

Fanconi Anemia

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Abstract: Fanconi anemia (FA) is the most common inherited bone marrow failure disorder characterized by cytopenias, somatic defects and increased propensity to develop malignancies. Chromosomal breakage analysis is the gold standard diagnostic test for this disease but it may produce false negative results. Therefore, genetic analysis is necessary for confirmation and complement group identification that will help in prenatal diagnosis. Phenotypic presentation of FA is quite variable. 20-30% patients are physically normal. Affected patients may exhibit anomalies of multiple organs or just a few café au lait spots. Therefore diagnosis of this disease remains challenging. Similar to its phenotype the pathophysiology of this disease is also complicated and still not completely understood. The basic defect is in the DNA repair mechanism. The 21 complementation genes responsible for DNA repair are hampered at any one or multiple levels. The chromosomes from the cells of these patients therefore show spontaneous breakages. The incidence of this disease throughout the world is very rare about 1-2/350000 persons. It is more common in Jews. In Pakistan incidence of FA is still not known and many of the physicians and surgeons don't have in depth knowledge as they consider it as a rare disease. However, because of the custom of inter marriages its incidence may be surprisingly high. Therefore in depth knowledge of this disease is necessary.

The aim of this review article is to provide information to the physicians dealing such patients so that they should be timely diagnosed and managed accordingly.

Keywords: Fanconi anemia, Aplastic anemia, Mitomycin C, Chromosomal breakages, Skeletal defects, Café au lait spots.

INTRODUCTION

Fanconi Anemia is an inherited disorder characterized by progressive bone marrow failure, congenital somatic abnormalities and increased propensity to develop acute leukemia and solid tumors [1]. It was first described by a Swiss Pediatrician Guido Fanconi in 1927 in three brothers with pancytopenia, short stature, skin hypo and hyper pigmentation [2]. Till now 21 complementation groups have been identified. FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCI, FANCL, FANCM, FANCN, FANCO, FANCP, FANCO, FANCR, FANCS, FANCT, FANVU and FANCV [3, 4]. These genes are responsible for DNA repair and work together in a common pathway known as the Fanconi anemia pathway. 80-90 % cases of FA are due to mutations in complementation groups A, C and G with > 60-70% in FANCA A group. FANCA gene is located on 16q24.3. It consists of total 43 exons. Location of other FANCA genes along with frequency is mentioned in Table 1. Mutations in FANCC and FANCG account for another 25% of cases [5, 6].

INHERITANCE

About 98% cases are autosomal recessive while 1-2% that has mutation in the FANCB gene is inherited in X linked recessive manner [7].

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Table 1. Summary of Fanconi Anemia Genes Identified.

FANCA Subtype	Gene	Chromosome	Frequency
A	FANCA	16q24.3	60-10%
B	FANCB	Xp22.31	2
C	FANCC	9q22.3	10-15
D1	FANCD/BRCA2	13q12-13	2
D2	FANCD2	3p25.3	3
E	FANCE	6p21-22	2
F	FANCF	11p15	2
G	FANCG	9p13	10-15
I	FANCI	15q25-26	2
J	FANCI	17q22.3	2
L	FANCL	2p16.1	Rare
M	FANCM	14q21.3	Rare
N	FANCN	16p12.1	Rare
O	FANCO	17q22	Rare
P	FANCP	16p13.3	Rare
Q	FANCO	16p13.12	Rare
R	FANCR	15q15.1	Rare
S	FANCS	17q21	Rare
T	FANCT	1q32.1	Rare

EPIDEMIOLOGY

It is common in all ethnic groups worldwide. Its frequency worldwide is estimated to be 1 in 350,000. It is more common in Ashkenazi Jews and Afrikaans of South Africa with carrier frequency of 1 in 89 and 1 in 83 respectively [7].

CLINICAL FEATURES

FA patients usually exhibit congenital anomalies but about 25-40% patients are totally asymptomatic. Most common and important clinical features are hematological abnormalities. Patients usually present within the 1st decade of life with signs of progressive bone marrow failure like bleeding, recurrent infections and anemia, median age being 7 years. Currently FA is the most common cause of constitutional aplastic anemia [8]. Risk of developing myelodysplastic syndrome (MDS) and acute leukemia (AML) increases with age. As compared with general population the risk of AML in these patients is 800 folds higher. Monosomy 5 and 7 are common in FA patients presenting with MDS/AML [9]. In addition to hematological cancers solid tumors also occur at higher rates in these patients [10].

Multiple congenital abnormalities have been reported in FA. Most common being the skeletal defects like thumb hypoplasia, radial ray, hyperplasia of radia, congenital hip dislocation and vertebral anomalies. Other common abnormalities include skin pigmentation defects (café au lait spots), microphthalmia, microcephaly, ear malformations, hearing impairment, low set ears, urogenital defects e.g. horse shoe kidney, pelvic kidney or absence of one kidney etc. and cardiac defects [11]. Fig. (1 a – c) are of a 17 year old fanconi anemia patient showing microphthalmia, café au lait spots and absence of Rt thumb.



Fig. (1). Classical Phenotypic Features of Fanconi Anemia.

- a) Café Au lait Spots (Reproduced from [41]).
- b) Fanconi Facies (Microphthalmia, Small Face).
- c) Absence of Thumb (Reproduced from [41]).

The risk of solid tumors like head and neck squamous cell carcinomas or gynecological cancers is also increased in FA [12]. This increased risk is secondary to dysregulated FA pathway [13]. Hepatic tumors are also very common probably secondary to androgens. There is no evidence regarding increased risk of malignancies in carriers of FA. However, those carriers that have inherited BRCA2 mutations have increased propensity to develop any malignancy [14].

Endocrine problems like short stature, osteoporosis is also common in these patients. These abnormalities may aid in diagnosing FA before bone marrow failure develops.

PATHOPHYSIOLOGY

The 21 complementation genes identified are all involved in DNA repair pathway that is specialized to resolve DNA interstrand crosslink lesions (ICL). These ICLs inhibit the replication and transcription of DNA. Different complementation genes work together in a pathway known as the fanconi pathway [15]. Eight of the upstream complementation genes (FANCA/B/C/E/F/G/L/M) form a large nuclear complex which is known as Core complex (CC) [16]. In about 90% patients mutations are present in these eight upstream genes. Most of the proteins in this core complex have no enzymatic activity rather they just acts as a scaffold. However, PHD-type RING finger domain of FANCL interacts with an E ubiquitin enzyme UBE2T that catalyzes mono ubiquitination of FANCD2 and FANCI. The ubiquitinated FANCD2/FANCI is then recruited to the chromatin in the form of heterodimer that identifies the stalled replication forks and participates in DNA repair [17].

Furthermore, four FA genes (FANCD1/BRCA2, FANCI/BRIP1, FANCN/PALB2, and FANCO/RAD51C)

that are known breast cancer susceptibility genes connect FA proteins to homologous recombination repair. The breast cancer gene BRCA1 is identical to FANCD1. FA genes also take part in other repair mechanisms like excision repair and translesion process [17].

Mutation in any of these genes and their products therefore hinders core complex formation and hence DNA repair mechanism is hindered. This failure of DNA repair mechanism in turn leads to bone marrow failures, susceptibility to develop malignancies and multiple congenital anomalies.

DIAGNOSIS

Because of the heterogeneous presentation of FA it is not easy to diagnose it on clinical grounds only. Therefore, diepoxybutane (DEB)/ mitomycin C (MMC) test for chromosomal breakage analysis should be done of every aplastic anemia patient. Cells from FA patients will exhibit chromosomal breakages when exposed to these interstrands cross linking (ICL) agents [18]. This is considered as gold standard diagnostic test but it can produce false positive results as in Nijmegen breakage or Blooms syndrome or false negative results as in case of mosaicism. About 10-30% FA patients are mosaic due to genetic reversion in hematopoietic stem cells. Therefore the definitive diagnosis is based on complementation identification for subtyping [16].

MOSAICISM

Somatic as well as reverse mosaicism has been observed in the lymphocytic population of patients with FA [19]. As per literature one in four to five patients develop reverse mosaicism during their life span. This can lead to normal blood counts and improved survival of these patients [20].

COMPLEMENTATION GROUPS AND PHENOTYPE

Phenotypic presentation of Fanconi anemia is very heterogeneous. Around 30% patients are completely asymptomatic with no congenital malformations while on the other hand patient present with bone marrow failure, malignancies at a very young age. It has been studied that severity of FA patients vary with different complementation groups [21]. The reason behind it may be that different complementation genes are involved at different stages of DNA repair pathway. Patients with FANCA gene mutations usually develop bone marrow failure at a later stage while patients with FANCD1 (mutated in BRCA) and FANCN (mutated in PALB2) present with very early onset (2-3 years) leukemia and solid tumors like Wilms tumor and medulloblastoma. According to 20 year perspective by IFAR, mutations in intron 4 and exon 14 of FANCC is associated with early onset bone marrow failure and poor survival as compared to mutations in FANCG and FANCA [22, 23].

DIFFERENTIAL DIAGNOSIS

It includes VACTER/VACTERL and other inherited bone marrow failure syndromes associated with congenital anomalies like Diamond Black fan anemia (DBA), Nijmegen breakage syndrome, Thrombocytopenia with absent radii (TAR) [24, 25].

VACTERL association is a rare inherited disorder affecting multiple organs. It is an acronym for (V) vertebral abnormalities (A) anal atresia (C) cardiac (heart) defects (TE) tracheal-esophageal abnormalities, including atresia, stenosis and fistula (R) renal and radial abnormalities (L) (non-radial) limb abnormalities (S) single umbilical artery [24].

Diamond Blackfan anemia is another rare inherited disorder characterized by macrocytic anemia, severe reticulocytopenia and defective erythroid maturation. Patients usually present within 1st year of life [26].

Thrombocytopenia with absent radii (TAR) is another rare autosomal recessive disorder that presents at birth or early infancy. As the name indicates patients have thrombocytopenia and radial ray defects but unlike in FA their thumbs are present bilaterally [27]. TAR is also not associated with increased propensity for developing tumors.

Nijmegen breakage syndrome is a rare autosomal disorder characterized by immunodeficiency, microcephaly and hypersensitivity to ionizing radiations [28]. These patients may present with hematological abnormalities. Furthermore, chromosomal breakages are also observed in these patients' lymphocytes when exposed to MMC or DEB. Therefore, genetic testing for this syndrome must be done if there is doubt in diagnosis for FA [29].

MANAGEMENT

Most commonly used treatment options for alleviation of cytopenias includes androgens, growth factors and stem cell transplant. Androgens like oxymetholon are male hormones that are effective in treating bone marrow failure to some extent in few patients. It is effective in 75% patients [30]. However, long term use of androgens is associated with multiple side effects that limits its utility. Side effects include hirsutism, altered liver enzymes and increased propensity to develop hepatomas [31]. As per some studies use of androgens may affect the outcome of bone marrow transplant in these patients [30, 32].

Hematopoietic stem cell growth factors alone or in combination with androgens are beneficial in improving the neutrophil count in few patients. However, these should be discontinued if there is any evidence of clonal evolution [32].

In majority of the fanconi patients the above mentioned therapy usually fails due to its limited efficacy and many side effects. Bone marrow transplantation is therefore the only curative option for fanconi associated bone marrow failure [33]. As this is a chromosomal instability disorder therefore these patients are offered non irradiation conditioning regime with low dose cyclophosphamide and fludarabine [34]. Until a suitable matched donor is available patient should be offered supportive care with transfusions, antifungal and antiviral therapy. Sibling donors are also screened for physical abnormalities and chromosomal breaks and genetic testing

before proceeding for BMT. Cord blood transplant has also been carried out successfully in these patients [35].

GENETIC AND PRENATAL COUNSELING

Molecular testing or sub typing of FA patients should be done in every case for counseling the parents about disease inheritance and future pregnancies.

PREIMPLANTATION GENETIC COUNSELING

This helps the couple of an affected child to conceive a normal baby. During this single cell from the blastocyst is analyzed for concerned mutations and HLA typing is also performed simultaneously [36].

FUTURE PROSPECTIVE

Current management of FA includes androgens, supportive care (blood products and antibiotics) and stem cell transplantation. Both of these are used to manage only hematological issues. No treatment is available or established currently for the cure of this disease. Different FA clinical trials are undergoing, focusing on DNA repair pathway mechanism, gene editing, development of antioxidants, inhibiting apoptosis by reducing levels of TNF α (Tumor necrosis alpha) and use of CRISPR [37].

Out of these CRISPR-Cas9 (clustered, regularly interspaced short palindromic repeats)-Cas9 (CRISPR associated protein 9) gene editing is the main focus of researchers. It involves a protein-RNA complex composed of an nuclease known as Cas9 bound to a guide RNA molecule that has been designed to recognize a particular DNA sequence [38]. The RNA molecule guides the Cas9 complex to the genomic site that requires repair [37]. Here Cas 9 nuclease produces a nick and moves the mutated gene which is then replaced by normal DNA through homology directed repair (HDR) [39, 40].

CONCLUSION

FA is a heterogeneous condition that can present with a variety of congenital defects. FA requires a multidisciplinary approach in managing patients. Furthermore, a high index of suspicion should be present, because patients with FA often initially present to general pediatricians or other subspecialists. Early detection and diagnosis is essential to optimize multidisciplinary subspecialty care, as screening of each organ system is essential to optimize the patient's quality of life.

CONFLICT OF INTEREST

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