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# Molecular basis of Fanconi anemia

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**Abstract**

***Background and Objective.*** **Fanconi anemia (FA) is an autosomal recessive disease characterized by pancytopenia, congenital malformation and high predisposition to developing malignancies. The phenotypical heterogeneity is associated with genetic heterogeneity: at least 8 complementation groups (FA-A to FA-H) have been identified, each group presumably corresponding to a separate disease gene (*FAA* to *FAH*). The *FAA* and *FAC* genes, which account for 75-80% of the patients, have been cloned. Their protein products have no significant homology to any known protein, or to each other, and may therefore represent elements of a new pathway. This review describes some of the recent contributions to the understanding of the molecular basis of FA.**

***Evidence and Information sources.*** **The authors of the present review have been working in the field of FA for several years. In the present review they have critically examined articles published in journals covered by the Science Citation Index and Medline.**

***State of Art and Perspective(s).*** **A variety of biochemical and cellular approaches have been used to determine the molecular defect of FA. Evidence for a possible role of the defective proteins in cell cycle regulation, apoptosis, or DNA stability have been reported. Recently, it has been demonstrated that FAA and FAC proteins bind each other and form a complex found in similar abundance in both cytoplasm and nucleus, suggesting a possible function in nucleus activities. Knowledge of the mutation spectrum occurring in the FA genes may contribute significantly to pathogenesis studies in FA and help to design mutation screening strategies. Further functional studies and the cloning of other FA genes will provide insights into the biological basis of FA and information for developing specific therapies for the disease.**



Key words: Fanconi anemia, complementation groups, FAA gene, *FAC* gene, mosaicism

In the last few years there have major achievements concerning Fanconi anemia (FA). This review describes some of the recent contributions to the understanding of the molecular basis of FA.

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## Clinical aspects and cellular phenotype

### *Clinical heterogeneity*

Fanconi anemia is an autosomal recessive disease characterized by a variety of clinical symptoms and cellular features.[1] FA is a rare disease with an estimated prevalence of 1-3 per million.[2] However, prevalence may vary with geographic region and ethnic structure of the population. In white Afrikaners a birth incidence of at least 1 in 22,000 has been attributed to founder effect.[3] A high FA incidence has also been reported in Italy with areas of major density in the South, especially in Campania.[4]

Clinical manifestations are extremely pleomorphic and include bone marrow failure, congenital malformations, and predisposition to cancers.[5] An important clinical hallmark, with a mean age of onset around 5 years, is a progressive pancytopenia initially characterized by thrombocytopenia and subsequently by granulocytopenia and anemia.[6,7] Multiple congenital malformations have previously been reviewed.[8-10] Clinical data from 370 patients of the *International Fanconi Anemia Registry* (IFAR) showed that more than half patients exhibit growth retardation, short stature, and abnormal skin pigmentation (*cafe-au-lait* spots). Skeletal malformations, particularly dysplasia of the radius and the thumb, represent a typical finding of the syndrome and are reported in about 50% of the patients. Other frequent abnormalities include ocular, kidney, and cardiac defects. Microcephaly is present in 36% of the patients while mental retardation accounts for 26% of the subjects. Hypogenitalism affects 20% of the male who show gonadal hypoplasia and defective spermatogenesis. Clinical features can be variably associated: 39% of the patients exhibit the most severe trait with both hematological and congenital defects, whereas only anemia or malformations affect 30% and 24%, respectively. A minor group of patients are clinically asymptomatic.[8]

The complex clinical trait of FA creates difficulties in differential diagnosis with other syndromes including, VACTERL association with hydrocephalus, Baller-Gerold, thrombocytopenia with absence of radii, Diamond-Blackfan syndrome, and dyskeratosis congenita.[11-15] Misdiagnosis raises the question of whether these syndromes are distinct disorders or phenotypic variations of the same disease.

Myelodysplastic syndromes (MDS) and acute

myeloblastic leukemia (AML) are the most severe complications in FA.[7,16] Actuarial risk of MDS and AML was 52% by 40 years of age. However, a variety of solid tumors, such as basal cell carcinomas, gastrointestinal and gynecological cancers, are also observed.[17,18] The average age for developing cancers is 15 years for leukemia, 16 years for liver tumor, and 23 years for other tumors.[19]

### *Cellular phenotype*

Similar to clinical manifestations, cellular phenotype shows a wide spectrum of features. FA cells exhibit elevated levels of spontaneous chromosomal aberrations with damage mainly of chromatids, including gaps, breaks, and interchanges.[20] Because of chromosomal instability, FA has been classified as a DNA repair disease and is often compared with other syndromes, such as ataxia telangiectasia, xeroderma pigmentosum, trichothiodystrophy, Cockayne syndrome, and Bloom syndrome.[21-27] The aspect that differentiates FA among these disorders is the hypersensitivity of cells to cross-linking agents, such as diepoxybutane (DEB) and mitomycin (MMC).[28,29] In the presence of these drugs, FA cells show an increased sensitivity to chromosomal damage. Clonal cytogenetic abnormalities, more frequently involving chromosomes 1, 7, and 11 are often associated with myelodysplastic syndrome or acute myeloblastic leukemia.[7,30-32] FA cells show also an abnormal response to oxygen with reduced growth at standard oxygen levels, suggesting a defect in response to oxygen toxicity.[33-36] Prolongation or complete arrest of G2 phase alters cell cycle progression in FA cells.[37-38] Since affected cells exhibit a normal G1/S transition, the FA proteins are likely to regulate the transition from G2 to M phase.

### *Diagnosis*

Although the diagnosis of FA is complicated by a considerable spectrum of clinical manifestations, the hypersensitivity of FA cells to cross-linking agents can be used as a cellular marker. A highly sensitive and specific test, named DEB test, has to be performed whenever FA diagnosis is suspected.[39] The 7% of clinically asymptomatic individuals are diagnosed only on the basis of a positive DEB test, which is therefore pathognomonic for the disease. However, the test is not sufficiently sensitive for FA carrier detection. Alternatively, diagnosis can be performed by delayed passage through the G2 phase of the cell cycle.[40]

### *Therapy*

Without curative support, spontaneous survival is poor, with death occurring during the second decade of life from aplastic anemia, leukemia or other cancers. Treatment of FA is similar to that of acquired aplastic anemia. FA patients respond transiently to therapy with androgens and cytokines, granulocyte and granulocyte-macrophage colony-stimulating factors (GM-CSG and G-CSF).[41,42] Androgen therapy was found to be associated with an increased risk of liver tumors.[43] In patients with bone marrow failure, blood transfusions may compensate for the lack of hematopoietic stem cells.

Bone marrow or cord blood transplantation represent the only treatments which restore definitively normal hematopoiesis.[44] Since only solid tumors and no lympho myeloproliferative malignancies have been observed in the post-transplant course, transplantation provides patients with a normal hematopoietic system, but it does not correct congenital defects in other tissues. For patients lacking a histocompatible stem cell donor, therapy is limited to supportive care and therefore alternative strategies, such as gene therapy, are being developed.

## Genetics

### *Complementation groups*

The most peculiar characteristic of the cellular phenotype of FA cells is a high sensitivity to cross-linking agents. When lymphoblastoid cell lines derived from unrelated patients are fused, the hybrids can be studied for cross linking sensitivity. They may be either resistant and therefore have a normal phenotype (complementation) or sensitive and be phenotypically FA cells (no complementation). Patients whose cell lines do not complement each other in a fusion hybrid, belong to the same complementation group. To determine the extent of genetic heterogeneity in FA, somatic cell hybrids have been analyzed. The first two complementation groups were designated FA-A and non FA-A.[45] As a continuation of this analysis, a further genetic heterogeneity was described in non FA-A with evidence for at least another three new subtypes, FA-B, FA-C, and FA-D.[46] This study was based on the analysis of only seven cell lines so that, when a European collaborative study (EUFAR, *European Fanconi anemia research*) was established with the aim to characterize a hundred FA patients, evidence for a fifth (FA-E) complementation group was reported.[47] To assess a further variability within subtype E, in which cell lines classified as non-ABCD were included, some fusion hybrids among FA-E cells indicated three new groups (FA-F, FA-G, and FA-H), providing evidence for a minimum of eight distinct complementation groups (Figure 1).[48]

The relative prevalence of FA-A and FA-C has been estimated at an average of about 65-69% and 12-18%, respectively but may widely vary according to ethnic background.[49-50] In Italy, 11 out of 12 patients analyzed by cell fusion studies were assigned to group FA-A, suggesting an unusual high relative prevalence of this subtype.[51] In the Netherlands, the majority (67%) of patients were found to belong to subtype FA-C.[52] Only a few cell lines have been assigned to FA-B and FA-D whereas only a single patient is known to belong to each of the other groups FA-E to FA-H.

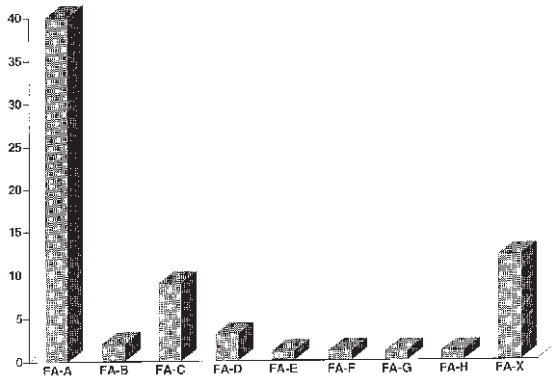


**Fig. 1. Number of FA patients assigned to each of the complementation groups FA-A to FA-H.46,50-52 FA-X indicated FA-non ABCD patients of unknown subtype, whose cell lines are able to correct the FA phenotype when fused with subtypes A, B, C, and D.**

### *FA genes*

Each of the eight complementation groups presumably corresponds to a distinct disease gene (*FAA* to *FAH*).[49] This assumption is true at least for *FAA* and *FAC* which have been cloned and for *FAD* which maps on a different chromosome, 3p22-26.[53]

*FAC* was the first gene to be identified by functional complementation cloning, which consisted of transfecting recipient FA-C lymphoblastoid cell lines with a cDNA library and successive selection in medium supplemented with DEB and MMC.[54] Since only cells that received the wild-type *FAC* gene were able to survive in these restricted conditions, the *FAC* gene was rescued from selected clones. The *FAC* gene is 4,566 bp and encodes a 63-kD protein of 558 amino acids. Differentially processed forms of 2.3, 3.2, and 4.6 kb, which differ in both the 5' and 3' untranslated regions (5'UTR and 3'UTR), are transcribed (Figure 2). The 5'UTR is characterized by the presence of two different exons, alternatively spliced, and the 3'UTR differs in size because of the recognition of alternative polyadenylation signals.[54] The functional significance of these mRNA splice variants remains unknown. The *FAC* gene, greater than 120 kb (Savoia, *unpublished data*) with 14 exons, maps to chromosome 9q22.3, between two genes involved in diseases with high predisposition to cancers, xeroderma pigmentosum and nevoid basal cell carcinoma syndrome (Gorlin syndrome).[46,55,56] The *FAC* homologues from mouse, rat, and bovine have also been isolated.[57-58] The murine Fac gene encodes a protein with a 78% similarity to the human polypeptide and, when expressed in human FA-C cells, it is able to correct the hypersensitivity to cross-linking drugs.[57]

The *FAA* gene was identified by both functional and positional cloning.[59-60] The latter strategy consists of two steps: first a gene is mapped to a subchromosomal region and then coding sequences from that region are isolated and analyzed for the presence of genetic alterations. The *FAA* gene was localized at chromosome 16q24.3 at an interval of 1 Mb by linkage analysis in a panel of families belonging to complementation group FA-A and identified by mutation analysis in candidate genes.[61-62] The gene has an open reading frame of 4,365 bp encoding a 16-kD protein of 1,455 amino acids. The predicted protein has possible nuclear localization signals and a partial leucine zipper consensus sequence, homologies consistent with a function of *FAA* in the nucleus. Northern blot analysis displays an extensive hetero-



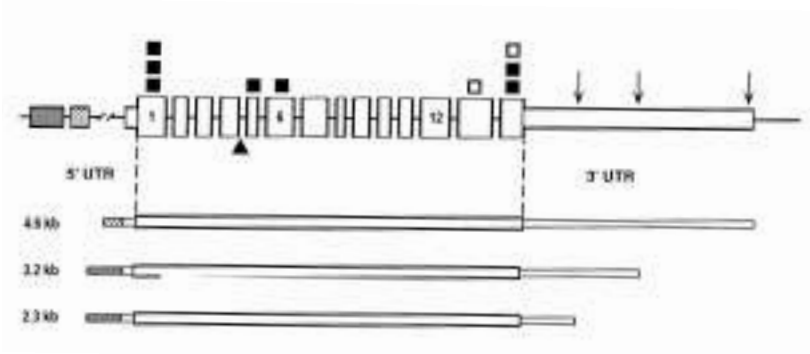


**Figure 2. Genomic organization and mutations of the *FAC* gene. The gene is at least 120 kb in size and maps to chromosome 9q22.3. The coding region is split up into 14 exons. The differentially processed forms of 2.3, 3.2, and 4.6 kb share the same open reading frame but differ in both the 5' and 3' UTR. The 5'UTR is characterized by the presence of two alternatively spliced exons (hatched and stippled areas), and the 3' UTR differs in size because of the recognition of alternative polyadenylation signals (arrows). Five nonsense mutations, a base deletion (322delG in exons 1) and a nucleotide insertion (1806insA in exon 14) are likely to determine truncated forms of the FAC proteins (solid squares). Two missense mutations (L496R and L554P in exons 13 and 14, respectively) and a donor splice mutation have also been identified. The latter, IVS4+4A→T was found in the majority of FA Ashkenazi-Jewish.**

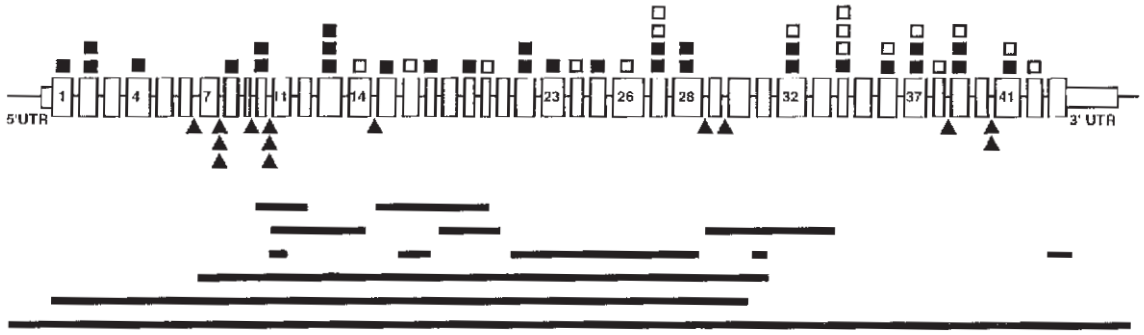


**Figure 3. Genomic organization and mutations of the FAA gene. The gene contains 43 exons spanning approximately 80 kb on chromosome 16q24.3. The seventy-four mutations so far detected are spread over the gene. Nonsense substitutions and frameshift mutations due to small insertions or deletions are indicated by solid squares. Missense mutations are represented by open squares. Several alterations in the donor and acceptor splice sites have been identified (triangles), some of which are associated with alternative splicing or exon skipping. Evidence for deletions of the *FAA* gene have been reported (bars). Only two deletions have so far been characterized at the genomic level: one of at least 120 kb encompass the entire FAA gene, the other of 5.0 kb spans from intron 17 to intron 21. The latter is likely to be the result of an unequal crossover between Alu repeats. FA mutations are listed in the *Fanconi Anemia Mutation Database* (*http://www.rockefeller.edu/fanconi/mutate/default.html*)**

geneity of *FAA* with transcripts of several sizes.[59-60] In particular, besides a prominent mRNA of 4.7 kb, there are transcripts of 7.5, 3.0 and 2.0 kb in size. In addition, differentially processed forms due to alternative splicing have been characterized (d'Apolito, *unpublished data*). Similar to *FAC*, the functional significance of the messenger heterogeneity is unknown. The genomic structure of the gene revealed that the coding sequence is contained in 43 exons spanning approximately 80 kb of genomic DNA.[63]

### *Mutation analysis*

In the *FAC* gene ten pathogenic mutations have so far been identified (Figure 2).[54,64-71] Except for two aminoacid substitutions, the other sequence variations would predict premature termination of the FAC protein. In particular, the L554P mutation completely abolished the activity of the FAC protein as demonstrated by functional complementation analysis using site-directed *in vitro* mutagenesis.[72] However, a wider spectrum of *FAC* mutations has to be expected since only one mutation has been identified in several compound heterozygous patients.

The most common mutation is IVS4+4A→T, a splice site mutation leading to a frameshift deletion of exon 4, which has been found in the majority of FA Ashkenazi-Jewish patients.[66,73,74] This mutation was also found to be homozygous in dizygotic twins with the VACTERL association, suggesting that appropriate investigations should be undertaken for differential diagnosis.[75] Another common alteration is 322delG, a frameshift deletion found in Northern European individuals.[67-70] The genotype-phenotype correlation revealed that the former was associated with severe clinical manifestation, whereas the latter was unexpectedly associated with a milder phenotype.[67] In fact, cell lines carrying the 322delG mutation express a FAC isoform of 50 kD which is an in frame amino terminal truncated protein able to partially correct the FA phenotype.[76]

Seventy-four different mutations have thus far been described in the *FAA* gene (Figure 3).[59,60,77-79] They include missense, nonsense, splicing, and frameshift mutations, which are widely distributed over the gene, suggesting that mutational hot spots are not involved. Most of the mutations are expected, as in FAC, to cause a premature termination of the *FAA* protein. The mutation screening also detected a consistent number of variants that are probably merely polymorphisms.[77-79] It has been suggested that *FAA* is hypermutable, and that slipped-strand mispairing is important for the generation of germline and somatic mutations in a variety of cancer-related genes.[77] However, a substantial proportion of mutations was not detected, at least in the European population.[78,79] This might be partially explained by the possible existence of rearrangements in the *FAA* gene, which might not be detectable by common screening procedures. In fact, in an Italian patient a more detailed analysis led to the characterization of the first two genomic deletions, one encompassing the entire gene and another spanning from introns 17 to 21.[80] Since the latter is due to a recombination between different *Alu* elements, found numerous in *FAA*, unequal crossover may be responsible for deletions and insertions.

The *FAA* mutations are widely distributed over the *FAA* gene and besides 1263delF and 3788-3790del

they were found only in single patients. This situation hampers a detailed genotype-phenotype correlation study, which would otherwise be helpful in determining possible associations with a mild or severe form of the disease. A further difficulty is due to a wide clinical heterogeneity in FA not only among patients from different families of the same complementation group A but also among patients within one family.[51]

Mutations in the *FAA* and *FAC* genes account for an estimated 75-80% of all FA patients. Therefore, the classification of patients in subtypes by cell fusion, which is extremely laborious and time-consuming, may be replaced by screening for mutations in both genes. The search for mutations can be preceded by linkage analysis on chromosomes 16q24.3 and 9q22.3 to exclude unclassified families from subtypes FA-A and FA-C, respectively.[78] Strong allelic association was detected with microsatellites at 16q24.3 in the Afrikaner FA patients, suggesting that a founder mutation has contributed to the high incidence of FA in this population.[59-61]

### Mosaicism

Mosaicism, resulting from post-zygotic mutation, is a condition in which an individual has 2 or more cell lines of different genetic constitution. Approximately 25% of patients with Fanconi anemia have spontaneously occurring mosaicism due to two different populations of lymphocytes, one of which is hypersensitive to cross-linking agents while the other normally responds to these agents.[81] If in one FA allele any event led to a normal copy of the gene, this event would confer wild type phenotype on the cell. This condition has already been described in other pathologies, such as Duchenne muscular dystrophy, Bloom's syndrome, and adenosine deaminase deficiency.[82-84]

The molecular basis of the phenotypic reversion in FA has not yet been completely determined. However, evidence for two different mechanisms have been reported: mitotic recombination and somatic mutation. In one patient, a mitotic intragenic crossing over between the maternally and paternally inherited FAC chromosomes has determined the segregation of a wild-type allele.[81] In another patient, a *de novo* insertion downstream of the germline *FAA* mutation restores the open reading frame possibly leading to a protein altered only for few amino acids (Savoia, *unpublished results*). Although mosaicism is under investigation, it is sometimes associated with a relatively mild hematological course of the disease with therefore several consequences and implication for diagnosis, bone marrow transplantation and gene therapy.

### Molecular biology

FAA and FAC proteins have no significant homology to any known polypeptide, or to each other, and may therefore represent elements of a novel pathway. A variety of biochemical and cellular approaches have been used to determine the defective function in FA by studying different biological aspects thought to underline the clinical and cellular features of the disease. Since several approaches could exhibit epiphenomena of the basic defect, the research has been focusing directly on FA genes and their products. The study of gene expression and regulation, the characterization of proteins, the development of animal models are some of the aspects that may provide insights into the molecular defect of the disease.

The *FAA* and *FAC* genes are ubiquitously expressed, although at different levels.[54,59] In human adult tissues, the highest expression was detected in testis, thymus, and bone marrow which are actively dividing and differentiating tissues (d'Apolito, *unpublished data*). Similarly, transcription is elevated in human fetal tissues. However, while the *FAC* transcripts were found in similar abundance in all tissues, *FAA* mRNA level was higher in liver and kidney than in brain and lung, suggesting that the *FAA* and *FAC* expression is under the control of different mechanisms at least in fetal tissues (d'Apolito, *unpublished data*). Consistent with these data, the expression of the mouse homologous *FAC* gene during development was found coupled with cellular proliferation and differentiation and the congenital malformations of FA patients.[85]

The analysis of the promoter regions of both FAA and *FAC* identified highly GC-rich regulatory sequences, a feature of several housekeeping genes characterized by wide expression in human tissues.[63,86] Functional analysis in *FAC* showed a complex transcriptional regulation possibly under the control of two separate promoters.[86] Further studies, leading to the identification of the trans-acting factors, will provide advances in the knowledge of the FA genes. Recently, it has been described that the p53 binds the *FAC* promoter and, if overexpressed, it regulates the transcription positively.[87] However, although p53 modulate the expression, it is not a prerequisite for the *FAC* transcription, suggesting a possible role only under certain conditions.

FAC is a soluble cytoplasmic protein associated with a multimeric complex.[88-91] It has been found to bind a member of the chaperone family, GRP94, and to cyclin-dependent kinase, cdc2.[92-93] FAA has also been detected in the cytoplasmic compartment and recently, it has been demonstrated that FAA and FAC proteins interact with each other and that, whereas unbound FAA and FAC are localized particularly in the cytoplasm, FAA-FAC complex is detected in both cytoplasm and nucleus.[94-95]

Analysis of mutations in FA patients has played an important role since it may identify functional domains. The C-terminal exon is necessary for the biological function of the FAC protein, which is not able to restore the cross-linking hypersensitivity when lacking at least the five C-terminal amino acids or when the fifth last leucine is replaced by proline.[69,72]

This indicates that the C-terminus is important for FAC activity, for instance in the binding with other proteins. In fact, the L554P mutant failed to interact to both FAA and cdc2 proteins.[93,95]

The development of a mouse model represents a powerful tool to understand the function of genes and to test new therapies. Two murine models have been created by knockout strategies.[96-97] Both mutants showed similar phenotype: absence of developmental abnormalities and hematological defects, increased chromosomal aberrations in response to cross-linking agents, and G2 cell-cycle arrest. Homozygous male and female mice had compromised gametogenesis with markedly impaired fertility. The knockout mice replicates some human disease features, but they did not show pancytopenia during the first year of life. In addition, mice showed a hypersensitivity to interferon-γ (IFN-γ), a feature that may be associated with the bone marrow failure in FA.[97]

## Possible function of the FA proteins

The basic defect in FA is not known. However, on the basis of clinical and cellular features and recent studies, some of the possible pathways, such as cell-cycle regulation, apoptosis or genomic stability, defective in FA are being delineated.

Evidence for a defect in cell cycle is supported by the prolonged G2 phase and the reduced proliferation that is enhanced by treatment with chemical cross-linking agents observed in FA cells. Studies of *FAC* expression during cell cycle showed that transcription is increased during S phase, is maximal at the G2/M transition, and declines during M phase.[93] In addition, the FAC protein binds cdc2, a cyclin-dependent kinase that together with cyclins A and B directs the G2 progression.[98] The G2 arrest and reduced proliferation of FA cells can be partially correct by overexpression of SFHAR, a member of the cyclin family necessary for cell cycle progression.[99] Tissues with cells in active proliferation exhibit an increased *FAC* expression that, if repressed, correlates to cell growth inhibition at least in hematopoietic progenitors.[85,100] Similar to other genetic diseases characterized by genomic instability, such as ataxia telangectasia or Li-Fraumeni, FA may be the result of a defect in cell cycle regulation.[101,102]

Altered apoptosis has been indicated as another possible defect in FA. Congenital malformation and hematological abnormalities might be due to the tendency of FA cells for apoptosis especially after treatment with cross-linking agents that may cause DNA damage.[103] In fact, *FAC*-retroviral transduced cells show significantly lower levels of apoptosis, so that *FAC* appears to be an inhibitor of apoptotic death.[104] Among the possible mechanisms regulating apoptosis, the p53 independent-pathway seems not to be affected, even if controversial results have been reported.[105-107] The *fas* activation by IFN-γ signals, another pathway controlling apoptosis, was thought to be defective because of the hypersensitivity to IFN-γ of hematopoietic progenitors from knockout mouse.[97] In fact, overexpression of FAC protein partially reduce the number of these cells in apoptosis.[108]

Since FA cells are characterized by chromosomal instability and hypersensitivity to DNA cross-linking agents, a defective DNA repair has been suggested as a possible mechanism responsible for FA. An abnormally high production of intragenic deletions in the hypoxanthine phosphoribosyl-transferase (*HPRT*) gene has been reported to be an important feature of FA phenotype, suggesting that FA proteins play a role in the maintenance of genomic integrity.[109] Among the different causes responsible for genomic rearrangements, the action of a site-specific mechanism has been suggested to be involved in FA cells.[110] In another recent study, elevated levels of homologous recombination seem to contribute to genomic instability.[111] For these reasons, it might be that the FA defect lies in a pathway that involves recombinational events during DNA replication or repair processes.

Although the molecular defect of FA remains unknown, it might be that FA proteins have a similar function, or work together, for instance, as part of a multimeric complex or participating in a common pathway.[112] The recent report on the interaction between FAA and FAC proteins and consequent translocation of the complex to the nucleus support the model suggested by D'Andrea and Grompe.[113] In FA, a signal transduction pathway is defective. FAC, as a *sensor* protein, interacts in the cytoplasm with FAA. This *translocator*, transferred to nucleus, is able to activate an *inducer* for the expression of proteins involved in the pathogenesis of FA.

## Perspectives

The cloning of the *FAA* and *FAC* genes has provided an opportunity to understand the molecular basis of FA because only, with these gene, can new approaches be taken to explore the role of the altered proteins. Although the FA defect remains unknown, some progress has been made in several key areas, including gene expression, characterization and interaction of proteins, animal models and determination of pathways defective in FA. However, together with the clarification of the role of FAA and FAC proteins, the cloning of all the FA genes will be represent an important prerequisite in the understand pathogenesis of FA. Both functional and positional strategies are technically difficult: the former tends to yield many false-positive cDNA clones whereas the latter is hampered by the need of a large sample of families eligible for linkage analysis. This limitation can be overcome by an alternative approach leading to gene localization by mediated chromosome transfer, as reported for FAD gene.[53]

The identification of the genes also allows the screening for mutations. Knowledge of the mutation

spectrum in the *FAA* and *FAC* genes may contribute significantly to pathogenesis studies in FA. The identification of pathogenic alterations in FA patients allows also for the assignment of patients to the respective complementation group and thus removes laborious complementation studies. For patients who do not belong to FA-A or FA-C, complementation analysis is still the only way to assess their subtype. In addition, since FA is a clinically heterogeneous disease and diagnosis on the basis of symptoms alone is difficult and often unreliable, knowledge of the mutations allows for an accurate diagnosis and for rapid prenatal identification of an affected fetus. Although the diagnosis is facilitated by the study of induced chromosomal instability, the DEB test is not reliable in individuals with mosaicism and in testing carriers from general population. In both cases, screening for mutations is a powerful approach for molecular analysis.

The cloning of the *FAA* and *FAC* gene is leading to the establishment of protocols for gene therapy. FA is a good candidate disease for this new therapeutic trial because *in vitro* transfected cells have a selective growth advantage on FA cells. In addition, different target cells, either stem cells or cord blood and peripheral blood cells, can be easily purified and in vitro manipulated for gene transfer. Retroviral vectors were able to fully complement the cellular defect of both FA-C and FA-A cells.[114,115] However, several problems remain unsolved, including the integration of the gene, the level of integration necessary for the correction of the disease, the long-term expression of the transfected gene, the selective growth advantage of transfected cells, and the function of the FA proteins.

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*AS was responsible for the conception of this review article. All the authors contributed to the analysis of the literature and writing of the paper. The authors wish to thank Achille Iolascon for helpful discussions and comments and Adriana Zatterale of the Italian Registry of Fanconi anemia (RIAF) for important contributions.*

### Disclosures

*Conflict of interest: none.*

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### Manuscript processing

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## References

1. Fanconi G. Familiar constitutional panmyelocytopathy, Fanconi's anemia (FA). I. Clinical aspects. Semin Hematol 1967; 4:233-40.
2. Joenje H, Mathew C, Gluckman E. Fanconi anemia research: current status and prospects. Eur J Cancer 1995; 31A:268-72.
3. Rosendorff J, Berstein R, Macdougall L, Jenkins T. Fanconi anemia: another disease of unusually high prevalence in the Africans population of South Africa. Am J Med Genet 1987; 27:793-7.
4. Sansone R, Sansone G. The Italian contribution to the study of Fanconi's anemia. Pathologica 1990; 82:473-8.
5. Buchwald M, Joenje H, Auerbach AD. Fanconi anemia. In: Scriver C, Beaudet AL, Sly WS, Vallz D, eds. The Metabolic and Molecular Basis of Inherited Disease. 7th ed. New York: McGraw-Hill, 1997.
6. Alter BP, Young NS. The bone marrow failure syndromes. In: Nathan DG, Oski FA, eds. Hematology of Infancy and Childhood, I. 4th ed. Philadelphia: Saunders; 1993. p. 216-316.
7. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. Blood 1994; 84:1650-5.
8. Auerbach AD, Rogatko A, Schroeder-Kurth TM. International Fanconi anemia Registry: relation of clinical symptoms to diepoxybutane sensitivity. Blood 1989; 73:391-6.
9. Alter BP. Fanconi's anemia and its variability. Br J Haematol 1993; 85:9-14.
10. Giampietro PF, Adler-Brecher B, Verlander PC, Pavlakis SG, Davis JG, Auerbach AD. The need for more accurate and timely diagnosis in Fanconi anemia: a report from the International Fanconi Anemia Registry. Pediatrics 1993; 91:1116-20.
11. Addison M, Rice MS. The association of dyskeratosis congenita and Fanconi anemia. Med J Aust 1965; 1:797-9.
12. Nowell P, Bergman G, Besa E, Wilmoth D, Emanuel B. Progressive preleukemia with a chromosomally abnormal clone in a kindred with the Estren-Dameshek variant of Fanconi's anemia. Blood 1984; 64:1135-8.
13. Hedberg VA, Lipton JM. Thrombocytopenia with absent radii. A review of 100 cases. Am J Pediatr Hematol Oncol 1988; 10:51-64.
14. Farrell SA, Paes BA, Lewis ME. Fanconi anemia in a child previously diagnosed as Baller-Gerold syndrome. Am J Med Genet 1994; 50:98-9.
15. Rossbach Hc, Sutcliffe MJ, Haag MM, Grana NH, Rossi AR, Barbosa JL. Fanconi anemia in brothers initially diagnosed with VACTERL association with hydrocephalus, and subsequently with Baller-Gerold syndrome. Am J Med Genet 1996; 61:65-7.
16. Auerbach AD, Allen RG. Leukemia and preleukemia in Fanconi anemia patients. A review of the literature and report of the International Fanconi Anemia Registry. Cancer Genet Cytogen 1991; 5:1-12.
17. Swift M, Zimmerman D, McDonough ER. Squamous cell carcinomas in Fanconi's anemia. JAMA 1971; 216:325-6.
18. Somers GR, Tabrizi SN, Tiedemann K, Chow CW, Garland SM, Venter DJ. Squamous cell carcinoma of the tongue in a child with Fanconi anemia: a case report and review of the literature. Pediatr Pathol Lab Med 1995; 15:597-607.
19. Alter BP. Fanconi's anemia and malignancies. Am J Hematol 1996; 53:99-110.
20. Sasaky MS, Tonomura A. A high susceptibility of Fanconi's anemia to chromosome breakage by DNA cross-linking agents. Cancer Res 1973; 33:1829-36.
21. Sasaky MS. Is Fanconi's anaemia defective in a process essential to repair of DNA cross-link? Nature 1975; 257:501-3.
22. Wevrick R, Buchwald M. Mammalian DNA repair genes. Curr Opin Genet Dev 1993; 3:470-4.
23. Taylor AM, Byrd PJ, McConville CM, Thacker S. Genet-

ic and cellular features of ataxia telangiectasia. Int J Radiat Biol 1994; 65:65-70.
24. German J. Bloom's syndrome. Dermatol Clin 1995; 13:7-18.
25. Friedberg E. Cockayne syndrome. A primary defect in DNA repair, transcription, both, or neither. Bioessays 1996; 18:731-8.
26. Ellis NA. DNA elicases in inherited human disorders. Curr Opin Genet Dev 1997; 7:354-63.
27. Copeland NE, Hanke CW, Michalak JA. The molecular basis of xeroderma pigmentosum. Dermatol Surg 1997; 23:447-55.
28. Auerbach AD, Wolman SR. Susceptibility of Fanconi's anemia fibroblasts to chromosome damage by carcinogens. Nature 1976; 261:494-6.
29. Isida R, Buchwald M. Susceptibility of Fanconi's anemia lymphoblasts to cross-linking and alkylating agents. Cancer Res 1982; 42:4000-6.
30. Berger R, Le Coniat M, Schaison G. Chromosome abnormalities in bone marrow of Fanconi anemia patients. Cancer Genet Cytogenet 1993; 65:47-50.
31. Alter BP, Scalise A, McCombs J, Najfeld V. Clonal chromosomal abnormalities in Fanconi's anaemia: what do they really mean? Br J Haematol 1993; 85: 627-30.
32. Fundia A, Gorla N, Larripa I. Spontaneous chromosome aberrations in Fanconi's anemia patients are located at fragile sites and acute myeloid leukemia breakpoints. Hereditas 1994; 120:47-50.
33. Joenje H, Arwert F, Eriksson AW, et al. Oxygen-dependence of chromosomal aberrations in Fanconi's anemia. Nature 1981; 290:142-3.
34. Schindler D, Hoehn H. Fanconi anemia mutation causes cellular susceptibility to ambient oxygen. Am J Hum Genet 1988; 43:429-35.
35. Joenje H, Gille JJP. Oxygen metabolism and chromosomal breakage in Fanconi anemia. In: Schroeder T, Auerbach AD, Obe G, eds. Fanconi anemia: clinical, cytogenetic and experimental aspects. Berlin: Springer-Verlag, 1989. p. 174-82.
36. Pagano G, Korkina LG, Degan P, et al. *In vitro* hypersensitivity to oxygen of Fanconi anemia (FA) cells is linked to *ex vivo* evidence for oxidative stress in FA homozygotes and heterozygotes. Blood 1997; 89: 1111-2.
37. Kaiser TN, Lojewki A, Dougherty C, Juergens L, Sahar E, Latt SA. Flow cytometric characterization of the response of Fanconi's anemia cells to mitomycin C treatment. Cytometry 1982; 2:291-7.
38. Dutrillaux B, Aurias A, Dutrillaux AM, Buriot D, Prieur M. The cell cycle of lymphocytes in Fanconi anemia. Hum Genet 1982; 62:327-32.
39. Auerbach AD. Fanconi anemia diagnosis and the diepoxibutane (DEB) test. Exp Hematol 1993; 21:731-3.
40. Seyschab H, Friedl R, Sun Y, et al. Comparative evaluation of diepoxybutane sensitivity and cell cycle blockage in the diagnosis of Fanconi anemia. Blood 1995; 85:2233-7.
41. Guinan EC, Lopez KD, Huhn RD, Felser JM, Nathan DG. Evaluation of granulocyte-macrophage colony-stimulating factor for treatment of pancytopenia in children with Fanconi anemia. J Pediatr 1994; 124: 144-50.
42. Rackoff WR, Orazi A, Robinson CA, et al. Prolonged administration of granulocyte colony-stimulating factor (filgrastim) to with Fanconi anemia: a pilot study. Blood 1996; 88:1588-93.
43. Touraine RL, Bertrand Y, Foray P, Gilly J, Philippe N. Hepatic tumours during androgen therapy in Fanconi anaemia. Eur J Pediatr 1993; 152:691-3.
44. Gluckman E, Auerbach AD, Horowitz MM, et al. Bone marrow transplantation for Fanconi anemia. Blood 1995; 86:2856-62.
45. Duckworth-Rysiecki G, Cornish K, Clarke CA, Buchwald M. Identification of two complementation groups in Fanconi anemia. Somat Cell Mol Genet 1985; 11:35-41.
46. Strathdee CA, Duncan AMV, Buchwald M. Evidence for at least four Fanconi anemia genes including FACC on chromosome 9. Nature Genet 1992; 1:196-8.
47. Joenje H, Lo Ten Foe JR, Oostra AB, et al. Classification of Fanconi anemia patients by complementation analysis: evidence for a fifth genetic subtype. Blood 1995; 86:2156-60.
48. Joenje H, Oostra A, Wijker M, et al. Evidence for at least eight Fanconi anemia genes. Am J Hum Genet 1997; 61:940-4.
49. Buchwald M. Complementation groups: one or more per gene? Nature Genet 1995; 11:228-30.
50. Jakobs PM, Fiddler-Odell E, Reifsteck C, Olson S, Moses RE, Grompe M. Complementation group assignments in Fanconi anemia fibroblast cell lines from North America. Somat Cell Mol Genet 1997; 23:1-7.
51. Savoia A, Zatterale A, Del Principe D, Joenje H. Fanconi anaemia in Italy: high prevalence of complementation group A in two geographic clusters. Hum Genet 1996; 97:599-603.
52. Joenje H. European Fanconi Anaemia Research Group. Fanconi anaemia complementation groups in Germany and the Netherlands. Hum Genet 1996; 97:280-2.
53. Whitney M, Thayer M, Reifsteck C, et al. Microcell mediated chromosome transfer maps the Fanconi anemia group D gene to chromosome 3p. Nature Genet 1995; 11:341-3.
54. Strathedee CA., Gavish H, Shannon WR, Buchwald M. Cloning of cDNAs for Fanconi's anemia by functional complementation. Nature 1992; 356:763-7.
55. Gibson RA, Buchwald M, Roberts RG, Mathew CG. Characterization of the exon structure of the Fanconi anemia group C gene by vectorette PCR. Hum Mol Genet 1993; 2:35-8.
56. Hahn H, Wicking C, Zaphiropoulos PG, et al. Mutations of the human homolog of *Drosophila* patched in nevoid basal cell carcinoma syndrome. Cell 1996; 85: 841-51.
57. Wevrick C, Clarke CA, Buchwald M. Cloning and analysis of the murine Fanconi anemia group C cDNA. Hum Mol Genet 1993; 2:655-62.
58. Ching-Ying-Wong J, Alon N, Buchwald M. Cloning of the bovine and rat Fanconi anemia group C cDNA. Mamm Genome 1997; 8:522-5.
59. FAB Consortium. Positional cloning of the Fanconi anemia group A gene. Nature Genet 1996; 14:324-8.
60. Lo Ten Foe JR, Roimans AA, Bosnoyan-Collins L , et al. Expression of cloning of a cDNA for the major Fanconi anemia gene, FAA. Nature Genet 1996; 14:320-3.
61. Pronk JC, Gibson RA, Savoia A, et al. Localization of the Fanconi anemia complementation group A gene to chromosome 16q24.3. Nature Genet 1995; 11:338-40.
62. Savoia, Piemontese MR, Savino M, et al. Linkage analysis of Fanconi anemia in Italy and mapping of the complementation group A gene. Hum Genet 1997; 99:93-7.
63. Ianzano L, d'Apolito M, Centra M, et al. The genomic organization of the Fanconi anemia group A (FAA) gene. Genomics 1997; 41:309-14.
64. Murer-Orlando M, Llerena JC, Birjandi F, Gibson RA, Mathew CG. FACC gene mutations and early prenatal diagnosis of Fanconi's anaemia. Lancet 1993; 342: 686.
65. Gibson RA, Hajianpour A, Murer-Orlando M, Buchwald M, Mathew CG. A nonsense mutation and exon skipping in the Fanconi anaemia group C gene. Hum Mol Genet 1993; 2:797-9.

66. Whitney MA, Saito H, Jakobs PM, Gibson RA, Moses RE, Grompe M. A common mutation in the FACC gene causes Fanconi anaemia in Ashkenazi Jews. Nature Genet 1993; 4:202-5.
67. Verlander PC, Lin JD, Udono MU, et al. Mutation analysis of the Fanconi anemia gene FACC. Am J Hum Genet 1994; 54:595-601.
68. Lo Ten Foe JR, Rooimans MA, Joenje H, Arwert F. Novel frameshift mutation (1806insA) in exon 14 of the Fanconi anemia C gene, *FAC*. Hum Mutat 1996; 7: 264-5.
69. Lo Ten Foe JR, Barel MT, Thub P, Digweed M, Arwert F, Hoenje H. Sequence variations in the Fanconi anaemia gene, *FAC*: pathogenicity of 1860insA and R548X and recognition of D195V as a polymorphic variant. Hum Genet 1996; 98:522-3.
70. Gibson RA, Morgan NV, Goldstein LH, et al. Novel mutations and polymorphisms in the Fanconi anemia group C gene. Hum Mutat 1996; 8:140-8.
71. Lo Ten Foe JR, Kruyt FAE, Zweekhoest MBM, et al. Exon 6 skipping in the Fanconi Anemia C gene associated with a nonsense/missense mutation (775C→T) in exon 5. Hum Mutat 1997; 1: S25-7.
72. Gavish H, dos Santos CC, Buchwald M. A Leu554-toPro substitution completely abolishes the functional complementing activity of the Fanconi anemia (FACC) protein. Hum Mol Genet 1993; 2:123-6.
73. Whitney MA, Jakobs P, Kaback M, Moses RE, Grompe M. The Ashkenazi Jewish Fanconi anemia mutation: incidence among patients and carrier frequency in the at-risk population. Hum Mutat 1994; 3:339-41.
74. Verlander PC, Kaporis A, Liu Q, Zhang Q, Seligsohn U, Auerbach AD. Carrier frequency of the IVS4+4A→T mutation of the Fanconi anemia gene *FAC* in the Ashkenazi Jewish population. Blood 1995; 86:4034-8.
75. Cox PM, Gibson RA, Morgan N, Brueton LA. VACTERL with hydrocephalus in twins due to Fanconi anemia (FA): mutations in the *FAC* gene. Am J Med Genet 1997; 68:86-90.
76. Yamashita T, Wu N, Kupfer G, et al. Clinical variability of Fanconi anemia (type C) results from expression of an amino terminal truncated Fanconi anemia complementation group C polypeptide with partial activity. Blood 1996; 87:4424-32.
77. Levran O, Erlich T, Magdalena N, et al. Sequence variation in the Fanconi anemia gene FAA. Proc Natl Acad USA 1997; 94:13051-6.
78. Savino M, Ianzano L, Strippoli P, et al. Mutations of the Fanconi anemia A gene (FAA) in Italian patients. Am J Hum Genet 1997; 61:1246-53.
79. Wijker M, Morgan NV, Herterich S, et al. Heterogeneous spectrum of mutations in the Fanconi anemia group A gene. Eur J Hum Genet 1998; in press.
80. Centra M, Memeo E, d'Apolito M, et al. Fine exon-intron structure of the Fanconi anemia group A (FAA) gene and characterization of two genomic deletions. Genomics 1998; in press.
81. Lo Ten Foe JR, Kwee ML, Rooimans MA, et al. Somatic mosaicism in Fanconi anemia: molecular basis and clinical significance. Eur J Hum Genet 1997; 5:137-48.
82. Klein CJ, Coovert DD, Bulman DE, et al. Somatic reversion/suppression in Duchenne muscular dystrophy (DMD): evidence supporting a frame-restoring mechanism in rare dystrophin-positive fibers. Am J Hum Genet 1992; 50:950-9.
83. Ellis NA, Lennon DJ, Proytcheva M, Alhadeff B, Henderson EE, German J. Somatic intragenic recombination within the mutated locus BLM can correct the high sister-chromatid exchange phenotype of Bloom syndrome cells. Am J Hum Genet 1995; 57:1019-27.
84. Hirschhorn R, Ruixi Yang D, Punk JM, Huie ML, Jiang CK, Kurlandsky LE. Spontaneous *in vivo* reversion to normal of an inherited mutation in a patient with adenosine deaminase deficiency. Nature Genet 1996; 6:290-5.
85. Krasnoshtein F, Buchwald M. Developmental expression of the Fac gene correlates with congenital defects in Fanconi anemia patients. Hum Mol Genet 1996; 5:85-93.
86. Savoia A, Centra M, Ianzano L, de Cillis GP, Zelante L, Buchwald M. Characterization of the 5' region of the Fanconi anaemia group C (FACC) gene. Hum Mol Genet 1995; 8:1321-6.
87. Liebetrau W, Budde A, Savoia A, Grummt F, Hoehn H. p53 actives Fanconi anemia group C gene expression. Hum Mol Genet 1997; 2:277-83.
88. Yamashita T, Barber DL, Zhu Y, Wu N, D'Andrea AD. The Fanconi anemia polypeptide FACC is localized to the cytoplasm. Proc Natl Acad Sci USA 1994; 91:6712-6.
89. Youssoufian H. Localization of Fanconi anemia C protein to the cytoplasm of mammalian cells. Proc Natl Acad Sci 1994; 91:7975-9.
90. Youssoufian H, Auerbach AD, Verlander PC, Steimle V, Mach B. Identification of cytosolic proteins that bind to the Fanconi anemia complementation group C polypeptide *in vitro*. Evidence for a multimeric complex. J Biol Chem 1995; 270:9876-82.
91. Youssoufian H. Cytoplasmic localization of *FAC* is essential for the correction of a prerepair defect in Fanconi anemia group C cells. J Clin Invest 1996; 97:2003-10.
92. Hoshino T, Youssoufian H, Wang J, et al. The molecular chaperone GRP94 binds to a central domain within the group C Fanconi anemia protein [abstract]. Blood 1996; 88(Suppl 1):1734a.
93. Kupfer GM, Yamashita T, Naf D, Suliman A, Asano S, D'Andrea AD. The Fanconi anemia polypeptide, *FAC*, binds to the cyclin-dependent kinase, cdc2. Blood 1997; 90:1047-54.
94. Kruyt FAE, Waisfisz Q, Dijkmans LM, et al. Cytoplasmic localization of a functionally active Fanconi anemia group A-green fluorescent protein chimera in human 293 cells. Blood 1997; 90:3288-95.
95. Kupfer G, Naf D, Suliman A, Pulsipher M, D'Andrea AD. The Fanconi anemia proteins, FAA and *FAC*, interact to form a nuclear complex. Nature Genet 1997; 17:487-90.
96 Chen M, Tomkins DJ, Auerbach W, et al. Inactivation of Fac in mice produces inducible chromosomal instability and reduced fertility reminiscent of Fanconi anaemia. Nature Genet 1996; 12:448-51.
97. Whitney MA, Royle G, Low MJ, et al. Germ cell defects and hematopoietic hypersensitivity to gamma-interferon in mice with a targeted disruption of the Fanconi anemia C gene. Blood 1996; 88:49-58.
98. Hirana T, Koeffler HP. Role of the cyclin-dependent kinase inhibitors in the development of cancer. Blood 1995; 86:841-54.
99. Digweed M, Gunthert U, Schneider R, Seyschab H, Friedl R, Sperling K. Irreversible repression of DNA synthesis in Fanconi anemia cells is alleviated by the product of a novel cyclin-related gene. Mol Cell Biol 1995; 15:305-14.
100. Segal GM, Magenis RE, Brown M, et al. Repression of Fanconi anemia gene (FACC) expression inhibits growth of hematopoietic progenitor cells. J Clin Invest 1994; 94:846-52.
101. Diller L, Kassel J, Nelson CE, et al. p53 function as a cell cycle control in osteosarcoma. Mol Cell Biol 1990; 10:5772-81.
102. Kastan M, Zhan Q, El-Deiry, et al. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia telangiectasia. Cell 1992; 71:587-97.

103. Ridet A, Guillouf C, Duchaud E. Deregulated apoptosis is a hallmark of the Fanconi anemia syndrome. Cancer Res 1997; 57:1722-30.
104. Cumming RC, Liu JM, Youssoufian H, Buchwald M. Suppression of apoptosis in hematopoietic factor-dependent progenitor cell lines by expression of the *FAC* gene. Blood 1996; 88:4558-67.
105. Kruyt FA, Dijkmans LM, van den Berg TK, Joenje H. Fanconi Anemia genes act to suppress a cross-linker-inducible p53-independent apoptosis pathway in lymphoblastoid cell lines. Blood 1996; 87:938-48.
106. Kupfer GM, D'Andrea AD. The effect of the Fanconi anemia polypeptide, *FAC*, upon p53 induction and G2 checkpoint regulation. Blood 1996; 88:1019-25.
107. Rosselli F, Ridet A, Soussi T, Duchaud E, Alapetite C, Moustacchi E. p53-dependent pathway of radio-induced apoptosis is altered in Fanconi anemia. Oncogene 1995; 10:9-17.
108. Rathbun RK, Faulkner GR, Ostroski MH, et al. Inactivation of Fanconi anemia group C gene augments interferon-gamma-induced apoptotic responses in hematopoietic cells. Blood 1997; 90:974-85.
109. Papadopulo D, Guillouf C, Mohrenweiser H, Moustacchi E. Hypomutability in Fanconi anemia cells is associated with increased deletion frequency at the HPRT locus. Proc Natl Acad Sci USA 1990; 87:8383-7.
110. Laquerbe A, Moustacchi E, Fuscoe JC, Papadopoulo D. The molecular mechanism underlying formation of deletions in Fanconi anemia cells may involve a site specific recombination. Proc Natl Acad Sci USA 1995; 92:831-5.
111. Thyagarajan B, Campell C. Elevated homologous recombination activity in Fanconi anemia fribroblasts. J Biol Chem 1997; 272:23328-33.
112. D'Andrea A. Fanconi anemia forges a novel pathway. Nature Genet 1996; 14:240-2.
113. D'Andrea A, Grompe M. Molecular biology of Fanconi anemia: implication for diagnosis and therapy. Blood 1997; 90:1725-36.
114. Machl AW, Planitzer S, Kubbies M. A novel, membrane receptor-based retroviral vector for Fanconi anemia group C gene therapy. Gene Ther 1997; 4:339-45.
115. Fu K-L, Lo Ten Foe JR, Joenje H, Rao KW, Liu JM, Walsh CE. Functional correction of Fanconi group A hematopoietic cells by retroviral gene transfer. Blood 1997; 90:3296-303.