The Molecular Biology of Fanconi Anemia

Hannah Tamary MD1,2, Raanan Bar-Yam BSc2, Michal Zemach MD2, Orly Dgany PhD2, Lea Salomon MSc2 and Isaac Yaniv MD1

1Institute of Hematology-Oncology, Schneider Children’s Medical Center of Israel, Petah Tiqva, Israel
2Pediatric Hematology-Oncology Laboratory, Felsenstein Medical Research Center, Rabin Medical Center (Beilinson Campus), Petah Tiqva, Israel

Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

Key words: Fanconi anemia, genes, mutations, DNA repair proteins, aplastic anemia.

Abstract

Fanconi anemia is a rare autosomal recessive disorder characterized clinically by congenital abnormalities, progressive bone marrow failure, and a predisposition to malignancy. FA cells are sensitive to DNA cross-linking agents. Complementation analysis of FA cells using somatic cell fusion has facilitated the identification of eight complementation groups, suggesting that FA is a genetically heterogeneous disorder. Six genes (FANCA, FANCC, FANCD2, FANCE, FANCG, FANCF) have been cloned so far. The majority of affected patients belong to FA group A. Of the 32 unrelated patients with FA that we studied, 6 carried the FANCC mutations and 15 the FANCA mutations. Among the Jewish patients, ethnic-related mutations were common. Recent cumulative evidence suggests that the FA proteins are repair proteins. FANCC, FANCA and FANCG bind and interact in a protein complex found in the cytoplasm and nucleus of normal cells. FANCD2 exists in two isoforms; the long active form, FANCD2-L, is absent from FA cells of all complementation groups. FANCD2 co-localizes with BRCA1 in nuclear foci, probably as part of a large genomic surveillance complex. Studies using FANCA and FANCC knockout mice suggest that bone marrow precursors express interferon-γ hypersensitivity and show progressive apoptosis. The definition of the molecular basis of FA in many affected families now enables prenatal diagnosis.

IMAI 2002:4:819–823

Clinical course of FA

The clinical course of FA has been extensively reviewed [1]. The common physical findings include abnormal skin pigmentation, growth retardation, radial ray or other skeletal malformations, microphthalmia, and renal or urinary tract malformations. The large range of organ systems affected implicates the FA genes in a general developmental process mandatory for normal human embryogenesis. About 40% of patients have no major physical anomalies.

The hematologic complications of FA include progressive bone marrow failure that usually develops in the first decade of life. Often thrombocytopenia or leukopenia appears before full pancytopenia. Erythropoiesis is usually fetal-like, with macrocytosis as well as high antigen and increased hemoglobin F levels.

Acute myeloblastic leukemia develops in at least 10–15% of patients with FA, and myelodysplastic syndrome in about 5% (average age 15 years). In addition, patients receiving androgen therapy for bone marrow failure are prone to liver tumors (average age 16 years). Later (average age 23 years), cancer of several organs, including the skin, gastrointestinal tract, and gonadal system, may develop. The skin and gastrointestinal tumors are usually squamous cell carcinomas. Before the advent of bone marrow transplantation, many FA patients died of bone marrow failure even before they could develop cancer, so the actuarial risk of cancer may be even higher.

The clinical picture of FA is highly variable. Some patients present with a relatively mild phenotype, normal skeletal development, and subclinical hematopoietic abnormalities, surviving to the third or fourth decade. Others have a more severe phenotype, with skeletal abnormalities and early onset of bone marrow failure and cancer.

Diagnosis of FA

Cells from patients with FA exhibit increased spontaneous chromosomal aberrations and hypersensitivity to DNA cross-linking agents such as MMC and DEB. Similar spontaneous, chromosomal

FA = Fanconi anemia

MMC = mitomycin C

DEB = diepoxybutane
changes are observed in other inherited chromosome instability syndromes, such as Bloom's syndrome and ataxia telangiectasia, but they are not DEB-induced. The DEB test is highly sensitive and specific for FA, and serves as a diagnostic criterion. New diagnostic approaches have resulted from the cloning of FA genes. FA cells also have several other phenotypic abnormalities, such as defects in cell cycle regulation and apoptosis.

Cloning of the FA genes

Clonal analysis of FA cells using somatic cell fusion has facilitated the identification of eight complementation groups, suggesting that FA is a genetically heterogeneous disorder [9,10]. This genetic heterogeneity has been largely verified by molecular cloning of the FA genes, each complementation group representing a distinct gene. Six genes have already been cloned: FANCA [3], FANCC [4], FANCD2 [5], FANCE [6], FANGF [7], and FANCG [8]. Of the first 100 FA patients classified by the European Fanconi Anemia Research Program, the majority (n = 71) belonged to the FA-A group [2] (Table 1). Worldwide prevalence varies according to ethnic background: for example, most patients in the Afrikaans-speaking population of South Africa belong to group A, whereas in the Ashkenazi-Jewish population (Eastern European origin), group C is most frequent [2].

Fanconi anemia C gene

- **FANCA gene and protein**

The FANCA gene was the first FA gene to be cloned by functional complementation of an Epstein-Barr virus-immortalized FA-C cell line [4]. As predicted by the complementation test, the FANCC cDNA corrects the MMC and DEB sensitivity of FA-C cell lines but does not correct the MMC sensitivity of FA cells derived from other FA groups. FANCC gene has been characterized [11] (Table 1). The FANCC protein shows no homology to any protein of known function. It is primarily a soluble cytoplasmic protein, but a nuclear complex of FANCA and FANCC and other FA proteins has also been detected [2].

- **FANCC mutations**

Mutation analysis of the FANCC gene in western countries has revealed a relatively small number of characteristic mutations [2]. In most patients, the mutations are clustered in three regions of the gene: exon 1, intron 4 and exon 14. The IVS4+4A→T mutation predominates in patients of Ashkenazi Jewish ancestry, accounting for more than 80% of cases of FA in this population [12]. The carrier frequency of this mutant allele in a selected Jewish population was determined to be 1.1% [13]. The 322delG mutation is found in patients of North European ancestry, particularly Holland. The relative prevalence of mutations in exon 14 and the high across-species conservation of this exon indicate that the carboxy terminal region of FANCC most likely contains a critical functional domain. Augmented mRNA expression has been observed in the skeletal system, suggesting a more specialized function of FANCC in bone development [14].

- **Genotype-phenotype analysis for FANCC**

In general, patients with mutations in intron 4 (IVS4-4A→T) or exon 14 (R548X, L534P) have a significantly earlier onset of hematologic abnormalities and poorer survival than patients with exon 1 mutations (322delG or Q13X,20). However, Japanese patients with the same IVS4-4A→T mutation have a milder phenotype [15]. The reason for this variability has not been elucidated. The molecular basis of the milder phenotype in patients with exon 1 mutations is still unclear. The observation that cell lines with the 322delG mutation express a truncated isoform of FANCC, resulting in partial correction of MMC sensitivity, whereas cell lines with intron 4 mutations lack this isoform [16].

FANCA gene and protein

The FANCA gene was cloned by two techniques independently: functional complementation of an EBV-immortalized FA-A cell line [17] and positional strategy [2] (Table 1). The FANCA protein contains a nuclear localization signal at its N-terminus and a partial leucine zipper motif between amino acids 1069 and 1090. The importance of the leucine zipper region remains unclear. The ACC protein contains a nuclear localization signal at its N-terminus and a partial leucine zipper motif between amino acids 1069 and 1090. The importance of the leucine zipper region remains unclear. The ACC protein contains a nuclear localization signal at its N-terminus and a partial leucine zipper motif between amino acids 1069 and 1090. The importance of the leucine zipper region remains unclear.

- **FANCA mutations**

More than 100 private and semi-private mutations have been identified so far in the FANCA gene [18–23]. A high proportion (roughly one-third) are deletions, owing to the frequent occurrence of duA repeats at the deletion breakpoints [24,25]. Somatic mosaicism due to reversion of the pathogenic allele to wild-type has been described in FANCA and FANCC [26].

- **Genotype-phenotype analysis**

Analysis of the FANCA mutations by the European Fanconi Anemia Research Program suggested that complete loss of the FANCA protein...
is associated with a severe phenotype, whereas alteration of the protein is associated with a milder phenotype, with later age at onset of aplastic anemia [27].

**Fanconi anemia G gene**
The FANCG gene was recently cloned and found to be identical to the previously cloned human XRCC9 gene [8] (Table 1). The FANCG protein is an orphan protein that contains an internal leucine zipper as its only recognizable motif. More than 20 mutations have been identified so far. All types of mutations have been found, with the exception of large deletions. One missense mutation in a possible leucine zipper motif may affect FANCG binding of FANCA [28]. A relatively early occurrence of acute lymphoblastic leukemia has been seen in patients in group G compared with patients in groups A and C [2].

**Fanconi anemia group E gene**
The FANCE gene was recently cloned [6] (Table 1).

**Fanconi anemia group F gene**
The gene mutated in Fanconi anemia group F was identified by complementation cloning. FANCF has no introns and encodes a 374 amino acid polypeptide with homology to prokaryotic RNA binding protein ROM [7] (Table 1).

**Fanconi anemia D2 gene**
Complementation group D is heterogeneous, consisting of two distinct genes, FANCD1 and FANCD2. Recently, FANCD2 was positionally cloned [5] (Table 1), and found to have two isoforms. It has no known functional domains but, unlike other known FA genes, is highly conserved in *Caenorhabditis elegans* and *Drosophila*.

**The mutational spectrum in Israeli FA patients**
We studied 32 unrelated Israeli patients with FA (22 Jewish and 10 Arab) (Table 2), who were either treated at the Hematology Clinic of Schneider Children’s Medical Center or referred to us from different pediatric hematological units throughout the country. Six bore FANCC mutations and 15 had FANCA mutations. Among the Jewish patients, ethnic-related mutations were common: IVS4-14 in the Ashkenazi Jews, 2172-2173insG and 4375delT in the Moroccan Jews [30], 890-893delT was found in the Jewish-Tunisian patients and

![Figure 1. FA pathway of genomic stability. The products of 5 FA genes – FANCA, FANCC, FANCE, FANCF, FANCG – assemble into a nuclear complex. Complex formation is essential to the attachment of a single ubiquitin moiety to form D2L (long form). Active D2L binds to BRCA1 at the nuclear foci.](image)

2474C>G in the Jewish-Indian patients. In seven of the nine Arab patients the FA mutations were not identified, however neither the FANCC nor the FANCA genes are apparently involved.

**Cloning of the FA genes and pathogenesis of the disease**
The availability of FANCC knockout mice has provided some clues to the pathogenesis of bone marrow failure. The study of FA genes recently led to the development of a model of molecular interactions.

**Animal models of FA**
Mice models with targeted disruptions of the FANCC and FANCA genes have been developed. However, the resulting mutants exhibit only part of the FA human phenotype. Cells derived from these animals show the classic hypersensitivity to bifunctional DNA cross-linking agents. Mice also display hypogonadism and reduced fertility [29]. Hematopoiesis, which is typically compromised in FA patients, appears to be unaffected in mice, but the repopulation capacity of the FANCC mutant stem cells upon serial transplantation is reduced. In addition, although no peripheral blood abnormalities were detected, an age-dependent decrease in burst-forming unit-erythroid and colony-forming unit granulocyte macrophage progenitors was found. In addition, the hematopoietic progenitor cells revealed a distinct hypersensitivity to interferon gamma [30]. Increased cell susceptibility to IFN-γ leads to fas-induced apoptosis, and the cells derived from the FANCC knockout mice exhibited a high level of Fas expression at a low INF-γ concentration [31]. It has recently been shown that functional correction of FA-C cells with FANCC suppresses the expression of INF-γ-inducible genes [32]. This suggests that INF-γ hypersensitive-

---

**Table 2. FA Mutations in Israeli patients**

<table>
<thead>
<tr>
<th>Complementation Group</th>
<th>Mutation</th>
<th>No. of alleles involved</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>FANCC</td>
<td>IVS4+4A&gt;T</td>
<td>12</td>
<td>Ashkenazi-Jewish</td>
</tr>
<tr>
<td>FANCA</td>
<td>2574C&gt;G</td>
<td>4</td>
<td>Indian-Jewish</td>
</tr>
<tr>
<td></td>
<td>2172-2173insG</td>
<td>14</td>
<td>Moroccan-Jewish</td>
</tr>
<tr>
<td></td>
<td>890-893delT</td>
<td>3</td>
<td>Tunisian-Jewish</td>
</tr>
<tr>
<td></td>
<td>4257delT</td>
<td>3</td>
<td>Moroccan-Jewish</td>
</tr>
<tr>
<td></td>
<td>IVS42-2GC</td>
<td>2</td>
<td>Arab</td>
</tr>
<tr>
<td></td>
<td>Val229Ie</td>
<td>2</td>
<td>Arab</td>
</tr>
<tr>
<td></td>
<td>Del exon-51</td>
<td>2</td>
<td>Arab</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IFN-γ = interferon gamma
Molecular Biology

ity may be the major pathogenic mechanism underlying the development of progressive aplastic anemia in patients with FA. The relationship between this phenotype and the cellular response to DNA cross-linking is still obscure.

Molecular interactions of FA proteins
Cumulative evidence over recent years shows that FA proteins participate in a novel cellular pathway. FANCC, FANCA and FANCG bind and interact in a protein complex found in the cytoplasm and in the nucleus of normal cells [33,34].

The prospects for understanding the FA pathway have greatly improved with the identification of FANCd2 [4]. Higura et al. [35] suggested that the FANCd2 protein exists in two isoforms: the primary translation product (short FANCd2-S) and a higher molecular weight form (long FANCd2-L). The long isoform has a single ubiquitin moiety attached to a highly conserved residue at position 561. FANCd2-L is absent from FA cells of all complementation groups (except D1), but it reappears after correction of the FA genetic defect.

In wild-type cells, FANCd2 and BRCA1, a DNA damage-response agent and the major breast cancer susceptibility protein, have been found together in the same nuclear foci. Although the manner in which FA proteins intermingle with the BRCA1-associated proteins is not clear, the FA protein is apparently a repair protein. It seems that BRCA1 facilitates FA downstream reactions, possibly in combination with other DNA repair proteins that are associated with BRCA1 in a large genome surveillance complex [35].

FA like xeroderma pigmentosum and hereditary non-polyposis colorectal cancer is, therefore, a caretaker-gene disease [2] featuring genomic instability in combination with a strong predisposition to cancer (Table 3).

Cloning of FA genes: implications for diagnosis
Although the DEB test is highly sensitive and specific for the diagnosis of FA, it fails to identify heterozygote carriers of mutant FA genes. Detection of the majority of the FA genes has allowed for prenatal diagnosis. The identification of common mutant alleles in each ethnic group contributes to rapid diagnosis.

Genotype/phenotype correlations are also important for FA management. If a severe phenotype is diagnosed, patients may be treated more aggressively with bone marrow transplantation or gene therapy.

Cloning of FA genes: implication for therapy
Allogeneic BMT from a human leukocyte antigen-matched sibling donor offers the only possibility of cure for the hematologic manifestations of FA (aplasia or bone marrow dysplasia). Low doses of cyclophosphamide and radiation must be used to avoid severe toxicity due to the chemo- and radiosensitivity in patients with FA [36]. Data from multiple institutions (over 150 patients) suggest an overall 2 year survival rate of 66% [37,38]. Despite success in treating FA aplasia by stem-cell replacement, some survivors show late development of secondary malignancies, particularly of the head and neck [37].

Most patients do not have an HLA-identical donor and are dependent upon the identification of suitably matched non-sibling relatives or unrelated donors. A study of 69 FA patients who underwent BMT from alternative donors showed a 3 year survival rate of 33%, graft failure being the most serious complication. Preliminary results based on the addition of fludarabine to the preparative regimens are encouraging [39].

An alternative in the absence of a histocompatible donor is the retroviral transfer of a FA gene into the hematopoietic stem cell. In current gene therapy trials, retroviral vectors expressing wild-type FANCC or FANCA are used to transduce the peripheral blood leukocytes of FA patients [2]. Once in the patient's bloodstream, the gene-correction stem cells presumably initiate and support profi- cient hemopoiesis. A major obstacle to successful gene therapy is the poor efficiency with which the rare hematopoietic stem cells are transduced. To increase the number of these cells, many centers have initiated programs for the collection and cryopreservation of hematopoietic stem cells from FA patients before the onset of aplasia. Subsequent difficulties may include unstable expression of the transgene due to gene silencing and immunologic attack of the transduced cell, which expresses a protein that the body might identify as foreign.

Although useful, gene transfer studies, like BMT, cannot ameliorate the developmental abnormalities or cancer risk in non-hematopoietic tissues in patients with FA.

Conclusions and future trends
Cloning of the FA genes has opened a window to our understanding of the molecular basis of Fanconi anemia. It is now known that the FA complexes function upstream of FANCd2, which, in modified form, acts together with the BRCA1 gene in the DNA-damage response pathway. The FA core complex might act as a sensor of DNA damage, leading to activation of FANCd2, which could be a crucial effector molecule in the circuit. Nevertheless, many questions remain unanswered: How does the FA core complex respond to DNA damage? How does modification of the FANCd2 target this molecule to BRCA1-containing foci? What precisely does FANCd2 do upon translocation to these sites?

Table 3. Caretaker-gene diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of suspected genes</th>
<th>Molecular process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td>1</td>
<td>DNA damage response</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td>1</td>
<td>DNA unwinding</td>
</tr>
<tr>
<td>Werner syndrome</td>
<td>1</td>
<td>DNA unwinding</td>
</tr>
<tr>
<td>Xeroderma pigmentosum</td>
<td>7</td>
<td>Nucleotide excision/transcription-coupled repair</td>
</tr>
<tr>
<td>Hereditary non-polyposis colorectal cancer</td>
<td>5</td>
<td>Mismatch repair</td>
</tr>
<tr>
<td>Hereditary breast/ovarian cancer</td>
<td>3</td>
<td>DNA damage response repair, recombination, transcription</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>7</td>
<td>?</td>
</tr>
</tbody>
</table>

BMT = bone marrow transplant
HLA = human leukocyte antigen
Acknowledgement. We are indebted to Prof. A. Abramov, Dr. D. Attias, Prof. I. Barak, Dr. A. Koren and Prof. G. Rechavi for granting us access to their patients. We also thank Gloria Ginza and Hanni Penn for their editorial and secretarial assistance.

References

Correspondence: Dr. H. Tamary, Institute of Hematology-Oncology, Schneider Children's Medical Center, 14 Kaplan St., Petah Tiqa 49202, Israel.
Phone: (972-3) 925-3669
Fax: (972-3) 925-3042
email: hhtamary@post.tau.ac.il