Chapter 1: Diagnosis and Evaluation

Introduction

Fanconi anemia (FA) is primarily inherited as an autosomal recessive disorder, though about 2% of all cases (1 of 16 known genotypes) are inherited as an X-linked recessive condition.

This chapter will explore the underlying molecular and genetic processes by which FA contributes to conditions such as bone marrow failure, leukemia, squamous cell carcinoma, endocrine abnormalities, and mild-to-severe birth defects [1-3]. In general, these conditions arise from genetic mutations that cause chromosome instability and reduce the cell's ability to repair damage to DNA. At publication, FA-associated mutations have been identified in 16 genes. A few patients with FA do not have mutations in the known genes; thus, more genes likely await discovery.

The oft-cited estimate of an FA carrier frequency of 1 in 300 was recently revised to be 1 in 181 for North America and 1 in 93 for Israel. Specific populations have founder mutations with increased carrier frequencies (less than 1 per 100), including Ashkenazi Jews (*FANCC*, *BRCA2/FANCD1*), northern Europeans (*FANCC*), Afrikaners (*FANCA*), sub-Saharan Blacks (*FANCG*), and Spanish Gypsies (*FANCA*) and others as detailed in *Chapter 17* [4].

Scientific Background

FA genes and the DNA damage response pathway
The products of at least 16 FA-associated genes interact in a unified response that unfolds in the cell after exposure to DNA damage, i.e., a "DNA damage

response pathway" (Figure 1 and Table 1) [5-7]. As this pathway includes the two main breast cancer-associated genes, *BRCA1* and *BRCA2/FANCD1*, it will be referred to here as the FA/BRCA pathway.

Helpful Words and Phrases

Genotype refers to a specific set of variations in genes or the genetic makeup. It can also be used to describe a particular cancer.

An **autosomal recessive** disorder shows up clinically when a person inherits two copies of an abnormal gene: one copy from the mother and another from the father. It's **recessive** because the person must inherit both copies to develop the condition. The affected gene is located on one of the chromosomes numbered 1-22, which are known as **autosomes**.

An **X-linked recessive** condition means that females must inherit two copies of an abnormal gene for the disease to develop, whereas males need only inherit one copy. That is because males have one X chromosome; females have two.

The **carrier frequency** is the proportion of individuals who carry in their DNA a single copy of an abnormal gene for an autosomal recessive disorder. Carriers usually do not develop the disorder, but can pass a copy of the abnormal gene onto their children.

A **founder mutation** is a genetic change that is present in a population over several generations.

Biallelic mutations are genetic changes found in both copies (alleles) of the same gene.

Hypomorphic mutations are changes that cause the gene product to only lose partial function.

Because each patient generally has just one FA gene containing biallelic mutations [1], patients can be assigned to complementation groups FA-A to FA-Q. These groups are defined by the absence of a normal gene product in the cells, even if the specific mutation(s) in that gene is/are not known. In 2013, the first patient (female) was reported with biallelic mutations in the *BRCA1* gene, which plays an important role in DNA repair and has been heavily associated with breast and ovarian cancer susceptibility. This patient had one hypomorphic missense mutation (known as p.Val1736Ala), and exhibited short

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1 With one notable exception: There has been a single published report of a *FANCM* patient who also has two germ-line mutations in *FANCA* [8].

stature, microcephaly (an abnormally small head), developmental delay, early-onset ovarian cancer, and significant toxicity from chemotherapy. Nonetheless, though *BRCA1* is an essential part of the FA/BRCA pathway, it is currently not considered to be a true FA gene [9].

A simplified model for the roles of the FA proteins in the DNA damage response to interstrand cross-links at stalled replication forks is shown in Figure 1 (for reviews, see [5, 10-12]). After FANCM and the FA-associated protein FAAP24 detect the DNA damage, the proteins produced from eight FA genes (*FANCA/B/C/E/F/G/L/M*) form the FA core complex, which facilitates activation of the pathway by monoubiquitination of the FANCD2 and FANCI proteins. These two activated proteins bind to form a dimer (ID2), which stabilizes the stalled replication fork and then in turn interacts in nuclear repair foci with the downstream FA gene products in the FA/BRCA DNA damage repair pathway. Damage repair is then achieved by the late FA proteins in cooperation with proteins from other DNA repair pathways (not shown in Figure 1). The FA/BRCA pathway has been elucidated almost in its entirety through the study of FA genetics and by biochemical studies in FA cells. In addition, germ-line (heritable) mutations in at least six of the downstream FA genes, *FANCD1/BRCA2, FANCJ/BRIP1, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4,* and *FANCQ/XPF*, have been associated with breast/ovarian, pancreatic, and other cancers in heterozygote individuals. In these individuals, loss of the second, wild-type allele occurs during their lifetime in a somatic (nonreproductive) cell and subsequently leads to malignant (cancerous) transformation [13-15].

Good to Know

DNA forms a double-helix structure that looks like a twisted rope ladder. When this ladder is unwound so that it can be copied to make additional ladders, it forms a Y-shaped area called a **replication fork**.

The replication process can be interrupted by cross-links, which occur when another molecule binds to two positions on the same side of the ladder **(intrastrand cross-links)** or on opposite sides of the ladder **(interstrand cross-links)**.

Two cross-linking chemicals used in screening tests for FA are mitomycin C (MMC) and diepoxybutane (DEB).

Figure 1. DNA damage response pathway, linking the FA and BRCA pathways.
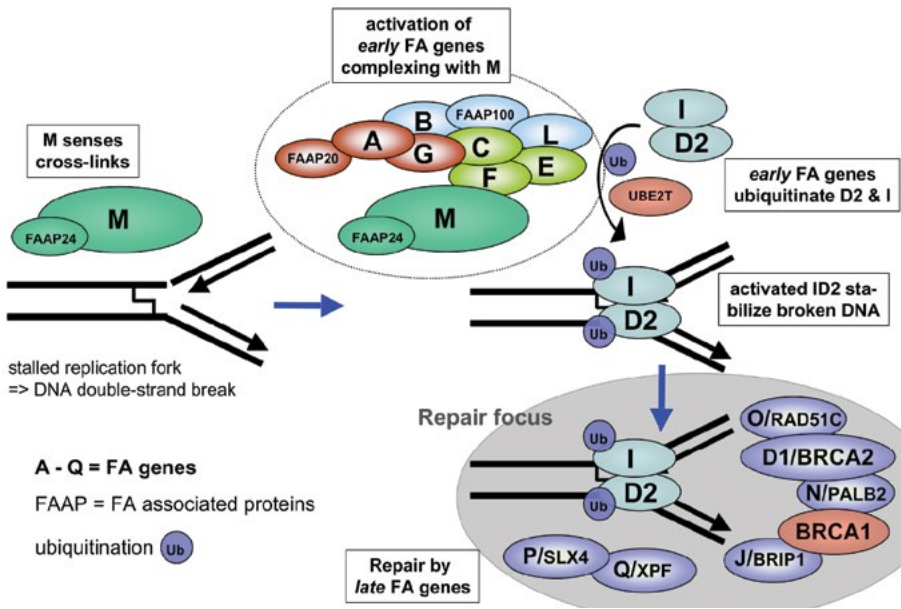
Complementation Groups

Historically, a **complementation group** is defined by a "reference cell line" (i.e., a lineage of laboratory-grown cells with a well-studied genotype) that is not functionally corrected by fusion with other cells of the same complementation group, because both cells have defects in the same recessive gene(s) and therefore cannot "complement" (i.e., correct) each other.

Cells from patients with FA can be classified into sub-categories known as **complementation groups** without knowing which defective genes or DNA mutations the patient carries. This can be done by fusing the patient's cells with the reference cells for a particular complementation group, or by expressing a normal ("wild-type") cDNA for the specific gene that defines the complementation group. This is usually performed in a laboratory using viral or plasmid vectors.

Each complementation group is defined by the defect(s) in both alleles, or copies, of a particular FA gene. That is, individuals who belong to FA complementation group A (known as FA-A) have at least one loss-of-function mutation in each allele of the *FANCA* gene, whereas individuals who belong to complementation group B (FA-B) have a mutation in the X-chromosomal *FANCB* gene. With the exception of *FANCB*, patients belonging to the same complementation group are therefore said to have "biallelic recessive mutations" in the same FA gene.

Table 1. Fanconi anemia genes and gene products.

Gene	Locus	Genomic DNA kB	cDNA kB	No. of Exons	Protein kD	Amino Acids	Approximate % of Patients	Genetics
FANCA	16q24.3	80	5.5	43	163	1455	60	AR
FANCB	Xp22.31	30	2.8	10	95	859	2	XLR
FANCC	9q22.3	219	4.6	14	63	558	14	AR
FANCD1 (BRCA2)	13q12.3	70	11.4	27	380	3418	3	AR
FANCD2	3p25.3	75	5	44	162	1451	3	AR
FANCE	6p21.3	15	2.5	10	60	536	1	AR
FANCF	11p15	3	1.3	1	42	374	2	AR
FANCG (XRCC9)	9p13	6	2.5	14	70	622	9	AR
FANCI (KIAA1794)	15q25-26	73	4.5	38	150	1328	1	AR
FANCJ (BRIP1)	17q22.3	180	4.5	20	150	1249	3	AR
FANCL (PHF9/ POG)	2p16.1	82	1.7	14	43	375	<1	AR
FANCM (Hef)	14q21.3	250	6.5	22	250	2014	<1	AR
FANCN (PALB2)	16p12.1	38	3.5	13	130	1186	<1	AR
FANCO (RAD51C)	17q25.1	42	1.3	9	42	376	<1	AR
FANCP (SLX4)	16p13.3	26.6	5.5	15	200	1834	<1	AR
FANCQ (XPF/ ERCC4)	16p13.12	39.2	6.8	11	104	916	<1	AR

Good to Know

A **heterozygote** individual is a person who carries two different copies of a gene: one normal copy and one mutated copy. **Wild-type** refers to the natural, non-mutated copy of a gene.

Complementation analysis by somatic cell methods refers to the study of a patient's nonreproductive cells to determine to which complementation group (defined by a single FA gene) the patient might belong.

Flow cytometry is a laboratory technique in which single cells in solution are used to diagnose blood cancers and other conditions. This technique can separate, count, and evaluate cells with distinct characteristics.

A **Western blot** is a laboratory technique that allows identification of proteins in cell extracts based on their size and movement in an electric field.

**Scientific techniques used for diagnostics**

Although next-generation sequencing is currently available as a standard routine diagnostic procedure for patients in most developed countries (discussed in detail in *Chapter 2*), complementation analysis by somatic cell methods has been the mainstay for distinguishing specific genetic lesions/complementation groups and is still used in a number of countries. In this technique, cells from an FA patient are tested by various methods in culture to identify the gene that corrects the FA cell's hypersensitivity to DNA damaging agents. Initially, this was done by fusing FA cells possessing unknown defects with reference cells from patients possessing a known FA gene defect or defined complementation groups, resulting in hybrid cells in which the unknown FA cells grew normally if they were corrected (or "complemented") with the known FA genes [5].

A more modern method—the only one clinically-certified in the U.S. (albeit used both clinically and in research worldwide)—is the transduction/infection of the unknown FA cells with retroviral vectors expressing normal wild-type FA proteins [16]. The patient cells can be either Epstein-Barr virus-transformed lymphoblast cell lines, primary or transformed skin or bone marrow fibroblasts, or primary T-cells from peripheral blood or bone marrow. Read-out of the retroviral complementation analysis can be any type of cellular or biochemical assay. The clinically-certified approach uses flow cytometry to determine correction of G2/M arrest in cells treated with DNA-damaging agents [17]. Each retrovirus contains only one of the FA genes. While the early FA genes

(*FANCA*, *-B*, *-C*, *-E*, *-F*, *-G*, and *-L*, though not *–M*) are easier to express, vectors also exist for the other genes and have been used for research purposes.

Alternatively, or if correction does not occur with these vectors, a Western blot can be performed to identify FANCD2 or mono-ubiquitinated FANCD2 protein and thus to determine whether the mutated FA gene is 1) upstream, which would involve the core complex needed for the ubiquitination of FANCI and FANCD2, or which would occur if *FANCD2* itself is mutated and absent; or 2) downstream, which would involve *FANCD1/BRCA2*, *FANCJ/BRIP1*, *FANCN/PALB2*, *FANCO/RAD51C*, *FANCP/SLX4*, or *FANCQ/XPF* [18]. As shown in Figure 2A, mutations in the FA core complex early genes lead to a single band of FANCD2 protein (D2-S = short), while mutations in the late FA genes are associated with normal monoubiquitination of FANCD2 and therefore have both the D2-L (= long) and the D2-S bands. Here, the classification of a patient as having defects in a late FA gene can be based on the hypersensitivity of cells in the chromosomal breakage test after crosslinker exposure and the normal FANCD2 Western blot. At least one of the mutations in *FANCD2* patients is hypomorphic and associated with residual protein function [19]. Therefore, residual FANCD2 protein can be detected in Western blots of all *FANCD2* patient cells using longer exposures. Defects in FANCI are associated with reduced FANCD2 protein levels [20], albeit monoubiquitination of the residual FANCD2 protein can be detected (Figure 2B).

Figure 2. FANCD2 Western blots for identifying the defect in the FA/BRCA pathway.
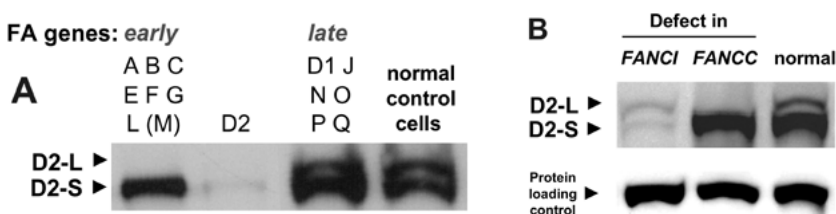
Any of these complementation group analyses should be confirmed for clinical purposes and for genotype-phenotype correlations by finding the mutation(s) in the FA gene identified in these studies and confirming the complementation group.

Diagnosis

Diagnostic screening

Good to Know

Hematopoietic stem cells are unique blood cells found in the bone marrow and umbilical cord. These cells are immortal based on their ability for self-renewal and can develop into any of the various types of blood cells found in the body.

In a person with **somatic hematopoietic mosaicism**, some cells in the blood system are genetically different from others. In FA, mosaicism is mainly used to describe cells where a spontaneous mutation reverts the defective FA gene back to the normal DNA sequence, either in stem cells or in T-lymphocytes.

The experience of hematologists familiar with FA suggests that while most individuals with the condition present early in life, a significant number of patients present beyond childhood. They may have been undiagnosed or misdiagnosed, and may not have been diagnosed until they presented with leukemia or a solid tumor or even as a "normal" stem cell transplant donor. For some patients, somatic hematopoietic mosaicism may have resulted in a less severe hematologic phenotype masking the diagnosis. The imperative is to have an index of suspicion that will lead to an early diagnosis of FA in all patients. It is essential that prospective parents be given the opportunity and benefit of reproductive choices through genetic counseling to avoid the significant consequences of the failure to diagnosis FA in a timely fashion. Indeed, genetic counseling is crucial because of the 25% risk of FA in each subsequent pregnancy (50% for the X-linked *FANCB*). Without being judgmental or proscriptive, early diagnosis provides the opportunity for family planning, prenatal diagnosis, and preimplantation genetic diagnosis, if desired by the couple/family (for more information, see *Chapter 17*).

Currently there is no established preventative treatment option to delay or avoid the clinical manifestations of FA, especially the bone marrow failure or the development of malignancies. Nevertheless, there are several benefits to consider by an early/timely diagnosis, including:

- *Avoiding medical complications from unrecognized subtle congenital abnormalities*

- *Ending the diagnostic odyssey that significant numbers of patients experience*

- *Enabling appropriate monitoring and management of hematologic disease [aplastic anemia (AA), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML)]*

- *Modifying radiation and chemotherapy protocols as needed to ameliorate the risk of severe side effects in patients where malignancies are the first clinical sign of FA*

- *Providing an opportunity to make life-style modifications to reduce risks (e.g., avoiding smoking, sunlight, alcohol exposure, or unhealthy workplace environments)*

- *Time to make family planning decisions in light of premature menopause and limited window of fertility*

For example, planned surgeries might be expedited so that they are completed before the development of significant cytopenias (i.e., abnormally low blood cell counts). Physicians can also offer targeted and intensified cancer surveillance, and early extensive surgery for solid tumors and thus avoid unnecessary and incrementally toxic chemo- and radiation therapy. In addition, experts can discuss a realistic prognosis prior to the onset of predictable adverse events. Early diagnosis also allows the patient time to consider the appropriate use of therapeutic options, including hematopoietic stem cell transplantation, androgens, hematopoietic growth factors, or supportive care while minimizing iron overload from red blood cell transfusions. Finally, the mutations can be identified prior to the next pregnancy in the family, thus giving parents time to consider their options.

Good to Know

The chromosome breakage (fragility) test is often the first test to diagnose a patient with FA. This test measures the types and rates of breakages and rearrangements found in the chromosomes of cells after challenge with DNA cross-linking agents. It also reveals how well the chromosomes can repair themselves after injury.

The tests noted here (e.g., chromosome breakage testing, mutation analysis) are described in more detail in Chapter 2, which also includes an algorithm for the laboratory diagnosis of FA. Generally speaking, the chromosome breakage test using peripheral blood T-lymphocytes is the accepted screening assay for FA. Primary skin fibroblasts have also been used as a readily available screening alternative, particularly if the T-cell analyses are ambiguous or

do not give a clear result that the patient has FA, especially when there is a question of mosaicism. The cross-linking agents mitomycin C (MMC) and/or diepoxybutane (DEB; due to its toxicity as a carcinogen, DEB is not available everywhere) are used to induce chromosome breakage, and FA cells are more sensitive than normal cells to these agents. Either or both agents are acceptable, as described in *Chapter 2*.

However, it is important to note that although a "diagnosis" of FA can be made by using a chromosome breakage assay as a screening method, this alone may lead to some confusion, as other genetic conditions can be associated with hypersensitivity, albeit less severe, to these agents. These genetic conditions, including Nijmegen breakage syndrome, are listed in *Chapter 2,* Table 1. Several important reasons for patients and families to identify the mutations in the affected FA gene are described in Table 2. A more detailed description of genetic counseling for FA is provided in *Chapter 17*.

Table 2. The benefits of genetic counseling and/or mutation identification.

Genetic Counseling and/or Mutation Identification
For the current patient • Understand appearance and consequences (genotype/phenotype) • Suggest complications and provide nuanced discussion of therapeutic alternatives (genotype/outcome) • Commence regular clinical review and surveillance • Confirm a sibling stem cell transplantation (SCT) donor does not have FA
For current family members • Identify undiagnosed affected siblings, who could be considered as SCT donors, and who need genetic counseling and surveillance for complications of FA • Identify carriers at risk for healthcare consequences, including cancer (e.g., Ashkenazi Jewish *FANCC* IVS5+4 A>T, *FANCD1/BRCA2*, *FANCJ/BRIP1*, *FANCN/PALB2*, *FANCO/RAD51C*, *FANCP/SLX4*, and *FANCQ/XPF* mutations)
For future children in the family • Prenatal diagnosis of an established pregnancy • In vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD) to create an unaffected child or identify an HLA-matched, unaffected sibling hematopoietic stem cell donor

Advances in molecular diagnostics coupled with reliable clinical information now permit genotype/phenotype correlations that can prove valuable in assisting the clinician with risk-appropriate care. New insights into the pathophysiology of FA and individual polymorphisms in detoxifying genes (e.g., aldehyde detoxification) [21] might be associated with better prognostic information and therapeutic options in the future.

Genotype/phenotype/outcome correlations
Correlations between genotype and other features can include birth defects (physical phenotype), hematologic outcomes (hematopoietic phenotype), and development of cancer (malignant outcomes), as shown in Table 3. In general, null mutations lead to a more severe phenotype (e.g., congenital anomalies, early-onset bone marrow failure, and MDS/AML) compared with the phenotypes associated with hypomorphic mutations. In particular, lower rates of anomalies are associated with mutations in *FANCA* and *FANCC* c.67delG (=322delG) [22-25]. Early bone marrow failure occurs more frequently in patients with *FANCA* null, *B*, *C* IVS5+4A>T, *F*, *G*, and *D2*19,22-25 mutations, while AML is clearly associated with *FANCD1/BRCA2*, *FANCN/PALB2, FANCA* null, and maybe *FANCG* mutations [23, 25-28]. Comparing the phenotypic consequences of homozygous IVS5+4A>T *FANCC* mutations in the clinically severely affected Ashkenazi Jewish patients and in the mildly affected Japanese patients revealed that unknown ethnic factors also play an important role in the clinical manifestations of FA [22,24,25,29]. The most common solid tumors in FA are head and neck squamous cell carcinomas (HNSCC) and vulvar/vaginal SCC [25,30,31]. These tumors **appear** to be more frequent in patients with FA genotypes who do not succumb to early death from bone marrow failure or AML, however, this may be due to a survival bias, rather than a genetic predisposition to these cancers. Specific additional solid tumors, most often medulloblastoma and Wilms tumor, are associated with mutations in *FANCD1/BRCA2* and *FANCN/PALB2* [27,28,32,33]. Table 3 will be expanded as more clinical data from large cohorts is linked to detailed genotypic information.

Table 3. Genotype/phenotype/outcome.*

Gene	Congenital Anomalies	BMF	AML	Brain/ Wilms Tumors	References
A null	+	+	+		23,25
A hypomorph	Fewer	Later	Later		23
B	+	+			34-36
C IVS5+4A>T (former IVS4)	+	+	+		22,24,29
C c67delG	Fewer	Later	Later		22,24,25,29
E	+				23,37
F	+	+			23,38
G		+	+		23,25
L	+	+			39,40
D2	+	+			19
D1/BRCA2	+++		+++	+++	25,26,32,33,41
N/PALB2	+++		+++	+	27,28

*Includes genes with reasonably well-documented associations.

Abbreviations: BMF, bone marrow failure; AML, acute myeloid leukemia; +, those with a
 relatively increased frequency compared with other genotypes; +++, those with a very high
 frequency compared to other genotypes.

Index of suspicion

The most frequent birth defects noted in patients with FA, in descending
frequency from approximately 50 to 20%, include skin hyperpigmentation;
hypopigmentation and café au lait spots; short stature; abnormal thumbs
and radii; and abnormal head, eyes, kidneys, and ears. Although there is
an inherent bias in anomalies featured in published case reports, the list of
anomalies compiled in Table 4 is a helpful guide for evaluating a patient whose
appearance suggests a diagnosis of FA. However, at least 25% of known FA
patients have few or none of these features [25,42].

Patients with FA may present with AA, MDS, single cytopenias, or macrocytic
red cells without another explanation. A diagnosis of FA should be considered
in all children and young adults with androgen-responsive or ATG/cyclosporine
A-non-responsive "acquired" aplastic anemia. It is absolutely imperative to
test for FA if a stem cell transplant is planned, as standard stem cell transplant

conditioning will result in significant morbidity and high mortality rates for patients with FA.

In addition, patients with FA are at a particularly high risk of developing specific solid tumors (e.g., head, neck, esophageal, and gynecological squamous cell carcinomas) as well as liver tumors, particularly in young patients without the usual viral or alcohol-related risk factors. Here, the role of human papillomavirus (HPV) infections as an additional risk factor in FA has not been unequivocally determined. However, numerous experiences with the two currently available HPV vaccines seem to indicate that vaccination is well tolerated in FA patients. This topic is discussed in detail in *Chapter 6*.

A diagnosis of FA must be considered and excluded in patients with MDS/AML, solid tumors, and other malignancies who experience excessive sensitivity to chemotherapy and/or radiotherapy. The presentation of specific cancers at an unusually young age and in patients who lack the usual risk factors for a specific type of cancer suggest the presence of genetic factors, such as FA, but also Li Fraumeni syndrome or other conditions, some of which are depicted in *Chapter 2,* Table 1. Somatic mosaicism in T-lymphocytes and even hematopoietic stem cells due to the reversion of an inherited mutation in an FA gene occurs in a minority of patients and should be considered in all young patients with the features described above. To reveal somatic mosaicism, DEB or MMC testing should then be performed using primary skin fibroblasts. The risk of head and neck squamous cell carcinoma is even higher in patients with FA who have received a bone marrow transplant, predominantly in those patients who experienced chronic graft-versus-host disease (GvHD) [43]. A significant number of patients with FA-associated cancers—approximately 25% of reported FA patients—were not aware that they had FA until they developed cancer (and/or significant complications from a standard cancer treatment) [30]. This finding substantiates the concern that older patients with FA may be significantly underdiagnosed. Table 4 provides a guide to determining which individuals should be screened for FA.

Often neglected, testing for FA should also be performed if spontaneous chromosome breaks are found during prenatal tests (e.g., chorionic villus sampling or amniocentesis), during the postnatal evaluation of genetic conditions, and possibly in males and females with unexplained infertility.

Table 4. Which patients should be evaluated for FA? [1,2]

Who?	Feature	Details
ANY PATIENT	Height	Short stature Microsomia
	Skin	Café au lait spots Skin pigmentation (hyper, hypo)
	Upper Limbs	**Radius:** absent, hypoplastic, absent or weak pulse **Thumb:** absent, hypoplastic, bifid, duplicated, rudimentary, attached by thread, triphalangeal, long, low set, digitized **Thenar-eminence:** flat, absent **Hand:** absent first metacarpal, clinodactyly, polydactyly **Ulna:** short, dysplastic
	Skeletal	**Head:** microcephaly, hydrocephaly **Face:** triangular, birdlike, dysmorphic, mid-face hypoplasia **Neck:** Sprengel, Klippel-Feil, short, low hairline, web **Spine:** spina bifida, scoliosis, hemivertebrae, coccygeal aplasia small, strabismus, epicanthal folds, hypotelorism, hypertelorism
	Eyes	Strabismus, cataracts, ptosis
	Renal	Horseshoe, ectopic, pelvic, hypoplastic, dysplastic, absent, hydronephrosis, hydroureter
	Gonads, male, urology	Hypogenitalia, undescended testis, hypospadias, micropenis, absent testis, infertility
	Gonads, female, gynecology	Hypogenitalia, bicornuate uterus, malposition, small ovaries, late menarche, early menopause, infertility
	Development	Mental retardation, developmental delay
	Ears	**Deafness:** conductive, sensorineural, mixed **Shape:** abnormal pinna, dysplastic, atretic, narrow canal, abnormal middle ear bones **Speech:** delayed, unclear
	Cardiopulmonary	**Congenital heart disease:** patent ductus arteriosus, atrial septal defect, ventricular septal defect, coarctation, situs inversus, truncus arteriosus
	Low birth weight	

Who?	Feature	Details
	Lower limbs	**Hips:** congenital hip dislocation
		Feet: toe syndactyly, abnormal toes, club feet
	Gastrointestinal	**Tracheoesophageal fistula**
		atresia: esophagus, duodenum, jejunum, imperforate anus, annular pancreas
		Malrotation
		Poor feeding
	Central nervous system	**Pituitary:** small, stalk interruption
		Structure: absent corpus callosum, cerebellar hypoplasia, hydrocephalus, dilated ventricles
FAMILY HISTORY	Any suspicion or patient	
VACTERL-H	Any patient	Especially if both radial ray and renal anomalies are present
HEMATOLOGY	Aplastic anemia	Any patient
	Acute myeloid leukemia	
	Myelodysplastic syndrome	
TUMORS	Head and neck SCC	"Young"- less than 50 years old
	Vulvar/vaginal SCC	
	Cervical SCC	
	Esophageal SCC	
	Brain Tumor	Midline, medulloblastoma
	Wilms tumor	
	Neuroblastoma	
	Retinoblastoma	
SIBLING OR CHILD OF FA PATIENT		
CYTOGENETICS	Abnormal chromosome tests (e.g., breaks) without DNA crosslinker in study done for non-FA purpose	

IMPORTANT NOTE: Any combination of the above should raise the level of suspicion and lead to peripheral blood testing for FA.

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