 (9). This disease is a consequence, in the majority of the cases, of biallelic mutations in the 22 genes that been determined to be involved in DNA repair and genome stability, termed complementation groups *FANCA-FANCW* (10,11). The primary inheritance pattern is autosomal recessive (genes *FANCA*, *FANCC*, *FANCD1/BRCA2*, *FANCD2*, *FANCE*, *FANCF*, *FANCG/XRCC9*, *FANCI*, *FANCL/BRIP1*, *FANCL*, *FANCM*, *FANCN/PALB2*, *FANCO/RAD51C*, *FANCP/SLX4*, *FANCO/ERCC4*, *FANCS/BRCA1*, *FANCT/UBE2T*, *FANCU/XRCC2*, *FANCV/REV7* and *FANCW/RFD3*) (11,12), except for *FANCB*, which exhibits X-linked recessive inheritance (13), and *FANCR/RAD51*, which presents a *de novo* autosomal dominant inheritance pattern (14,15).

All proteins encoded by the aforementioned genes participate in the FA/BRCA repair pathway, which detects damage that covalently binds the two DNA strands (interstrand

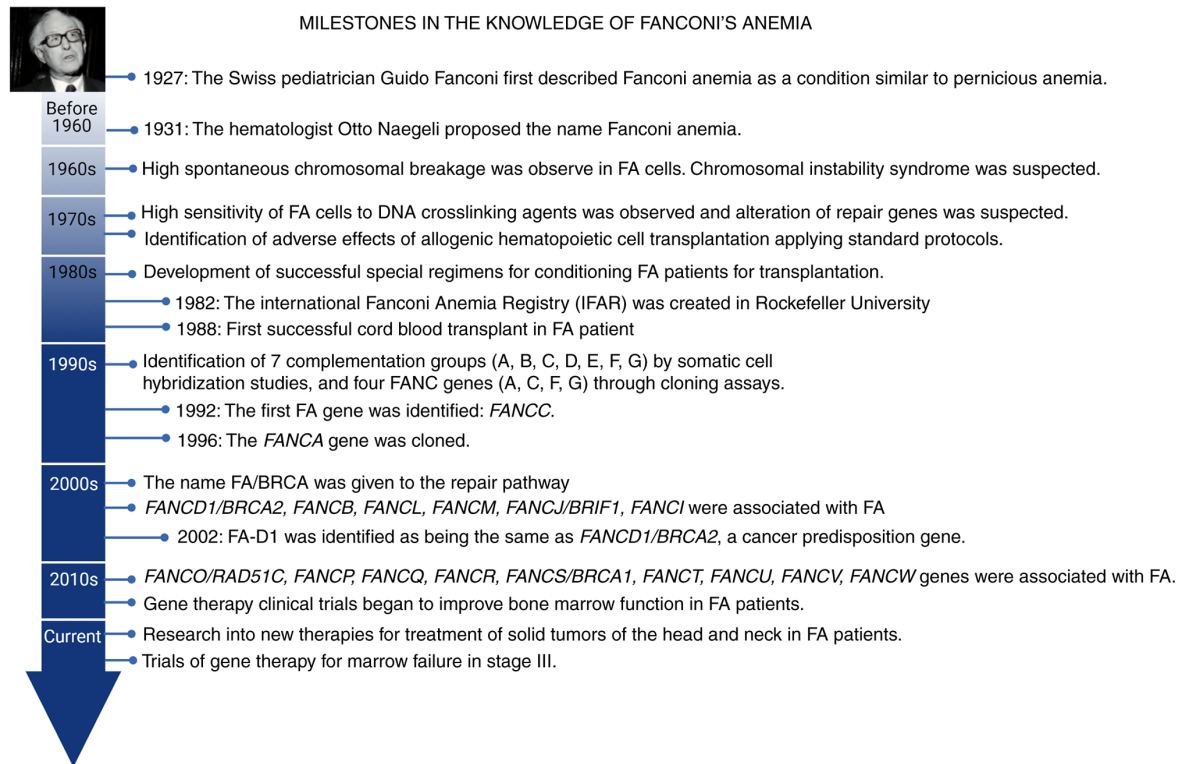


Figure 1. Principal milestones in the research and diagnosis of FA. The important events in the evolution of knowledge in the clinic, genetics and treatment of FA. Dr Guido Fanconi described FA in 1927; based on his experience, he hypothesized that patients' symptoms were very complex and could not be caused by a single genetic defect. He suggested a chromosomal alteration caused it, but this was ruled out in the 1960s. Here the search for the genetic defects responsible for the complexity of the disease begins and the development of therapies adjusted exclusively to patients with FA (25,38,50,73-76). Created with BioRender. FA, Fanconi anemia.

crosslinking; ICL) and coordinates their repair through homologous monoubiquitination and recombination (16).

ICLs are formed in DNA by the presence of exogenous agents, such as cancer chemotherapeutics, as well as by endogenous agents, such as alcohol metabolites, cigarette smoke, acetaldehyde and malondialdehyde (17). These lesions cause the blockade of transcription and replication forks, making it impossible to separate double-stranded DNA; at this point, the response to DNA damage and the homologous recombination repair processes that act in the S-phase are activated (11). Individuals without compromises in this group of genes manage to eliminate ICLs; however, patients with FA do not have the optimal machinery. Unrepaired ICLs lead to DNA breakage and nonhomologous end-joining of free ends, which are visible and countable on metaphase chromosomes, in a chromosome breakage study (17). Consequently, there is an accumulation of damage in the genome, chromosomal instability, a high risk of cancer and congenital malformations in the majority of those individuals affected (16). Recently, FA proteins were discovered to fulfill other noncanonical functions in maintaining the integrity of genetic information and cellular metabolism, which explains the complexity of the FA/BRCA pathway, and its dysregulation in the etiology of the phenotype (18).

The canonical function of FANCD proteins is to repair ICLs, which can be divided into three phases: i) damage recognition, AF core complex activation, and FANCD2 and FANCI monoubiquitination; ii) FANCD2-FANCI complex formation; and iii) activation of the DNA repair complex and repair (18,19) (Fig. 2).

In the first stage, FANCM, together with the non-AF protein FAAP24 and the DNA-binding cofactors histone-fold-containing FANCM-associated protein (MHF)1 and MHF2, recognize the lesion site (ICL) and immediately recruit FA and non-FA proteins to form the core complex (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FANCT/UBE2T, FAAP100 and FAAP20), which monoubiquitinates FANCD2 and FANCI (20-22). In the second stage, the FANCD2-FANCI complex or ID complex is formed, with ubiquitination that allows its displacement to the site of damage (focus formation) and the coordination of the repair of the ICL (23). Finally, in stage three, the recruitment of FA and other non-FA proteins that make DNA incisions on both sides of the ICL and unhook it (FANCP/SLX4 and FANCP/XPF), translesion DNA synthesis (FANCV/REV7 and other non-FA proteins), and repair by homologous recombination through the formation of Holliday junction intermediaries occur for the synthesis and final replacement of double-stranded sequences of the DNA (FANCD1/BRCA2, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCP/ERCC4, FANCR/RAD51, FANCS/BRCA1, FANCU/XRCC2, FANCV/REV7 and FANCW/RFWD3). During the S phase, the detection of ICLs leads to their repair, and proteins such as ATR-CHK1 activate the cell cycle control point to decrease the speed of DNA replication and allow repair to be finished (16,19).

Early diagnosis of FA allows anticipation of possible complications and thus affects the prognosis. Early diagnosis may be based on clinical suspicions and the positive findings

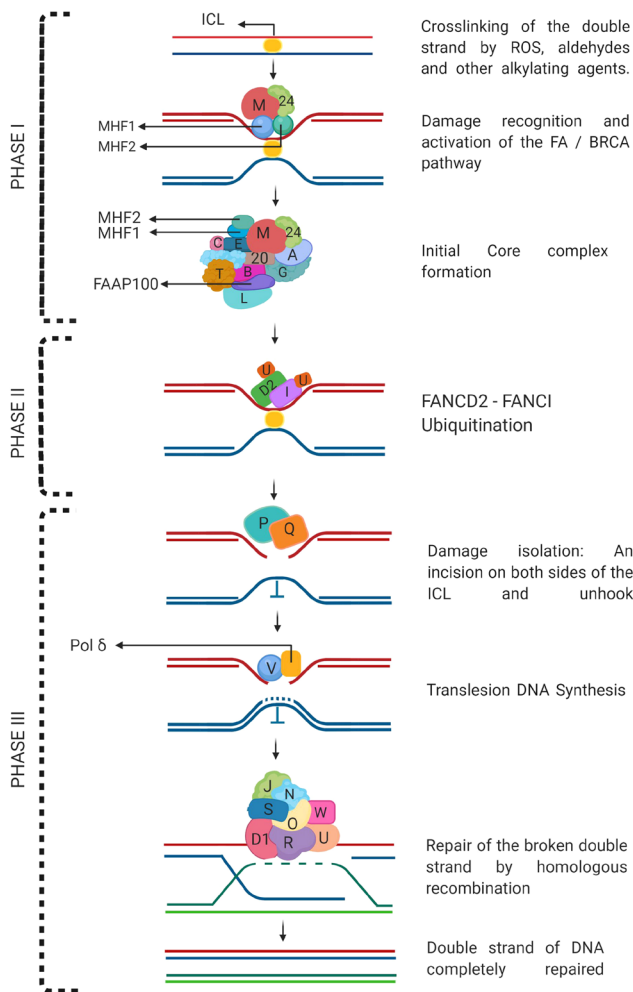


Figure 2. Schematization of the FA/BRCA repair pathway. Phase I corresponds to the recognition of ICL damage and the activation of the core complex. In phase II, the ubiquitination of FANCD2 and FANCI and their recruitment to the damage site are carried out. In phase III, the processes of ICL unhook, translesion DNA synthesis and homologous recombination occur. Capital letters identify each FANC protein, 20 and 24 are FAAP protein., Created with BioRender. FA, Fanconi anemia; ICL, interstrand crosslinking; MHF, histone-fold-containing FANCM-associated protein.

of genetic analysis, and the chromosomal breakage test is used to confirm the diagnosis; molecular tests such as western blotting, multiplex ligation-dependent probe amplification (MLPA) and gene sequencing studies using next-generation sequencing (NGS) are also used as diagnostic methods (24,25). This review presents the clinical, genetic and diagnostic aspects of FA for healthcare professionals.

2. Clinical presentation

Patients with FA present with congenital malformations, bone marrow failure that manifests as pancytopenia, and a predisposition to cancer. Practically all systems are affected by the disease; however, the clinical presentation has a variable expressiveness (2). Not all patients present malformations or pancytopenia at birth, and the first manifestation of FA in these individuals may be solid tumors, hematologic malignancies or other complications, such as infertility (2,26). Physical abnormalities are found in 75% of all patients with FA and may be accompanied by a low birth weight, short pre- and

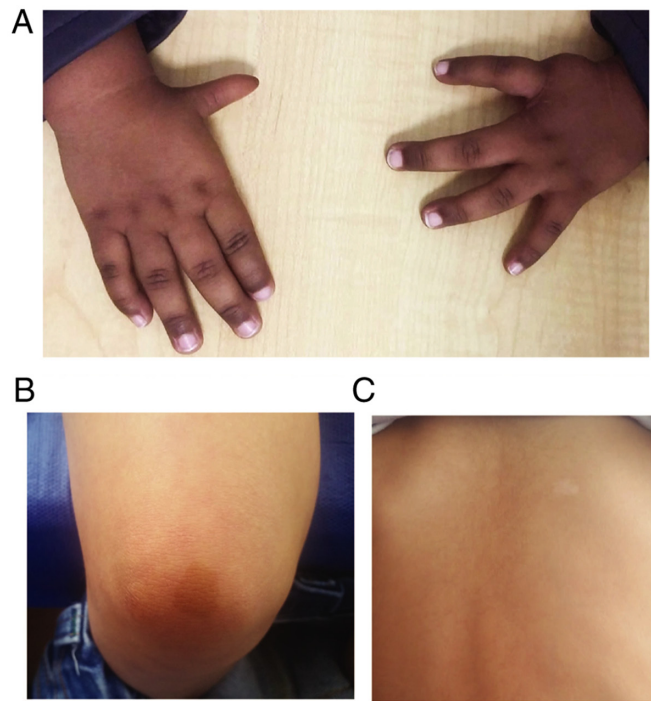


Figure 3. Phenotypic characteristics in patients with FA. (A) Typical radial ray abnormalities, bilateral thumb hypoplasia: The right hand has oligodactyly secondary to a Pollicization surgery, and the left hand has a hypoplastic thumb. (B and C) Typical skin pigmentation abnormalities: (B) Café-au-lait spot on knee. (C) Hypochromic spot on back. The photographs have come from patients analyzed at the Institute of Human Genetics, Pontificia Universidad Javeriana. FA, Fanconi anemia.

postnatal height and microcephaly (27,28). The other 25% of patients represent a challenge for clinicians because the absence of physical abnormalities can delay clinical suspicion, and therefore, the timely diagnosis of the disease (29). Below are the most frequent alterations found in the different organs.

Head and neck. The face of patients with FA has particular identifiable characteristics, such as a triangular face, bilateral epicanthic folds, micrognathia and middle facial hypoplasia. Ocular findings such as microphthalmia, cataracts, astigmatism, strabismus, hypotelorism, hypertelorism and ptosis have been described (30,31). The neck may be short with low implantation of the hairline, pterygium Colli, Sprengel deformity and Klippel-Feil anomaly (2).

Cardiac and gastrointestinal system. Cardiac malformations can be persistent arterial ducts, atrial or ventricular septal defects, coarctation of the aorta, common arterial trunk and situs inversus totalis (32). Only 5% of patients have gastrointestinal abnormalities such as tracheoesophageal fistula, esophageal, duodenal or jejunal atresia, imperforate anus, annular pancreas and intestinal malrotation (33).

Bone and limb defects. Limb defects are the most suggestive for diagnosis. However, limb defects are not observed in all patients, and may involve both the upper and lower extremities unilaterally or bilaterally (34). In the upper extremities, the radius, thumbs and hands (hypoplasia of the thenar and hypothear eminence) are the most affected regions and less

frequently, the ulna (Fig. 3A; most common malformations of the thumbs and palms of patients with FA). In the lower extremities, congenital dislocation of the hip, syndactyly and talipes have been reported (30).

In 2% of patients, spina bifida, scoliosis and abnormalities of the vertebrae (hemivertebrae, rib abnormalities, coccygeal aplasia) may be observed (2). Bone abnormalities have also been reported in the middle ear, associated with conductive hearing loss or alterations in the conformation of the auricular pavilion, which may be dysplastic or absent, low-set ears, narrow or absent internal auditory canal, absent tympanic membrane, microtia and/or fused ossicles (29).

Genitourinary system. Kidney malformations, such as horseshoe kidney, ectopic, hypoplastic, dysplastic or absence of one kidney in addition to hydronephrosis or hydroureter, have been reported in patients with FA (30). Males can present with hypospadias, micropenis, cryptorchidism, oligospermia or azospermia, and abnormal spermatogenesis is associated with infertility (26). Females may exhibit malposition of the uterus, bicornuate uterus and smaller ovaries. Up to 50% of women are infertile, and when they achieve pregnancy, they can have complications of rapid progression, such as bone marrow failure, preeclampsia and premature delivery (29).

Endocrinological system. In the endocrinological field, >60% of affected individuals may present with a short stature due to growth hormone deficiency, hypothyroidism, or glucose or insulin abnormalities. Due to the endocrinological abnormalities, affected individuals should be monitored with regard to their hormonal profile (35,36).

Cutaneous system. The alterations described in the skin are generalized hyperpigmentation, hypopigmentation and cafe-au-lait spots (Fig. 3B; Cafe-au-lait spots and hypopigmentation in patients with FA evaluated by our FA group). The areas of hyperpigmentation are primarily on the trunk, neck, groin and armpits, with a mottled pattern of large patches and diffuse boundaries (30).

Nervous system. Malformations such as small pituitary gland, an absent corpus callosum, pituitary stalk interruption syndrome, cerebellar hypoplasia, hydrocephalus and dilated ventricles in the central nervous system have been described. Additionally, some affected individuals may present with neurodevelopmental delays and intellectual disability (33).

Hematological system. The cells of patients with FA exhibit chromosomal instability generated by the presence of unrepaired damage during the S-phase; stagnation in the G2 phase or passage to mitosis without adequate DNA repair has been proposed as one of the mechanisms that induces the depletion of hematopoietic cells by cellular senescence and the presence of damage that eventually leads to bone marrow failure, myelodysplastic syndrome or acute leukemia (2,16).

The age of onset of bone marrow failure is very variable, even in the same family, and it rarely manifests in the lactation period. The average age of onset of hematological symptoms is 7 years (9). Bone marrow failure is one of the manifestations most commonly associated with FA, so in patients with

mild or imperceptible congenital malformations, the diagnosis tends to be delayed until the onset of cytopenia (37).

Generally, at the onset of the disease, thrombocytopenia or leukopenia are present, followed by anemia in fewer cases (38). In several cases, macrocytosis and increased fetal hemoglobin are observed. As bone marrow failure advances, progression to pancytopenia occurs; therefore, patients with persistent and idiopathic FA cytopenia should be suspected (39). According to Kutler *et al* (40), individuals with FA have a 90% risk of developing a hematologic abnormality by the age of 40.

Patients with FA have a high risk of developing myelodysplastic syndrome, which usually precedes acute myeloid leukemia; this condition is associated with chromosomal findings in the bone marrow, such as a gain of 3q, monosomy 7 or deletions in 7q (2,41).

As mentioned above, involvement of the hematopoietic system is the most common clinical feature in FA. The failure of the bone marrow occurs early in the life of those affected, and the median survival rate is 21 years if it is not treated early (9). Currently, the only curative treatment to restore the function of the hematological system is hematopoietic stem cell transplantation. However, this procedure requires the intervention of a trained team due to the risk of recurrence, graft-versus-host disease, and mortality (42). Recently, new treatment strategies, such as gene therapy have been implemented, in which the complications associated with hematopoietic stem cell transplantation may be avoided (38). One of these therapies uses lentiviral vectors to transfer a functional copy of a specific gene (the gene that is mutated) to autologous hematopoietic stem cells. These newly edited cells remove hematologic abnormalities in the patient and restore bone marrow cell function. The mobilization of the stem cells of the patient from the bone marrow into the peripheral blood has been proposed to collect CD34⁺ cells, correct the genetic alterations, and then infuse the cells into the patient (38). To date, these therapies have a good safety profile, but additional studies are required to investigate the possible long-term effects.

Solid tumors. In patients with FA, solid tumors have an accumulative incidence of 28% by the age of 40 years old (40,43). Solid tumors commonly occurring in the anogenital area, and the head and neck are 500 and 700 times more frequent in patients with FA than in the healthy population, especially in cases with transplanted hematopoietic stem cells (44). Tumors in the brain, in the liver (secondary to androgen treatment) and in the kidney (as Wilms tumor) can appear as other tumors (40,44). With this condition in mind, the patients should be monitored throughout a patient's life.

The most frequent carcinomas associated with FA are squamous cell carcinoma of the head and neck, preferentially located in the oral cavity, with the tongue being the most commonly affected region (45). Carcinomas appear at an earlier age than in the general population (20-40 years old) and the patients may exhibit exacerbated radiosensitivity to therapy (46,47). Patients with FA have a high risk of cancer associated with human papillomavirus, which is why they should be vaccinated (48).

The defects in DNA repair in these patients makes them extremely sensitive to chemotherapy and radiotherapy;

Table I. Syndromes with clinical characteristics common to FA.

| Diagnosis | Type of inheritance | Genes | Clinical factors common with Fanconi Anemia | Clinical factors not common to FA (Refs.) |
|---|---------------------|--|---|---|
| Diamond-Blackfan anemia | AD | <i>RPS7, RPS17, RPS19, RPS24, RPL5, RPL11, RPL35A</i> ^a | <ol style="list-style-type: none"> 1. Congenital aregenerative anemia, generally macrocytic with erythroblastopenia. 2. Short stature, Pierre-Robin sequence, urogenital, and thumb abnormalities. 3. Leukemia and increased risk of cancer. 4. Early age of diagnosis. | Pure red cell aplasia (54) |
| Shwachman-diamond syndrome | AR | <i>SBDS, EFL1</i> | <ol style="list-style-type: none"> 1. Hematological disorder: Thrombocytopenia and anemia, increased fetal hemoglobin. Some cases progress to bone marrow aplasia. 2. Presents as ichthyosis, bone abnormalities, such as metaphyseal dysostosis, and delayed motor neurodevelopment. | Pancreatic lipomatosis, exocrine pancreatic insufficiency (9) |
| Evans Syndrome (immune pancytopenia) | - | - | <ol style="list-style-type: none"> 1. Chronic hematological disorder, characterized by autoimmune hemolytic anemia, immune thrombocytopenic purpura, occasionally autoimmune neutropenia. 2. Manifests itself in childhood or adulthood. | Autoimmune disorder. Hemolytic anemia and thrombocytopenia of immunological origin (9,55) |
| Thrombocytopenia-absent radius syndrome | AR | <i>RBM8A</i> | <ol style="list-style-type: none"> 1. Bilateral absence of radius, thrombocytopenia, cardiac malformations. 2. Patients may present abnormalities in the ulna, humerus, phocomelia, and the lower extremities. | Thumbs are always present (56) |
| VACTERL association | - | - | Association of congenital malformations and at least three of the following: vertebral defect, anal atresia, heart defects, tracheoesophageal fistula, renal anomalies and anomalies in the extremities. | It does not present with microcephaly, or hematological affection (57) |
| Baller-Gerold syndrome | AR | <i>RECQL4</i> | <ol style="list-style-type: none"> 1. Association of coronal craniosynostosis, facial and radial axis anomalies, such as oligodactyly, aplasia, or hypoplasia of the thumb or radius. 2. Risk of cancer, predominantly osteosarcoma. | Craniosynostosis, with the coronal suture being the most commonly affected region. (58) |

^aRibosomal proteins. AD, autosomal dominant; AR, autosomal recessive; FA, Fanconi anemia.

therefore, the suggested therapy is surgical resection of the tumor. However, in cases where the diagnosis is belated and large tumor sizes are encountered in surgical management, the use of radiotherapy or chemotherapy is recommended as the only scheme or in association with surgery (43,49). Currently, the development of more secure and effective therapy protocols for the treatment of squamous cell tumors of the head and neck has been prioritized. Preclinical trials are being performed with drugs already approved for the treatment of cancer, with efficient cytotoxic and cytostatic activity that is nongenotoxic for the cells of patients with FA (50).

Fig. 3 presents the images of the most common alterations of 2 patients evaluated by our FA group. Fig. 3A corresponds to a 10-month-old male patient, with low height and weight at birth, bilateral thumb hypoplasia, ectopic right kidney, renal tubular acidosis, dysgenesis of the corpus callosum and colpocephaly, hypochromic spots, very brown skin and mild pancytopenia. Fig. 3B and C correspond to a 3-year-old male patient. Low height and weight, Left preaxial polydactyly, bilateral hypoplasia of thenar eminence, café-au-lait spots, hypochromic spots, mild VSD and aplastic anemia.

3. Differential diagnoses

As mentioned above, FA has variable expressiveness and can affect a range of systems. These characteristics overlap with several clinical manifestations of other syndromes, which often leads to a delay in an accurate diagnosis. Thus, according to the phenotype of the patient, the attending physician must consider different diagnoses.

At birth, malformations are the first signs that allow health professionals to suspect exposure to teratogens or congenital infection. Once acquired causes are ruled out, a genetic etiology must be considered. A complete systematic physical examination makes it possible to suspect a syndromic entity.

An example of differential diagnosis is esophageal atresia with or without tracheoesophageal fistula, which can be found at a low frequency in FA, and can also be related to the VACTERL association, and syndromes such as trisomy 21 and Klippel-Feil (51,52). However, in the case of a patient without a history of malformations with idiopathic bone marrow insufficiency, FA or other syndromes predisposing an individual to bone marrow failure or cancer must be considered, or even syndromes that affect DNA repair genes. FA is included in inherited bone marrow failure syndrome (IBMFS), which present certain common clinical signs that make diagnosis difficult. IBMFS typically includes cytopenia of at least one hematopoietic cell lineage that can progress to pancytopenia, in addition to an elevated risk of hematologic and solid tissue cancer (53). IBMFS also includes Blackfan Diamond anemia, congenital dyskeratosis, Shwachman-Diamond syndrome, amongst other, less familiar conditions (9,53,54-58). The clinical characteristics of each syndrome allow its diagnosis; however, phenotypic overlap between them frequently occurs, affecting the diagnosis and timely treatment (9,59). In these cases, it is recommended to use differential diagnostic methods to confirm a diagnosis of FA (9). Table I describes the symptoms of certain conditions that are differentially diagnosed for FA. Research on these types of clinical conditions avoids incorrect or under

diagnosis, and adequately determines the specific follow-up and prognosis of the affected individual.

4. Diagnostic methodologies

In the past, cases of FA were recognized based on the association of aplastic anemia and birth defects. However, overtime, the criteria have become more extensive, and a diagnosis is now established based on a test using hypersensitivity to clastogenic chemical agents such as diepoxybutane (DEB) or mitomycin C (MMC), where damage is associated with the formation of ICLs in DNA (8), or by the identification of pathogenic variants in the genes associated with FA through molecular studies. The most common tests for diagnosis are described below.

Cytogenetics. The cells of patients with FA exhibit exacerbated sensitivity to cyto-reduction regimens used for bone marrow transplantation and hypersensitivity to agents that cause DNA interstrand crosslinks (60). This characteristic is the basis for the chromosome breakage test, which exposes lymphocytes or fibroblasts from individuals with suspected FA to cisplatin, MMC or DEB *in vitro* (61,62).

The test consists of counting both spontaneous and induced ruptures in the metaphase chromosomes of the patients after exposure of the cells to the aforementioned agents, and comparing those ruptures with those of a healthy control individual with similar demographic characteristics. The number of chromosomal breaks per cell, the presence of radial figures, and the proportion of aberrant cells (one or more breaks/cell) are identified and recorded. A patient with FA will exhibit a significant increase in chromosomal breaks and radial figures compared with the control individual, although there may be variations in this value in patients with FA with somatic mosaicism (62,63). At present, cytogenetic tests are considered the gold standard in the diagnosis of FA (64). Fig. 4 shows the different fragility expression chromosomal events that must be evaluated to establish the diagnosis of FA.

In ~25% of cases of FA, patients may present with somatic mosaicism, which reduces or eliminates lymphocyte sensitivity to clastogenic agents. The presence of this condition causes difficulties in the identification of affected individuals utilizing the chromosomal breakage test (65). Somatic mosaicism in Mendelian hematopoietic disorders is the process in which one pathogenic mutation is reversed in a cell of a tissue, resulting in a group of cells with the defect corrected (66). In FA, this process occurs primarily in hematopoietic stem cells or lymphocyte progenitors. The corrected cells proliferate and clonally expand, improving the blood counts of the individual and thus reducing the incidence of bone marrow failure and hematological malignancy (67).

The cytogenetic test analyzes peripheral blood T cells; in this way, a high proportion of reversed T cells can lead to a false-negative result. Some individuals without FA may present with a proportion of T cells sensitive to DEB or MMC treatment, which could be interpreted as mosaicism, generating false positives (64). To resolve the overlap between patients with mosaic FA and non-FA patients, and to discriminate non-mosaics in patients with FA, it is proposed to use the chromosome fragility index (CFI), which calculates the quotient between the percentage of aberrant cells (cells with 1 or more

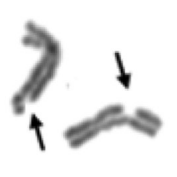

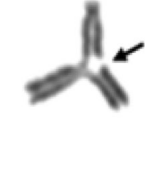
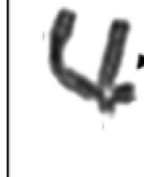

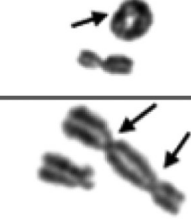
| Chromatid break | Isochromatid break | Triradial chromosome | Quadriradial chromosome | Polyradial chromosome | Ring and dicentric chromosomes |
|---|---|---|---|--|---|
|  |  |  |  |  |  |
| 1 Break | 1 Break | 2 Breaks | 2 Breaks | 4 Breaks | Two breaks per event |

Figure 4. Aberrant chromosomal events considered for the quantification of chromosomal breaks. The images have come from FA fragility tests of patients performed at the Institute of Human Genetics, Pontificia Universidad Javeriana.

breaks) and the number of breaks in multi-aberrant cells (cells with 2 or more breaks): [CFI = percentage of aberrant cells x (number of aberrations/ number of multi-aberrant cells)]; Fig. 4 shows the establishment of the number of breaks per chromosome or chromosomal event. For the Spanish population, a patient with suspected FA and a CFI >55 is considered to have FA, while within the group diagnosed with FA, when a patient has <40% aberrant cells, it is considered mosaic (64). In studies of patients lymphocytes, where they have been reported as normal or inconclusive and reversal mosaicism of their bone marrow mutation, but FA is suspected, a test of sensitivity to ICL-inducing agents in fibroblasts is recommended (62).

MLPA and array comparative genomic hybridization (array-CGH). In ~70% of cases of FA, the cause of the disease are pathogenic variants in the *FANCA* gene. Although the majority of these variants are produced by point mutations, up to 44% of cases can be caused by small deletions/insertions and large intragenic deletions. These types of variants have not only been described in the *FANCA* gene but also in other genes related to the disease (68).

MLPA allows the detection of intragenic deletions in *FANCA* genes and is recommended for the initial screening of patients when the proportion of large intragenic deletions amongst mutated alleles is high, as it happens for the *FANCA* gene (69). This test is useful to confirm or dismiss compound heterozygosis, which explains the phenotype of the patient. Additionally, the analysis for the search for large intragenic deletions can be performed by array-CGH, which allows establishing the extension of the deletions beyond the limits of any *FANCA* gene, and the exact points of breakage and loss in the chromosome (68). The array-CGH test is important as the additional loss of other genes involved in the deletion may contribute to the phenotype of the patient (68).

Molecular test. With the advent of NGS, the identification of new genes associated with FA has been achieved and has allowed the analysis of several genes involved in different diseases, where clinical diagnosis is not easy. Currently, 22 genes have been confirmed to cause FA.

Within the molecular test, clinical exome sequencing or the panel of genes specifically analyzes the exons of the

genes that are involved in the disease. However, despite having improved coverage and specificity, the molecular test has the disadvantage that not all panels include the same number of genes and have a low cover of intronic regions (70). Due to the above disadvantages, the specialist must ensure that the requested study contains all the genes associated with FA. For carriers or prenatal tests, the sequencing of a single gene or a specific mutation is indicated.

Once the pathogenic variant associated with FA has been identified, carriers in the family can be identified. The case of an autosomal recessive inheritance pattern will require the study of the parents to determine their carrier status and the risk to offspring. In the case of identifying a female carrier under the context of an X-linked recessive disease, the specialist should advise not only on the risks but also on the options for having healthy children, such as preimplantation diagnosis (2). In situations where the disease is considered sporadic, the patient must attend a consultation with his geneticist if they wish to have children.

5. Genetic counseling

Counseling is based around the inheritance pattern and the genes involved in the disease. Parental consanguinity should be questioned, as the autosomal inheritance pattern is most commonly associated with this syndrome (2).

In autosomal recessive FA, each child of a couple with a pathogenic variant has a 25% probability of inheriting both pathogenic variants and being affected, a 25% probability of inheriting both benign variants and being healthy, or 50% probability of being a carrier by inheriting a single pathogenic variant (heterozygous). When an affected individual is diagnosed with FA, there is a possibility of another asymptomatic affected individual amongst the siblings; here, cytogenetic tests should be used on all siblings to rule out the disease, as a timely diagnosis can improve the prognosis.

In autosomal dominant FA caused by a pathogenic variant in the *RAD51* gene, two cases have been reported, each with a *de novo* variant, so the risk of having this same disease for other family members is presumed to be very low (14,15). Although only 2 cases are known, the search should not be discarded, especially if clinical suspicion is high.

Considering to whom the counseling is given in the X-linked recessive inheritance is overriding because of the probability of transmitting the pathogenic variant changes. If genetic counseling is provided to an affected man with a healthy partner, their daughters will be carriers of the pathogenic variant, but not their sons, since they obtain the Y chromosome from their father. Carrier women have a 50% chance of transmitting the pathogenic variant in each pregnancy. According to this scenario, male children who inherit the pathogenic variant of the mother will be affected, whilst women will be healthy carriers.

Distinguishing heterozygous carriers from noncarriers has genetic implications, as they may have an increased risk of cancer. For example, carriers of heterozygous pathogenic variants in *FANCD1/BRCA2* have an increased risk of breast and ovarian cancer (65). Knowing the pathogenic variants of the family is a priority to identify carriers or other affected members.

6. Other issues regarding diagnosis

FA is a rare disease, general knowledge of which is still limited. At present, for several countries in the world, including in our country of Columbia, the behavior of this disease is not known in terms of its incidence, clinical characteristics, genetics and treatment, reflecting the insufficient knowledge in the medical community that exists in the clinic, despite the existence of specialized guidelines produced by the Fanconi Anemia Research Fund (initially created in 1999, now on the fifth edition, which was published in 2020) (71), and the abundance of pertinent literature (2,9,29,31,33,44,72).

In Colombia unlike in European countries, although the healthcare system allows access to cytogenetics and molecular studies, there is a significant lack of knowledge amongst healthcare professionals regarding the clinical manifestations associated with this condition. Therefore, referrals with specialists trained to diagnose orphan diseases are not prioritized. However, although the cytogenetic test is performed by several diagnostic centers, personnel trained in these types of tests are scarce, and there are no specialized reference centers for these studies in fibroblasts. Therefore, the cytogenetic group of Pontificia Universidad Javeriana in recent years has dedicated itself to investigating this disease in Colombia. In the workgroup experience with patients with FA, a delay has been observed in the diagnosis of several cases and the timely treatment of hematological complications, due primarily to the inadequate application of confirmatory cytogenetic tests for FA, confusion of the phenotype with other clinical entities, and the untimely evaluation of the patient under the clinical geneticist criteria. Thus, it is recommended that both the medical staff and the cytogenetic diagnosis should be periodically trained and updated, respectively.

7. Conclusions

FA is a hereditary disease in which there is a compromise of genes involved in DNA repair. When the cell cannot adequately repair its genetic information due to damage to this machinery, various clinical manifestations, such as bone marrow failure, congenital malformations and an increased predisposition to

cancer occur, which increases the morbidity and mortality of these patients. Before the era of in-depth molecular studies, multiple diseases with variable expressivity could not be clinically confirmed, which limited the efforts of the professionals to improve the prognosis of affected individuals. Cytogenetic and molecular studies allowed for confirmation of diagnoses of diseases with shared symptoms and has facilitated the development of knowledge of the phenotype-genotype relationship, as well as the pathophysiology of the diseases. Both clinical and paraclinical criteria support early diagnosis, prognosis evaluation, adequate monitoring and genetic counseling for individuals and families of patients with FA.

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Authors' contributions

OMM and ACP searched the literature, reviewed the articles and collected the relevant data from selected papers. OMM, ACP, AR wrote the manuscript. ACP and FSO reviewed the clinical articles. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Informed consents was signed by the parents of the patients whose images are presented (approval no. CIE-2014/150).

Patient consent for publication

Consent for publication was provided by the parents of the patients.

Competing interests

The authors declare that they have no competing interests.

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