# Chapter 2

## Diagnosis of Fanconi Anemia: Testing and Genetic Counseling

### Introduction

Fanconi anemia (FA) is a very rare genetic disorder that results from DNA repair defects arising from pathogenic variants in at least 23 genes (*FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BRIP1, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4, FANCR/RAD51, FANCS/BRCA1, FANCT/UBE2T, FANCU/XRCC2, FANCV/REV7, FANCW/RFWD3,* and *FANCY/FAP100*) discovered to play a role in the FA pathway (see Chapter 1). All pathogenic variants in these genes are autosomal recessive, except *FANCB,* which is x-linked, and *FANCR/RAD51,* which is autosomal dominant. The carrier frequency of FA is 1:181 in the general population in North America and 1:93 in Israel [1]. Specific populations have a founder effect with increased carrier frequencies (1 per 100 or less), for example Ashkenazi Jews (*FANCC* and *FANCD1/BRCA2*) [2, 3], Afrikaners (*FANCA*) [4], sub-Saharan Africans (*FANCG*) [5], Spanish gypsies (*FANCA*) [6], and South Asians from India and Pakistan (*FANCL*) *[7].* The wide spectrum of disease presentation in individuals with FA is closely tied to the relationship between clinical features of the disease and the underlying genetic cause. Early diagnosis and the characterization of patient-specific pathogenic variants  (historically called mutations) is

of utmost importance as this information may influence a patient's clinical management, especially for severe cases. This chapter discusses the importance of early diagnosis and the role of genetic counseling and specific cytogenetic and molecular tests used to diagnose FA. Also included are test interpretation considerations for accurate diagnosis that can aid clinical management and facilitate appropriate testing for family members.

# Clinical Manifestations and Evaluation for Diagnosis

Most patients with Fanconi anemia have manifestations either at birth or during childhood. The median age at diagnosis is 7 years [8, 9], although it is typically younger if the clinical phenotype is more severe [10]. Those without overt congenital differences may not be diagnosed until adulthood unless they develop bone marrow failure (BMF) (see Chapter 3) or a solid tumor (see Chapters 4 and 5).

## Physical Phenotype

The physical phenotype associated with FA is extremely heterogenous and multisystemic, but can offer clues for testing and early diagnosis [9]. The classical congenital abnormalities seen in patients with FA include those described in the VACTERL-H (Vertebral, Anal, Cardiac, Tracheo-esophageal fistula, Esophageal atresia, Renal, upper Limb and Hydrocephalus) association [11]. In a recent comprehensive literature review of FA cases, the proportion of FA patients who met criteria for VACTERL-H association (presence of at least 3 of the 8 common features) was 12% [12], which was similar to prior studies (5% to 30%) [13, 14]. Other abnormalities common to FA were recently grouped with the acronym PHENOS (skin Pigmentation, small Head, small Eyes, Nervous system, Otology, and Short stature) [13]. In the previously mentioned literature review, 9% of the patients with FA had ≥4/6 features of PHENOS [12]. The most frequent abnormalities described are: short stature, skin pigmentary changes, upper limb malformations, male genitalia abnormalities, microcephaly, ophthalmic and renal manifestations [9]; all, except for the male genitalia anomalies, are included in VACTERL-H or PHENOS. Although the majority of patients will have at least one abnormality, between 25-40% will have none, thus the absence of abnormal features does not rule out the diagnosis [13, 14]. The information listed in Table 1 can be used as a guide for evaluating a patient whose appearance suggests a diagnosis of FA. Any combination of the abnormalities listed in Table 1 should raise the level of suspicion for FA.

**Table 1. Manifestations that are indicators for Fanconi anemia screening.**

| Organ, system, or feature | Abnormality |
|---|---|
| Height | Short stature |
| Head | Microcephaly |
| Central nervous system | Small pituitary and stalk interruption; agenesis of corpus callosum; cerebellar hypoplasia; hydrocephalus; dilated ventricles; developmental delay |
| Eyes | Microphthalmia; epicanthal folds; almond-shaped fissures; ptosis; strabismus; cataracts |
| Otology | Hearing loss (conductive, sensorineural, or mixed); abnormal pinna; atretic, narrow canal; and abnormal middle ear bones |
| Facial | FA facies; triangular face; micrognathia; pointed chin; mid-face hypoplasia; facial nerve palsy; microsomia; hypertelorism; hypotelorism; cleft palate |
| Heart | Patent ductus arteriosus; atrial septal defect; ventricular septal defect; coarctation; situs inversus; truncus arteriosus |
| Gastrointestinal | Tracheoesophageal fistula<br>Atresias: esophageal, duodenal, jejunal<br>Anal malformations: imperforate or bifurcated anus<br>Annular pancreas<br>Intestinal malrotation |
| Renal | Horseshoe, ectopic, hypoplastic, dysplastic, absent, hydronephrosis, hydroureter |
| Male Genitalia | Undescended, small or absent testis; microphalus; hypospadias; micropenis; absent testis; infertility |
| Female Genitalia | Hypoplastic, absent or bicornuate uterus; gonadal dysgenesis; small ovaries; rectovaginal fistula; vaginal atresia; late menarche; early menopause; infertility |
| Upper limb | Thumb: absent, hypoplastic, triphalangeal, polydactyly<br>Radius: absent, hypoplastic<br>Thenar-eminence: hypoplastic, absent<br>Others: absent first metacarpal, clinodactyly<br>Ulna: short, dysplastic |
| Lower limb | Hips: congenital dislocation/dysplasia, malrotation<br>Feet: toe syndactyly, abnormal toes, club feet |
| Vertebral | Web, hemivertebrae; Klippel-Feil; scoliosis; kyphosis; coccygeal aplasia |
| Skin | Café au lait macules; generalized hypo- or hyperpigmentation |
| Bone marrow failure | Anemia; leukopenia; thrombocytopenia; aplastic anemia; myelodysplastic syndrome |
| Leukemia | Mainly acute myeloid leukemia |
| Squamous cell carcinoma | Head and neck; esophageal; anogenital (including vulvar, skin) |
| Other cancers | Skin basal cell carcinoma; medulloblastoma; neuroblastoma; Wilms' tumor; breast; lung |

# Diagnostic Testing

Any physician who suspects that a patient may have FA should refer the patient to a hematologist and/or clinical geneticist or genetic counselor who can arrange for diagnostic testing.

All laboratories involved in the testing (both the cytogenetic and molecular laboratories) should be accredited by a recognized regulatory body and certified to perform FA testing for clinical care. Recognized accreditation bodies in the United States, Canada, and Europe are as follows:

**United States**
- Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP) provide laboratory certification and accreditation.
- The American College of Medical Genetics and Genomics (ACMG) provides detailed guidelines for cytogenetic testing and the interpretation of the results of genetic testing [15].

**Canada**
- The Ontario Laboratory Accreditation and the Canadian College of Medical Genetics (CCMG) provide laboratory oversight and guidelines, respectively.

**Europe**
- The Belgian Accreditation Council (BELAC), the French Accreditation Committee (COFRAC), the Deutsche Akkreditierungsstelle (DAkkS), the Swiss Accreditation Service (SAS), and the United Kingdom Accreditation Service (UKAS) provide accreditation services.

As FA testing is highly specialized, particularly for evaluation of chromosome breakage in response to DNA damage, only laboratories with extensive experience should undertake this testing.

The recommended testing procedures are outlined in the flow chart in Figure 1. The flow chart presents one potential algorithm for testing, starting with chromosome breakage, and followed by molecular evaluation. However, as genetic testing has become increasingly utilized as a front-line diagnostic test for newborns and pediatric patients with multiple congenital anomalies, the order of testing in these cases may be reversed. Most important, both chromosome breakage and germline genetic testing (described in the following sections) should be applied to each patient for precise diagnosis.

## Chromosome Breakage Test in Peripheral Blood Lymphocytes

The chromosome breakage test is the first test that should be performed for an individual suspected of having FA. This assay is performed in a clinical cytogenetics laboratory, often

using a sample of the patient's peripheral blood. Lymphocytes isolated from the blood sample are treated with DNA cross-linking agents; the most commonly used for FA testing are diepoxybutane (DEB) and mitomycin C (MMC) and the chromosomes are examined for evidence of chromosomal breakage [16, 17]. Cells from individuals who do not have FA have relatively few chromosome breaks or other rearrangements detected. In contrast, cells from patients with FA typically show multiple chromosomal breaks and rearrangements per cell, including complex rearrangements such as radial figures. As detailed by the American College of Medical Genetics and Genomics guidelines for cytogenetic laboratories [18], the test results report should include the breakage and rearrangement rates, as well as the distribution of chromosomal breakage among cells or the average number of aberrations per cell with and without radial figures. Further, all tests should include at least two independent cultures (e.g., samples treated with different concentrations of MMC, or one sample treated with MMC and the second with DEB, or another relevant combination) to show that the results are reliable. Because some patients' specimens will have very low white blood cell counts, it may not be possible to set up two cultures for a given test. In such cases, a second specimen should be obtained from the patient, if possible, to confirm the findings obtained from the first culture.
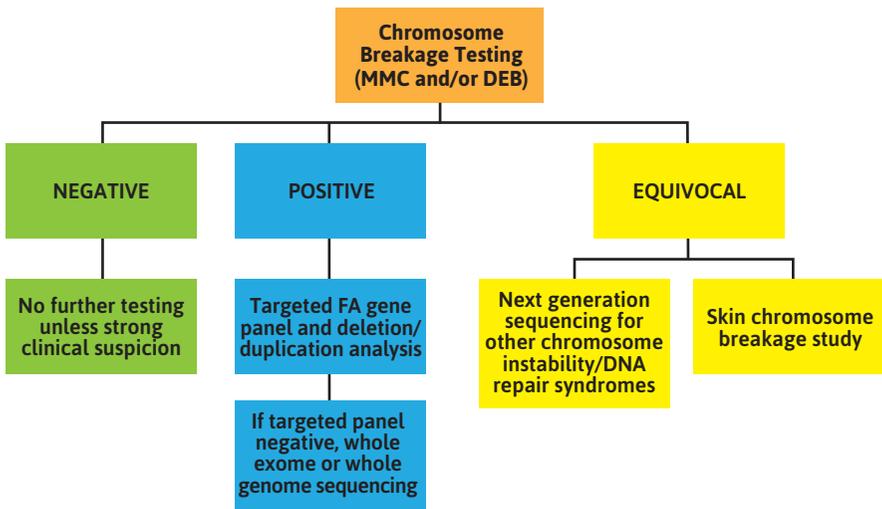


**Figure 1. Schematic representing a suggested algorithm for Fanconi anemia testing.**

The gold-standard test for diagnosing Fanconi anemia (FA) is the chromosome breakage test (CBT) using the DNA cross-linking agents mitomycin C (MMC) and diepoxybutane (DEB) (orange box). If a patient has a negative CBT (green boxes), no further testing is necessary unless there is strong clinical suspicion. In this case, a skin cell CBT should be performed. If the CBT has a positive result (yellow boxes), targeted FA gene panel should be performed. If the targeted panel is negative, whole exome or whole genome

sequencing can be performed. An equivocal or inconclusive result (blue boxes) will require next generation sequencing for variants that cause other chromosome instability syndromes, or a skin CBT for confirmation of FA.

The laboratory should also measure baseline chromosome breakage by evaluating cells that have not been treated with MMC and/or DEB. The measurements of baseline breakage can vary markedly among patients with different FA variants. For example, patients with variants in the *FANCD1/BRCA2* or *FANCN/PALB2* genes have very high levels of baseline breakage and unusual constellations of abnormalities compared with other groups of patients with FA [19]. The baseline breakage also may aid the differential diagnosis of other chromosome instability disorders that display specific types of chromosomal abnormalities, such as rearrangements of chromosomes 7 and/or 14, which commonly occur in ataxia-telangiectasia and Nijmegen breakage syndrome; telomeric rearrangements, which often occur in dyskeratosis congenita; and premature centromere separation, which are characteristic of Roberts syndrome and Warsaw breakage syndrome [20-25]. Finally, if the breakage is evaluated on G-banded chromosome preparations, it will be possible to rule out constitutional chromosome abnormalities that may provide an alternative diagnosis for the patient's clinical findings. The latter have been documented in approximately 1-2% of patients referred to rule out FA.

## Cell Cycle Analysis in Peripheral Blood Lymphocytes

Mitomycin C and/or DEB-induced chromosome breakage analysis is the most common first-line test for the diagnosis of FA. However, a few laboratories measure cell cycle kinetics, rather than chromosome breakage, in peripheral blood lymphocytes treated with mitogen and DNA cross-linking agents [26, 27]. Normal lymphocytes that do not have any DNA damage will progress through all the normal phases of the cell cycle without significant delay. However, cells that have DNA damage will stop at the S/G2 phase of the cycle to repair the damage before they progress to M phase. Because FA cells have more unrepaired damage after treatment with DNA crosslinking agents, a higher percentage of cells (generally 40% or more) from FA patients will be arrested during the S/G2 phase when compared to cells from individuals without FA. Some laboratories may use cell cycle analysis in conjunction with a chromosome breakage test for research purposes. Although cell cycle analysis is not currently used in the clinical setting, the principles and flow chart delineated for the chromosome breakage test should be applied. Positive, negative, and equivocal results should be followed as described for the chromosome breakage test results delineated in Figure 1.

## Interpreting Chromosome Breakage Test Results

Although chromosome breakage is considered the gold standard for diagnosis of FA, there is still the possibility that a test result is a "false positive" (the test is positive but the patient does not have FA) or that a test result is a "false negative" (the test gives a negative

result but the patient does have FA). Critical to the interpretation of the laboratory results is the establishment of positive and negative control ranges by the laboratory. In order to establish these ranges, the laboratory must have tested a sufficient number of patients (typically 30 or more) with a confirmed diagnosis of FA. Situations that may yield a false negative or false positive test result are described in the following sections.

### Positive Test Result

A patient is considered to have a positive test for FA if the lymphocytes display markedly increased chromosomal breakage and rearrangement after treatment with MMC and/or DEB compared with their baseline breakage. Typically, more than 90% of the metaphase cells examined in the MMC or DEB treated culture from an individual with FA will show increased breakage, and the rates and types of breakage observed will fall within the laboratory's established FA range. After a positive result, a genetic counselor can help coordinate the necessary follow up. Importantly, follow-up testing should be performed to identify the patient's pathogenic variant(s) using the molecular methods described in this chapter.

In some cases, a diagnosis of FA may be suspected only after the individual has been diagnosed with a cancer, such as leukemia or a solid tumor. The physician may suspect FA because this patient experiences severe side-effects from the therapy that is given to treat the cancer. Evaluating MMC and DEB chromosomal breakage test is warranted.

### Negative Test Result

A test result is considered negative if the metaphase cells from the MMC or DEB treated culture do not show increased chromosomal breakage or rearrangement, and if the rates of observed breakage are within the laboratory's established normal range. If the chromosome breakage test is negative and the clinical evidence that the patient may have FA is weak, no further studies are needed. By contrast, if the chromosome breakage test is negative but there is strong clinical evidence that the patient may have FA, then skin fibroblast testing should be performed to rule out the possibility of somatic mosaicism, as described below. In addition, there are multiple disorders that have some clinical features in common with FA and are associated with some form of chromosome instability [20-25]. Therefore, patients who have a negative chromosome breakage test should be evaluated by a clinical genetics service as additional genetic testing may be warranted.

### Equivocal Test Result

Test results are considered equivocal, or inconclusive, if the percentage of cells that display chromosomal breakage patterns characteristic of FA is lower than the laboratory typically sees for FA, or if there is increased breakage but the types of breakage are not characteristic of FA. The average number of breaks per cell may fall above the upper limit of the normal control range, but below the lower limit of the laboratory's FA range. Underlying causes of inconclusive results include mosaicism in the patient's peripheral

blood cells, hypomorphic alterations, and the possibility that the patient has a condition other than FA that manifests with increased chromosomal breakage.

### Mosaicism in Peripheral Blood Cells

Somatic mosaicism can occur in T-lymphocytes and hematopoietic stem cells due to the reversion of an inherited variant in an FA gene. Testing to detect mosaicism should be performed if the clinical evidence that the patient may have FA is strong, but the peripheral blood chromosome breakage test results were reported as negative or equivocal. Mosaicism can be diagnosed by sending a sample of the patient's skin, obtained via a skin biopsy, to a certified clinical cytogenetics laboratory, which can perform the MMC/DEB chromosome breakage test on fibroblast cells. The diagnosis of FA can be confirmed by a chromosome breakage test that reveals increased breakage in the fibroblasts, with the types of breaks and rearrangements characteristic of FA. Approximately 10-20% of patients with FA have a form of mosaicism in which the fibroblast cultures show increased breakage, while the lymphocytes do not. The percentage of normal cells in the blood of these patients may range from less than 50% to 100%. Over time, a patient with a low percentage of normal cells may develop a high percentage of normal cells, and this process may be associated with spontaneous improvement in the patient's blood cell counts. However, the mosaicism measured in peripheral blood lymphocytes may not reflect mosaicism in the bone marrow cells. This means that a patient with a high percentage of normal cells in the tested lymphocytes may have no (or a very low percentage of) normal cells in his or her bone marrow. As the bone marrow cells are involved in the development of leukemia, their status should not be generalized from the lymphocyte results. It is not possible to directly test the bone marrow cells using the same chromosome breakage tests used for lymphocytes; thus, it remains unclear whether the clinical course of the disease will be altered in patients who have normal cells in the peripheral blood. Importantly, the presence of mosaicism—in either the blood or the bone marrow—does not protect the individual from the development of clonal chromosome abnormalities within the population of cells that retain their FA gene variants, which may lead to the development of hematologic malignancies. In addition, mosaicism in the blood or bone marrow also does not protect against the development of solid tumors.

# Germline Genetic Testing

If the results from the chromosome breakage test are positive, genetic testing should be performed to identify the specific FA-causing variants. Genetic testing enables accurate diagnosis and improves clinical care for individuals with anticipated genotype/phenotype manifestations and for relatives who are heterozygous carriers of FA gene variants that confer increased risk for malignancy (see Carrier Cancer Risk section in this chapter).

Further, genetic analysis is useful for preconception screening, prenatal diagnosis, and required for preimplantation genetic diagnosis (see Chapter 7).

## Next Generation Sequencing

Until recently, a genetic test known as complementation analysis was the primary method available for determining which *FANC* genes were altered in a given patient. However, complementation analysis is labor-intensive, expensive, and time-consuming. Over the course of the past decade, the development and expansion of next generation sequencing (NGS) technologies, also referred to as massively parallel sequencing or multiplex testing, have transformed the field of genetic testing because they enable detailed analysis of numerous genes simultaneously. Following a positive chromosome breakage test, NGS panel testing for clinically available FA genes should be offered as the next step of testing.

Clinical labs have evolved to offer two types of panel tests: dedicated panels (laboratory pre-selected genes associated with a patient's phenotype) and custom panels (self-selection of desired genes from a large list). When selecting a panel, it is important to consider whether the test has been designed to address variant hotspots and/or gene regions known to present reporting challenges. As an example, the *FANCD2* gene is known to have two pseudogenes that can complicate the accuracy and interpretation of testing [28, 29]. Due to the rapidly evolving knowledge of FA, many laboratories have not yet been able to add the more recently discovered FA genes to their panels. Thus, the majority of panels currently available evaluate only a subset of the 23 known FA genes [30, 31].

In addition to sequencing, testing should always include copy number analysis that will identify large deletions, duplications and insertions [32]. This is critical as 35% of FA patients harbor large deletions that account for 18% of all FA pathogenic variants [33]. Due to the high rate of copy number variants, techniques that can detect gene deletions, duplications, and insertions, such as array comparative genomic hybridization (aCGH), multiplex ligation-dependent probe amplification (MLPA) or NGS-based copy number analysis, are an important part of the genetic testing process; and, it should be determined whether this type of assessment is included in the chosen test platform. Copy number variants (CNV) can be performed in tandem with panel testing or as a reflex test. In cases where the diagnosis of FA is in question, broader panels targeting a specific phenotype such as bone marrow failure or MDS/AML may be considered. Broad panels often are not comprehensive for each of the syndromes they analyze, so an FA-specific panel is still preferred when the diagnosis of FA is considered likely.

## Whole Exome and Whole Genome Sequencing

Whole exome sequencing (WES) is an NGS approach that is more expansive than the sequencing of targeted panels of genes. This technique aims to sequence all the exons and splice sites of all known genes, which represent approximately 2% of the human genome. An even more expansive NGS application is whole genome sequencing (WGS), which

analyzes the entire human genome. Clinical WGS recently has been made available; however, the analysis largely remains focused on exons and splice sites, as the ability to interpret the impact of variants outside of those regions is still limited. The high cost of such testing currently prohibits this as a frontline testing tool. It may be warranted to use WES for an individual with a diagnosis of FA based on a positive chromosome breakage test but without causative variants identified on a dedicated FA panel test.

Targeted panels can identify novel variants within known FA genes, but only tests such as WES or WGS can identify novel FA genes since they screen regions of the genome and beyond [29]. Additionally, WGS looks at regions within known FA genes that may not be covered by other methods, such as deep intronic or promoter variants, and, therefore, could detect novel variants in classic FA genes. While WES and WGS are beneficial for detecting variants in a larger area of the genome when compared to panel testing, these methods are not without risks and limitations (See Table 2). Critically, WES/WGS may identify a greater number of variants of uncertain significance, and may create ethical dilemmas in the event of findings not related to the patient's phenotype [29, 34]. These aspects should be presented to the patient/family in advance. Genetic counselors are experienced in conducting informed consent conversations and ordering broad sequencing tests like WES/WGS. They can assist with results interpretation and should be involved in results discussions with providers and families.

Each assay offers different advantages as well as limitations. Table 2 provides an overview of the benefits and limitations for dedicated gene panels, WES, and WGS sequencing.

**Table 2. Benefits and limitations of current next generation sequencing platforms.**

| Platform | Benefits | Risks/Limitations |
|---|---|---|
| Dedicated gene panel | • Clinically available genes associated with a patient's phenotype are analyzed in a single test.<br>• Certain regions may be addressed specifically to capture accurate data of known variant/mutation hotspots and sequencing challenges, e.g., *FANCD2* pseudogenes.<br>• Fast turnaround time and lowest cost option. | • Will not detect larger deletions/duplications if copy number analysis is not included. Also will likely fail to detect variants that are deep intronic or in the promoter of a gene.<br>• Variants of uncertain significance may be identified.<br>• Incidental discovery of a hereditary cancer risk in the family not associated with the underlying FA diagnosis is possible. |
| Whole exome sequencing (WES) | • All coding regions (exons) of the genome are sequenced in a single test.<br>• May provide value for patients not identified with causative variants by dedicated panel testing (may provide opportunity for gene discovery through research).<br>• WES can provide information for conditions other than FA if diagnosis is uncertain and if those conditions have specific clinical management. | • Cannot detect larger deletions/duplications or structural changes like translocations and inversions, distinguish pseudogene regions, or detect deep intronic variants. Overall coverage is poorer, and some exons are not analyzed effectively.<br>• May uncover findings not related to patient's diagnosis, with potential for greater number of uncertain variants than panel testing.<br>• More expensive than panel testing and slow turnaround time. |
| Whole genome sequencing (WGS) | • All coding and non-coding regions (exons and introns) of the genome are sequenced in a single test.<br>• May provide value for patients not identified with causative variants by dedicated panel testing or WES (may provide opportunity for gene discovery through research).<br>• WGS can provide information for conditions other than FA if diagnosis is uncertain and if those conditions have specific clinical management. | • Standards of what will define a clinical genome are still emerging. Assay cost, turnaround time and variant interpretation are still subject to further refinement to be clinically relevant. |

## Special Considerations with Genetic Testing

### Genetic Discrimination

Fear of discrimination is a common concern for patients when considering genetic testing. Genetic discrimination occurs when people are treated differently because they have a

genetic variant that increases the risk of an inherited condition. The Genetic Information Nondiscrimination Act (GINA) is a U.S. federal law designed to protect people from health insurance and employment discrimination. The GINA does not protect against this discrimination with other forms of insurance such as life, disability, or long-term care insurance.

## Variant Interpretation

A major challenge in the interpretation of genetic testing is the identification of variants of unknown significance (VUS). A VUS is a DNA alteration with an uncertain relationship to disease. Although healthy variation in the human genome is expected, the more of an individual's genome that is analyzed, the more likely it is to find sequence alterations that are novel and difficult to interpret. The American College of Medical Genetics and Genomics (ACMG) has recommended that a standard classification system be used to create a common language for clinical variant interpretation [35]. Based on specific criteria, a sequence change may be characterized in terms of its relationship to disease as one of the following: pathogenic, likely pathogenic, VUS, likely benign or benign. While pathogenic and likely pathogenic results are often sufficient to provide a genetic diagnosis, VUS findings should be interpreted with caution. Families should be encouraged to stay in contact with their genetics team annually for updates on the interpretation of their specific variant(s) and enter research studies that can assess pathogenicity.

## Variant Confirmation

Although sequencing platforms can detect the presence of genomic variants, they may or may not detect "phase." Phase refers to the positioning of variants affecting the same gene. *In cis* variants are located together on the same copy of a given gene; *in trans* variants are situated on opposite copies of the same gene. Thus, to confirm a diagnosis of autosomal recessive FA, parental testing should be offered to confirm that the variants are positioned *in trans*.

## Secondary Findings

As the number of genes in the analysis increases, so does the potential to identify additional findings that may or may not be related to the goal of testing. In addition to identifying the underlying genetic cause of an individual's FA, larger panels, WES, and WGS also may reveal a variant in a gene linked to other health risks. In this scenario, the unanticipated variant is called a secondary finding. For example, testing may detect two *FANCA* gene variants that explain the patient's FA phenotype, and also identify a single pathogenic *BRCA2* variant associated with Hereditary Breast and Ovarian Cancer Syndrome. Patients (and/or their parents or guardians in the case of children) should be told of this potential in advance. In the case of WES and WGS, the ACMG has compiled a specific list of genes for which reporting of secondary findings is recommended [36]. As a critical component toward the acknowledgement of a patient's right "not to know," it is

important to review the opportunity to opt out of receipt of secondary findings during an informed consent discussion for WES and WGS.

### Negative Molecular Test Results

Negative molecular test results should be carefully interpreted for an individual with a chromosome breakage test within the FA range. One explanation for a negative result is the presence of a variant(s) in an undiscovered *FANC* gene or a type of variant in a gene that cannot be identified with current technology; another possibility is somatic mosaicism [37, 38]. Analysis of an alternative sample type (such as fibroblasts) may be considered in individuals presenting with an FA phenotype and negative genetic studies on peripheral blood.

# Bone Marrow Analysis for Somatic Genetic Variation

## Chromosome G-Banding Analysis

Following the diagnosis of FA, a cytogenetic study of the chromosomes of the patient's bone marrow cells should be analyzed using standard G-banding methodology. The goals of such studies are to investigate for the presence of a clone with acquired chromosome abnormalities and, if present, to characterize the observed abnormalities. The identification of a clone, which by definition involves the presence of the same numerical and/or structural chromosomal abnormalities in multiple cells, is an indication of an abnormal hematologic process. The significance of the cytogenetic findings must be interpreted within the context of the clinical findings, bone marrow morphologic findings from hematopathology examination, and immunophenotyping. It also is important to note that the cells of patients with FA demonstrate chromosomal instability and it is likely that some cells will develop random, non-clonal abnormalities. The clinical laboratory performing the chromosome analysis should have expertise in cancer cytogenetics, be familiar with FA and the types of abnormalities associated with the disorder, and be able to distinguish non-clonal abnormalities (which are limited to single cells and do not represent an emerging malignant process) from clonal abnormalities (which can herald the development of a premalignant or malignant condition).

## Clonal Abnormalities

Myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and other hematologic malignancies are associated with clonal abnormalities; therefore, the observation of a clonal abnormality may herald the emergence of neoplasms or of a precancerous condition. Some clonal abnormalities in patients with FA may persist for a long time

without causing adverse consequences; others have been recognized as being associated with more rapid progression or more aggressive disease. In either case, clonal evolution and clonal expansion are frequently associated with disease progression. If no clonal abnormalities are observed in the patient's bone marrow, then the G-banding analysis should be repeated annually. If an abnormal clone is observed, then follow-up analyses should be performed more than once per year to monitor the behavior of the clone and evaluate for evolution or expansion. To fully interpret the results of the bone marrow chromosome analysis, a hematopathologist should provide morphologic evaluation and flow cytometry and immunophenotyping should be used to provide additional characterization of the abnormal cells.

Recurring clonal chromosome abnormalities may be found in patients with MDS, AML, and other cancers (see Chapter 3). Certain chromosomal abnormalities occur more frequently in patients with FA, including a gain of material from the long arm of chromosome 1 (1qG), gain of material from the long arm of chromosome 3 (3qG), and loss of chromosome 7 (7L). These abnormalities can occur alone or in combination with each other, or with other abnormalities involving other chromosomes [39-43]. One study found that gains of the long arms of chromosomes 1 and 3, and loss of chromosome 7 accounted for 75% of the clonal abnormalities observed in patients with FA [39]. The finding of the 3q gain, in particular, is specific to FA, and frequently is associated with cytogenetic evolution that includes monosomy 7 and leads to MDS. In fact, discovery of a 3q gain in a patient with apparent de novo MDS or AML should trigger the recommendation of breakage analysis.

## Fluorescence In Situ Hybridization

Because a given clonal abnormality, such as the gain of the long arm of chromosome 3, is often embedded within a more complex structural abnormality (for example, a very small amount of material from 3q may be translocated to another chromosome), it may be difficult to accurately characterize using G-banding alone. In such cases, fluorescence in situ hybridization (FISH), which employs fluorescently labeled chromosome region or gene -specific probes, can be a highly informative addition to G-banded chromosome analysis. Other subtle abnormalities may be completely overlooked without the use of FISH. While G-banding examines all chromosomes for abnormalities, FISH analysis typically examines cells for a small set of pre-specified abnormalities. Furthermore, G-banding is limited to the dividing cells and is rather labor intensive, which limits the overall number of cells analyzed. FISH analysis, on the other hand, can be used to quickly examine more than 100 cells. Thus, the two techniques of G-banding and FISH complement each other. Because the gain of 1q (1qG) and/or 3q (3qG), and loss of 7 (7L) comprise the majority of the clonal abnormalities seen in cells from patients with FA, it is recommended that, in addition to the G-band analysis of 20 metaphase cells, FISH analysis of 100 to 200 interphase cells be performed to detect low-level presence of a clone harboring one of these three abnormalities. Some laboratories use FISH analysis for a larger number of regions involved

in MDS and AML (e.g., 5q, 20q) in both FA and non-FA patients. Such FISH panels can be applied to either unstimulated peripheral blood or to bone marrow. The concordance between FISH results on blood and bone marrow in patients with FA has not yet been clearly established; however, some physicians and laboratories have started to perform FISH analyses on peripheral blood samples that are collected at time points in between the annual scheduled bone marrow testing. This intervening blood FISH study is being tested as a noninvasive means of monitoring, on a more frequent basis, for the emergence of an abnormal clone with 1qG, 3qG, or 7L.

## Genomic Microarray Testing

Genomic microarray testing is a relatively recent technique that has become a major tool for cytogenetics and/or molecular laboratories. Microarray techniques such as array comparative hybridization and/or single-nucleotide polymorphism analysis can identify regions of chromosomal loss and/or gain that may be too small, too ambiguous in banding pattern, or too complex to be identified by conventional chromosomal banding techniques. Sometimes there are so many abnormalities in a single cell, that a specific abnormality is essentially hidden. Microarray techniques are highly sensitive for detecting and identifying the origin of regions of chromosome loss and gain. For example, microarray techniques can rapidly detect and characterize the presence of a 3qG abnormality and provide specific information about the boundaries of the region that is gained. However, one limitation of this technique is that the clonal abnormality must be present in a sufficiently high percentage of cells (generally higher than 10%) to be detected. Unlike FISH and conventional G-banding analyses, microarray analysis does not provide information about individual cells, but rather provides results based on the total population of cells sampled. However, given the now wide availability of microarray testing, in the case of a complex bone marrow chromosome result, microarray analysis is recommended.

# Genotype/Phenotype Associations in Fanconi Anemia

Fanconi anemia is a genetically and clinically heterogeneous disease. In some cases, knowing the gene and specific variant(s) can be a critical component of identifying potential risk and attempting to understand clinical course. Medical management for most individuals with FA will be in accordance with their clinical presentation. However, for individuals with variants in genes that have altered phenotypes, genotype identification is essential for proper medical management and for prognostic purposes, particularly as genes with FA-like phenotypes may exclude classic FA symptoms. It is important to recognize that genotype/phenotype information often is based on a limited number of

cases and that outliers to the traditional phenotype have been observed. Several FA variants for which sufficient information is available is included below.

## FANCA

One study reported that individuals with homozygous null variants in the *FANCA* gene develop anemia at an earlier age, and have a higher incidence of leukemia than individuals with residual function *FANCA* variants [44]. However, a separate analysis revealed that the age of onset of anemia and incidence of leukemia was not altered in patients with homozygous null *FANCA* variants or in patients who express an abnormal form of the protein [45]. Specific variants may help predict phenotype such as the p.His913Pro and p.Arg951Gln/Trp variants that have been reported in association with a later onset of disease and slow hematologic progression [46].

## FANCB

Males with a truncating variant in the *FANCB* gene frequently present with overt findings consistent with VACTERL-H [47], although a milder phenotype has been reported for patients with missense variants or somatic mosaicism [48, 49]. Female *FANCB* carriers do not appear to have associated disease findings [50].

## FANCC

The International Fanconi Anemia Registry (IFAR) noted that individuals with variants in *FANCC* had an earlier age of onset of bone marrow failure and poorer survival compared to individuals with variants in *FANCA* or *FANCG* [51]. This finding was not reported by the European FA Research Group, which described the least severe hematologic course and fewer somatic abnormalities in the *FANCC* group when compared to *FANCA* and *FANCG* [44]. Multiple variants in the *FANCC* gene have been associated with specific phenotypes. Variants located in a region of the gene known as exon 15 (historically exon 14) have been reported in association with the development of blood abnormalities at an earlier age, more congenital abnormalities and poorer survival compared with individuals who have variants in exon 2 (historically exon 1) [51, 52]. The variant c.456+4A>T (formerly known as IVS4+4A>T) also was reported in association with a more severe disease presentation in Ashkenazi Jewish individuals [52, 53]. However, this variant has been reported in other populations [54, 55] and may not be associated with a severe phenotype in certain groups [56]. Several studies suggest that the c.67delG founder variant (formerly known as 322delG) is associated with milder symptoms, but exceptions have been observed [52, 53, 57]. A study in the Saudi population reported that the founder variant c.165+1G>T also may be associated with a mild form of the disease [58].

## FANCD1/BRCA2

A study published in 2002 reported that individuals with FA and pathogenic biallelic variants of the *BRCA2* gene may develop leukemia, acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL), at a much earlier age than expected [59]. They also are at risk of developing solid tumors of the brain (e.g., medulloblastoma, glioblastoma multiforme, astrocytoma) and kidney (e.g., Wilms tumor), which are not commonly seen in FA [60, 61]. If a patient has biallelic *FANCD1/BRCA2* variants, additional screening with brain magnetic resonance imaging (MRI) and kidney ultrasound should be considered [62]. While some studies in this population have demonstrated a severe phenotype, including multiple congenital abnormalities and a 97% risk of developing any malignancy by 5.2 years of age [60], there is a report of older individuals with milder or later onset disease [63, 64].

## FANCG

The European FA Research Group reported that individuals with pathogenic variants in *FANCG* had more severe cytopenia and a higher incidence of leukemia than patients with variants in other FA genes [44], but this pattern was not observed in the data set collected by IFAR [51].

## FANCM

Overall, more information is needed to better understand the *FANCM* phenotype. *FANCM* was proposed in 2005 to operate as an FA core complex gene and associated with an FA phenotype in a family with affected siblings [65]. Biallelic *FANCA* variants were later identified in the affected siblings, raising the question of *FANCM* as a canonical FA gene [66]. Biallelic loss of function *FANCM* variants have since been identified in individuals diagnosed with FA, and some authors suggest an alternate phenotype associated with an early-onset cancer syndrome rather than a classical FA phenotype, as their cohorts lacked bone marrow failure and congenital anomalies [67, 68]. In 2014 it was reported that a patient with compound heterozygous *FANCM* variants exhibited chromosome fragility, thumb and thenar eminence anomalies of the right hand, and bone marrow failure [69]. Reports of early-onset breast cancer and reduced fertility (primary ovarian insufficiency and mild to severe spermatogenesis in two families) in presumed biallelic carriers without an overt phenotype also have been published [68, 70, 71]. See section on cancer risk for carriers in this chapter.

## FANCN/PALB2

Variants in the *FANCN/PALB2* gene typically are associated with a more severe clinical presentation. Similar to the *FANCD1/BRCA2* phenotype, individuals with variants in *FANCN/PALB2* develop solid tumors and leukemia at an earlier age than patients with variants in other FA genes [72]. Commonly reported tumors include medulloblastoma,

Wilms tumor, AML, and neuroblastoma [72-74]. The cancer surveillance recommendations for patients with biallelic *FANCD1/BRCA2* variants also may be considered for individuals with *FANCN/PALB2* variants in the absence of consensus guidelines. Phenotypes outside of this spectrum have been reported [75] indicating that additional cases over time may further expand the phenotypic spectrum of *FANCN/PALB2*-associated FA.

## FANCO/RAD51C

Two families with an FA-like disorder and biallelic variants in *FANCO/RAD51C* have been reported [76, 77]. In both families, the affected individuals presented with significant congenital anomalies, including some that are atypical in classical FA such as palate anomalies, holoprosencephaly, and overlapping fingers. Hypersensitivity to diepoxybutane (DEB) and mitomycin C (MMC) and increased radial breaks confirmed the diagnoses of FA. The risk of hematologic features and squamous cell tumors remains unknown.

## FANCR/RAD51

While the majority of genes associated with FA require a pathogenic variant on both copies of an FA gene, only a single pathogenic variant in the *FANCR/RAD51* gene is needed to cause disease. In the two reported cases, the *FANCR/RAD51* variant appears to have been de novo in the proband, resulting in an FA-like phenotype that includes congenital abnormalities but has not been associated with hematologic disease or cancers thus far [78, 79].

## FANCS/BRCA1

The first confirmed case of biallelic *FANCS/BRCA1* variants was reported in a 28-year-old woman with stage IV papillary serous ovarian carcinoma and severe toxicity to cisplatin treatment, although diagnosis was not confirmed by chromosome breakage analysis [80]. A second case of a 23-year-old woman with ductal breast carcinoma was confirmed to have FA through chromosome breakage studies [81]. Both individuals presented with short stature, microcephaly, dysmorphology, and some degree of intellectual or developmental disability. A recent publication reported two families with four children having chromosome breakage studies consistent with FA, as well as homozygous truncating *BRCA1* variants. All four children had congenital abnormalities and growth deficiency; one child developed T-cell acute lymphocytic leukemia at 5 years and a second child developed neuroblastoma at 2 years. The remaining two children were cancer-free at 5 years and 15.5 years of age [82]. Another case confirmed by chromosome breakage was of a 2.5-year-old female with short stature, microcephaly, neurodevelopmental delay and dysmorphology but without a history of cancer [83]. None of the reported cases have developed bone marrow failure.

## FANCQ/ERCC4

In addition to the FA phenotype, biallelic variants in *FANCQ/ERCC4* have been linked to autosomal recessive Cockayne syndrome, xeroderma pigmentosum, and a single case of XFE progeroid syndrome. Affected individuals can present with a single phenotype or concomitant phenotypes, depending on how gene function is impacted [84-87].

## FANCR/RAD51

Monoallelic variants in FANCR*/RAD51* have been reported with autosomal dominant congenital mirror movements (CMM) [88, 89]. To date, FA and CMM phenotypes have not been reported in the same individual.

# Additional Genetic Counseling Considerations

The decision to proceed with any type of genetic analysis should be at the discretion of the patient or guardian. Genetic testing has benefits, risks, and limitations, which should be reviewed in advance so that an informed decision about testing can be made. The complex challenges of genetic testing necessitate a detailed conversation with a genetic counselor as misdiagnosis or misinterpretation of test results can have a significant impact on the individual and his or her family members. Patients should be counseled by an experienced genetic counselor at the time of diagnosis and at various points throughout their lives. A genetic consultation should include discussions of the following:

- The genetic testing process
- Family, medical, and pregnancy histories
- Inheritance of FA
- Reproductive options for the patients, parents, and relatives
- Research opportunities
- Community support and resources

## Inheritance of FA

Fanconi anemia is predominantly inherited in an autosomal recessive fashion, meaning that affected individuals harbor a disease-causing variant in both copies of the same FA gene. However, etiology for a small fraction of affected individuals is due to a single disease-causing variant in either the *FANCB* or *FANCR* gene. *FANCB* is inherited in an X-linked recessive pattern, meaning males with a single pathogenic variant in the *FANCB* gene have FA. *FANCR/RAD51* is inherited in an autosomal dominant pattern meaning both males and females with a single pathogenic variant in *FANCR* would be expected to have FA. The importance of the different forms of inheritance on recurrence risk is described in the section on "Genetic Testing of Family Members."

## Family History

A genetic counselor or family member should collect a three-generation family history. Family history can be helpful in identifying other family members with FA-related clinical features and in determining inheritance pattern. Ancestry and any family history of cancer should be noted since some FA genes have carrier cancer risks. In the event that patterns or clues are identified, testing may be targeted to a single gene or small number of genes of interest.

## Ancestry

Most disease-causing variants occur regardless of ancestral background. However, in certain groups, some variants, referred to as "founder mutations," are carried at an increased frequency. Founder variant information can be useful for a few reasons:

**Phenotype Predictions:**
- For example, the *FANCC* variant (c.67delG) that is common in Northern Europeans [52] and the *FANCA* variant (p.His913Pro) that appears to be common in the Sicilian population [46] are typically associated with a milder FA phenotype. Alternatively, the c.456+4A>T variant in the *FANCC* gene is associated with a severe phenotype in the Ashkenazi Jewish population [53], while this phenotypic severity is not necessarily seen in affected individuals within the Japanese population [56].

**Carrier Frequency Predictions:**
- While the carrier frequency in the United States general population is predicted to be approximately 1:181 based on the reported incidence of FA, the carrier frequency for FA is higher in certain populations such as Spanish Gypsies, Afrikaners, and Ashkenazi Jews due to known founder events [1]. This information is important for appropriate reproductive counseling for individuals with a personal or family history of FA when his/her partner's ethnic background increases their risk for being a carrier of FA.

**Targeted Genetic Testing:**
- Historically, founder variant information could be utilized in some cases as a first tier, more targeted genetic test.

# Genetic Testing for Family Members

Once an individual's genotype is known, family members can then undergo "targeted" analysis (also called carrier or single-site testing) to determine their carrier status and inform family planning. When possible, efforts should be made to first test the affected individual. However, if the proband is unavailable for dedicated FA testing, panel testing

on relatives to identify their carrier status is a reasonable approach. In this case, results interpretation may be complicated by variants of uncertain significance (VUS), and negative test results for an unaffected relative should be interpreted with caution.

Owing to the clinical variability of FA (even within the same family), all biological siblings of an affected person should undergo a chromosome breakage test. This is particularly important in the setting of transplant where a family member is identified as a potential donor. Pending the breakage study outcome, additional testing may be considered. Subsequent testing options are listed in the diagnostic testing section of this chapter.

As described earlier, most forms of FA follow an autosomal recessive inheritance pattern. Two forms deviate from this with one following X-linked inheritance (*FANCB*) and the other displaying autosomal dominance (*FANCR/RAD51*).

## Autosomal Recessive Inheritance

Autosomal recessive inheritance means that an individual must have two copies of a non-working gene to have symptoms of the condition. Biological parents of an affected child should be offered carrier testing. Doing so confirms that each parent carries one of the known variants and proves the variants are *in trans* (on separate gene copies). Each child from parents who are confirmed carriers has a 25% chance of having FA. Unaffected siblings (following negative chromosome breakage studies) have a 67% chance of being an FA carrier. Although rare, it is possible that a parent tests negative. Explanations for this include:

- The egg or sperm involved in the child's conception developed a spontaneous change (known as a *de novo* variant)
- Only a fraction of the parent's reproductive cells has the variant (known as germline or gonadal mosaicism)
- Uniparental disomy (UPD) in which one variant is present on both gene copies and was inherited from only one parent (thus far only reported in *FANCA* and *FANCP* cases) [90]
- Misattributed parentage (the child was adopted, was the product of a donor egg or sperm, paternity or maternity was not accurately reported)

Inheritance also is an important consideration when an individual with FA reaches reproductive age. While reduced fertility is reported, some individuals with FA have conceived biological children. The likelihood of having an affected child depends on the genetic status of a partner. Comprehensive testing that includes full sequencing and deletion/duplication analysis to identify *any* pathogenic variant in the causative *FANC* gene of the partner will best inform risks to future children. For example, when FA is attributed to the *FANCA* gene, comprehensive *FANCA* analysis is needed for the partner, rather than targeted testing for the known *FANCA* variants. Depending on the couple's genetic status, pregnancy outcomes are as follows:

- If the partner tests negative, the chance to have a child with FA is very low. All children will be carriers.
- If the partner tests positive in the same *FANC* gene, there is a 50% chance each child will have FA, and a 50% chance each child will be a carrier of FA.
- If both partners have FA and have variants in the same FANC gene, all (100%) of their children will have FA.

Should both partners have FA due to variants in different *FANC* genes, their children will be carriers for two different forms of FA. The chance for their children to be affected is very low, presuming they had negative carrier testing for one another's FA type. Extended relatives on both sides of the family should be offered carrier testing for the familial *FANC* variants.

## X-Linked Recessive Inheritance

With an X-linked condition, the disease-causing gene resides on the X chromosome. In FA, this inheritance applies to the *FANCB* gene. Women have two X chromosomes while men have one X and one Y chromosome. If a woman carries the causative variant, there is a 50% chance of passing on the variant in each pregnancy. Any sons who inherit the variant will be affected. Any daughters who inherit the variant will be carriers. If the mother of an affected boy has negative carrier testing, her son's FA is likely *de novo* although germline mosaicism cannot be excluded. As such, male siblings of an affected male should undergo chromosome breakage analysis. Maternal relatives in these families have an increased chance of carrying or having the condition. Any daughters born to affected men will be obligate carriers. Any sons will be unaffected, since they inherit a Y chromosome from their fathers.

## Autosomal Dominant Inheritance

Autosomal dominant inheritance means that an individual need only one non-working copy of a gene to have symptoms of the condition. In FA, autosomal dominant inheritance applies to the *FANCR/RAD51* gene. While affected individuals to date are reported with *de novo* variants (meaning the condition was not inherited from an affected parent), parents should still be offered testing. With negative testing, germline/gonadal mosaicism cannot be excluded. A small chance remains that siblings or a future pregnancy could be affected. All siblings, therefore, should be assessed by chromosome breakage. Anyone who tests positive has FA. Any sons or daughters of a person with autosomal dominant FA would have a 50% chance to have FA, and a 50% chance to be unaffected.

## Carrier Cancer Risk

Fanconi anemia and hereditary breast and ovarian cancer genes encode proteins that operate within a common pathway, called the FA DNA repair pathway. These proteins

function together to maintain genome integrity by repairing DNA damage (see Chapter 1) [91]. Variable cancer risks already have been linked to altered protein function of FA genes; however, it is critical for providers and families to understand how information about cancer risk in carriers is evolving and the association of rare variants with cancer susceptibility [92]. In fact, many FA genes are included in large clinical panel tests despite insufficient data to adequately define their cancer risks [93-95]. Currently, for genes listed in Table 3, there are management recommendations published by the National Comprehensive Cancer Network (NCCN) for individuals with positive test results, also known as pathogenic or likely pathogenic test results. The NCCN reviews existing literature annually and provides updated clinical practice guidelines for the detection, prevention, and risk reduction of adult onset cancers as new information is learned [96].

**Table 3. Established cancer risks in carriers warranting modified management.**

| FANC Gene | Established Cancer Risk in Carriers | Additional References |
|---|---|---|
| *FANCD1 (*BRCA2)* | Breast, ovary, prostate, pancreas, melanoma | [97-98] |
| *FANCJ (BRIP1; BACH1)* | Ovary | [96, 100, 101] |
| *FANCN (PALB2)* | Breast, pancreas | [96, 98, 101-105] |
| *FANCO (RAD51C)* | Ovary | [101, 106, 107] |
| *FANCS (*BRCA1)* | Breast, ovary, prostate, pancreas | [97, 98, 101, 108] |

*Hypomorphic variants noted.*

Outside of the five genes listed in Table 3, question about increased cancer risk in carriers of the remaining FA and FA-like genes is a topic of great interest and ongoing research. Over the years, data published from FA families have tried to capture the observed number of cancers in carriers versus an estimated cancer incidence in the general population. These studies, limited in scale and to self-reported data collection, did not indicate a significant difference that would warrant modified management [110-112].

All FA carriers should be encouraged to communicate their genetic status with primary providers and to reach out annually for potential health and cancer risk updates. It is equally important to ensure in advance that families are aware of the potential to uncover health risks affecting carriers, and the potential for discrimination of unaffected individuals based on positive findings. Referral to a genetic counselor who specializes in cancer predisposition is recommended for accurate risk assessment and a comprehensive discussion about testing, management options and family planning.

# Reproductive Planning

There are multiple reproductive options for parents of a child with FA and individuals with FA. Preconception genetic counseling is available for families to discuss these options in greater detail.

### Prenatal Diagnostic Testing

Prenatal diagnostic testing of fetal cells can be done at various times in the pregnancy to determine whether a fetus has FA. Prenatal testing also can be used to determine whether the fetus has the same human leukocyte antigens (HLA) as the sibling with FA. This process, known as HLA typing, reveals whether the child will be a suitable donor for the sibling with FA. Prenatal testing options include chorionic villus sampling and amniocentesis, which are typically available from the 10th and 15th weeks of pregnancy, respectively. The goal of both procedures is to obtain fetal cells for genetic testing or chromosomal breakage analysis. Targeted variant analysis should be performed on fetal DNA if the genetic variants are known, whereas chromosome breakage testing should be performed when the familial variants are not known. Both procedures are associated with a risk of miscarriage and should be discussed in detail with the center performing the procedure.

### Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis (PGD) is a genetic screen used to test embryos produced through in vitro fertilization (IVF). While PGD can attempt to select embryos without FA and those that are an HLA-match for an affected sibling, this technology is not a guarantee and the individual PGD center should inform families of their experience and accuracy. Parents considering PGD should be advised of the chances of selecting a healthy, HLA-matched embryo. Theoretically, for couples who have a child with autosomal recessive form of FA, there is a 75% chance that an embryo will not have FA, and a 25% chance that an embryo will be an HLA match, thus the odds that an embryo will be both unaffected with FA and an HLA match is 18.75% (3/16). Realistically, many couples need multiple rounds of IVF-PGD to achieve a clinical pregnancy resulting in a birth. Further, it is recommended that prenatal testing be performed for all pregnancies resulting from embryos produced through IVF-PGD to confirm the expected genetic status based on PGD.

### Other Reproductive Options

Other reproductive options include the use of donor gametes (egg or sperm), adoption, and unassisted pregnancy. Surrogacy also is an option, especially for women with FA who are concerned about the health implications of pregnancy.

# ◥ Summary

Close communication between physicians, genetic counselors, cytogenetics and molecular genetics laboratories, and hematopathologists is critical for the diagnosis of FA and the optimal care for patients with the disease. Early diagnosis of FA and the characterization of patient-specific FA variants is of utmost importance as this information may influence a patient's clinical management. It is critical that a clinically certified laboratory perform the diagnostic tests to ensure adherence to rigorous standards for quality control and quality assurance. All cytogenetic findings should be interpreted within the context of the patient's complete hematological profile and other clinical features to obtain a comprehensive assessment of the patient's status. It is strongly recommended that a genetic counselor or other genetics professional help guide the testing. Prior to the initiation of testing, the genetic counselor should confer with the laboratory director about the limitations of the testing methodology and analysis being used. Specifically, the genetic counselor and laboratory director should discuss the types of variants that can and cannot be detected, and the number of FA genes and other relevant genes that will be included in the testing. This information should be summarized by the genetic counselor and communicated to the patient and the patient's family.

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