Evaluation of Clinical and Genetic Features of 40 Patients with Fanconi Anemia: A Single-Center Experience

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ABSTRACT

Fanconi anemia (FA) is a rare inherited bone marrow failure syndrome that leads to congenital malformations, aplastic anemia, and cancer. FA is also classified as a chromosome breakage syndrome. FA cells are hypersensitive to diepoxybutane (DEB) and mitomycin C (MMC). To date, 23 genes have been identified to be responsible for FA. Forty patients diagnosed with FA between 2012 and 2022 were included in the study. Patients were evaluated in terms of growth retardation, congenital abnormalities, and cancer development. Reports of chromosome breakage tests, laboratory workup, imaging studies, and mutation analyses were reviewed for each patient. The median age at diagnosis was 101 months. Microcephaly (92.5%) was the most common clinical finding. The most common laboratory findings at the time of diagnosis were macrocytosis and thrombocytopenia (62.5% each), and 70% of the patients had bone marrow failure. DEB test was positive in 24 patients. FANCA (89.6%) was most commonly mutated, and four novel variants were identified in six patients. While leukemia was detected in two patients, none of the patients had solid tumors. Early diagnosis of FA is essential for the timely management of complications and improvement of patient outcomes. The diagnosis of FA is based on a combination of clinical manifestations, laboratory findings, and genetic testing.

Keywords: Aplastic anemia, Bone marrow failure, Children, Fanconi anemia

INTRODUCTION

Fanconi anemia (FA) is a rare multigenic disorder characterized by congenital abnormalities, bone marrow failure and, cancer predisposition, with an estimated incidence of 1 in 160,000-360,000.[1,2] FA was first described by Guido Fanconi in 1927 in three siblings with short stature, physical birth defects, hypogonadism, hyperpigmented skin lesions, and anemia.[3] Short stature, microcephaly, limb abnormalities (especially associated with thumb), hypo/hyper-pigmentation of the skin, and genitourinary and ocular abnormalities are common, occurring in up to 75 % of FA patients. In general, macrocytosis is the first presentation of hematologic impairment, followed by thrombocytopenia, pancytopenia, and aplastic anemia. Patients with FA may develop myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Also, solid tumors, particularly those affecting the head and neck region, skin, gastrointestinal system and genitourinary tract, are more common in FA patients than in healthy population.[4]

The maintenance of genomic stability is critical for survival, and its failure is usually associated with tumor development. The FA pathway is essential for DNA interstrand crosslink (ICL) repair. Driven by genomic instability, a defect in this pathway may result in chromosome breaks and rearrangements. The cells of FA patients are highly sensitive to ICL agents such as diepoxybutane (DEB) and mitomycin C (MMC).[5]

Table 1. Severity of Fanconi Anemia[4]

	Mild	Moderate	Severe
Absolute neutrophil count (ANC)	< 1,500/mm^3	< 1.000/mm^3	< 500/mm^3
Platelet count	50.000-150.000/mm^3	< 50.000/mm^3	< 30.000/mm^3
Hemoglobin level	≥ 8 g/dl	< 8 g/dl	< 8 g/dl

To date, 23 FA genes (FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BRIP1, FANCL, FANCM, FANCN/ PALB2, FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4/XPF, FANCR/ RAD51, FANCS/BRCA1, FANCT/UBE2T, FANCU/ XRCC2, FANCV/ REV7, FANCW/ RFWD3 and FAAP100) have been identified.[4,6] Mutations in FANCA are the most common (60-70%) among FA patients, followed by FANCC and FANCG (10% of cases each).[2] Although the primary inheritance pattern of FA is autosomal recessive, FANCB (X-linked recessive) and FANCR/RAD51 (de novo autosomal dominant) exhibit different inheritance patterns.

The most severe congenital defects commonly occur in patients with FANCC, FANCD1/BRCA2, FANCD2, FANCG, FANCI, and FANCN/PALB2 mutations.[7] FANCG is associated with severe aplastic anemia and leukemia.[8] Biallelic pathogenic variants in BRCA2 are associated with early-onset acute leukemia and solid tumors. It has been reported that, by the age of six years, the cumulative probability of developing any malignancy, including AML, medulloblastoma, and Wilms tumor, reaches 97%.[4]

This study aimed to review the clinical and molecular findings of patients with Fanconi anemia referred to our center.

PATIENTS AND METHODS

Patients with a clinical suspicion of FA and abnormal chromosome breakage test results, those genetically diagnosed with FA or those with both chromosome breakage test results and genetics consistent with FA were included in the study. All patients with FA were evaluated for developmental delay, and, skeletal, renal, cardiac and head abnormalities. Data were retrieved from medical records of the patients, including demographics, clinical findings and results of laboratory workup (chromosome breakage testing, imaging and molecular studies).

The term bone marrow failure (BMF) was used to indicate hematologic abnormalities other than MDS or AML.

Cytopenia was classified as mild, moderate and severe (Table 1).[4]

This study was approved by the Institutional Review Board of Gaziantep University (Approval number: 2023/306; October 04, 2023).

Genetic Analysis

DNA extraction from peripheral blood was performed according to the manufacturer's instructions (Maxwell RSC Blood DNA kit, Promega, USA). DNA concentration was evaluated spectrophotometrically by measuring absorbance at 260/280 nm using a Nanodrop 1000 apparatus (Thermo Fisher Scientific). The concentration of DNA samples for libraries was determined using Qubit 3.0 (Thermo Fisher Scientific). The sequencing libraries for exome sequencing were prepared according to the Twist Human Core Exome Kit protocol (Twist Bioscience, USA). Sequencing was performed on a NovaSeq system (Illumina, USA). Sequence data were analyzed using SOPHIA DDM (Switzerland). Several in silico prediction tools, including BayesDel, REVEL, MetaRNN, CADD, EIGEN, SIFT, Provean, MutationTaster, were used to determine the pathogenicity of the variants identified. The frequency of the individual variants in the general population was assessed using the Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org/about). Franklin and Varsome online search engines were utilized for these evaluations.

Table 2. Physical anomalies in our patients with FA

Physical anomaly	Number of patients	Physical anomaly	Number of patients
Microcephaly	37	Cardiac anomaly	8
Short stature	36	PDA	3
Eye anomaly	35	PFO	2
Micropthalmia	35	ASD	1
Thumb anomaly	26	Aortic root dilation	1
Low-set	21	Complex	1
Bifid	2	Genital system anomaly	5
Attached by thread	2	Cryptorchidism	3
Agenesis	1	Micropenis	1
Renal anomaly	11	Hypospadias	1
Ectopic kidney	8	Gastrointestinal system anomaly	2
Single kidney	1	Duodenal atresia	1
Dysplastic kidney	1	Anal atresia	1
Cutaneous findings	8	Skeletal system anomaly (other than thumb)	1
Hyperpigmentation	5	Scoliosis	1
Hypopigmentation + Hyperpigmentation	3	Other	1
		Cleft palate-lip	1

PDA= Patent ductus arteriosus, PFO= Patent foramen ovale, ASD= Atrial septal defect

ClinVar and literature data were used to compare the clinical impact of the variants with previous findings. The variants identified were categorized as per the American College of Medical Genetics (ACMG) 2015 criteria.[9] Confirmation of identified variants was performed through Sanger sequencing and segregation analyses.

Statistical Analysis

Collected data were analyzed using SPSS version 24 (IBM Corp., Armonk, NY). Quantitative (numerical) variables were presented as mean, standard deviation, median, maximum and minimum, while qualitative (categorical) variables were summarized as frequency and percentage.

RESULTS

Forty patients with FA (52.5% female) diagnosed between 2012 and 2022 were included in the study. 75% of the patients were children of consanguineous parents. The median age at diagnosis was 101 months (range: 7-229).

Microcephaly (92.5%) was the most prevalent clinical finding, followed by short stature (90%), and thumb abnormalities (65%). Twenty-six patients had a thumb abnormality, 21 (80.7%) had low-set thumbs, 2 (7.7%) patients had bifid thumbs, 2 (7.7%) patients had a thumb attached by a thread, and one (3.9%) patient had no thumbs.

Eleven (27.5%) patients had kidney abnormalities. Eight (72.7 %) of them had ectopic kidneys (four of these kidneys were also horseshoe-shaped), two patients had a single kidney, and one patient had dysplastic kidneys. Eight (20%) patients had cardiac abnormalities. Three patients had patent ductus arteriosus (PDA), two patients had patent foramen ovale (PFO), one patient had secundum atrial septal defect (ASD), one patient had aortic root dilation and one patient had a complex cardiac abnormality including ASD, ventricular septal defect (VSD), pulmonary stenosis and right ventricular hypoplasia. The physical abnormalities found in FA patients are listed in Table 2.

Table 3. All variants identified in this study

Gene name	Transcript	Variation	Number of patients
FANCA	NM_000135.4	c.2495_2497del (p.Phe832del)	9
		Exon 16-21 homozygous deletion	1
		Exon 37 homozygous deletion	1
		c.3520_3522del (p.Trp1174del)	4
		c.3348+1G>T	2
		c.479T>A (p.Met160Lys)	3
		c.3931_3932del (p.Ser1311Ter)	3
		c.2938G>C (p.Ala980Pro)	1
		c.3931_3932del (p.Ser1311Ter)	2
		c.4124_4125delCA	1
		Exon 1-2 homozygous deletion	2
		Exon 1-20 homozygous deletion	1
		c.1492delC (p.Leu498fs)	1
		c.2852+1G>A	2
		Exon 9-10 homozygous deletion	1
FANCB	NM_001324162.1	Exon 6-10 hemizygous deletion	1
BRCA2	NM_000059.4	c.426-1G>C	1
FANCI	NM_001376911.1	c.2108A>G (p.Asp703Gly)	1
SLX4	NM_032444.4	c.643C>T (p.Gln215Ter)	1

The most common laboratory findings at initial diagnosis were macrocytosis and thrombocytopenia (62.5% each). Growth retardation was the most common presentation (42.5%, n= 17), followed by complaints related to bone marrow failure (pallor, epistaxis) in 14 (35%) patients. Nine (22.5%) patients were asymptomatic and detected through family screening.

At the time of diagnosis, 28 (70%) patients had bone marrow failure (BMF), which was severe in 4 (14.3%) patients. One patient had MDS. During the follow-up period, 9 (22.5%) patients underwent allogeneic hematopoietic stem cell transplantation (HSCT), and two (22.2%) of them died due to transplantation-related complications within the first 100 days after transplantation. Two patients did not have a suitable donor for HSCT and were receiving androgen therapy.

Chromosome breakage tests [Diepoxybutane (DEB), Mitomycin C (MMC)] were performed for 37 (92.5%) patients. DEB test was positive in 24 (64.9%) patients and negative in 13 patients. Mitomycin C was performed in two of these DEB-negative patients and found positive. For the other 11 patients with a negative DEB test result, MMC testing was not requested because there was no center performing MMC test in our city at that time and there was a risk that samples sent out would lose viability and potentially yield negative test results. Instead, mutation analysis was requested from the patients due to the primary consideration of Fanconi anemia. All remaining patients were diagnosed with FA based on characteristic physical abnormalities and mutation analysis.

The FA mutation was studied in 95% of the patients. FANCA (89.6%) was the most common mutation, followed by FANCB, FANCI, BRCA2, and SLX4 (2.6% each) (Table 3). In our cohort, the most prevalent mutation in FANCA was c.2495_2497del (p.Phe832del). We identified four new variants in six of our patients with FA: NM_000135.4(FANCA):c.479T>A(p.Met160Lys) in 3 patients, NM_032444.4(SLX4):c.643C>T(p.Gln215Ter) in 1 patient, NM_000059.4(BRCA2):c.426-1G>C(p.(?) in 1 patient, NM_001376911.1(FANCI):c.2108A>G(p.Asp703Gly) in 1 patient (Table 4). A mutation in BRCA2 was de-

Table 4. Novel variants identified in this study and their ACMG classifications

Novel Variants	ACMG classification
NM_000135.4(FANCA): c.479T>A(p.Met160Lys)	variant of uncertain significance
NM_032444.4(SLX4):c.643C>T(p.Gln215Ter)	likely pathogenic
NM_000059.4(BRCA2):c.426-1G>C(p.(?)	likely pathogenic
NM_001376911.1(FANCI):c.2108A>G(p.Asp703Gly)	variant of uncertain significance

tected in the patient with a diagnosis of AML and a positive DEB test. The patient had undergone surgery for cleft palate and lip and had a family history of leukemia and breast cancer.

Among eight families, 19 (47.5%) patients had family members with FA. Six of these family members had a history of malignancy, including leukemia (n= 3), gastric cancer (n= 2) and oral squamous cell carcinoma (n= 1).

In our study, two (5%) patients had leukemia, and none of the patients had solid tumors. One patient had leukemia at the time of diagnosis, and the other patient experienced MDS transformation to leukemia during the search for an unrelated stem cell donor. Both of these patients had died.

DISCUSSION

Our study is one of the largest studies that reports both clinical and genetic findings of FA from a single center. In the current study, the median age at diagnosis was 9 years (range, 2-229 months). This finding is consistent with previous studies that reported a wide range of ages at diagnosis, with a median age of 10 years.[2]

Consanguineous marriage is a well-known risk factor for FA, and the prevalence of FA is higher in countries where consanguineous marriages are common. In our study, 75% of our patients were born to consanguineous parents. The prevalence of consanguinity in FA patients reported by Ben Haj Ali et al. and Altay et al. was 86.2% and 78% respectively. Thus, our findings are in line with previous studies that have reported a high prevalence of consanguinity among FA patients.[10,11]

In our study, microcephaly (92.5%) was the most common clinical finding, followed by short stature (90%), and thumb abnormalities (65%). Similarly, Kesici et al. reported these findings at a rate of 92.6%, 75.4%, and 53.1%, respectively. In our study, 11 (27.5%) of our patients had kidney abnormalities. This is consistent with the prevalence reported by Kesici et al. (30.9%).[12] In the literature, the reported incidence of congenital kidney abnormalities in patients with FA is around 30%.[13] Interestingly, cardiac abnormalities were more common among our patients (20%) compared to an incidence of 6% reported by previous studies in patients with FA.[4] This can be explained by the fact that patients with cardiac anomalies are referred to our center due to the large number of successful interventional procedures performed by the pediatric cardiologists at our center, and FA is diagnosed while investigating an underlying condition in these patients.

In our study, macrocytosis and thrombocytopenia were the most common laboratory findings at initial diagnosis, which is consistent with previous reports.[4,14]

Genetic testing is also an important tool for the diagnosis of FA, and it can identify the specific gene mutations that cause FA. Most patients with FA (approximately 60%-70%) have biallelic pathogenic variants in the FANCA gene, followed by FANCC (14%) and FANCG (10%). In our study, genetic testing was performed in 95% of the patients, and four new variants were identified in six patients. Two of these variants, NM_000135.4 (FANCA): c.479T>A (p.Met160Lys) and NM_001376911.1 (FANCI):c.2108A>G (p.Asp703Gly), have been classified as a "variant of uncertain significance (VUS)" according to the ACMG guidelines. It was

noted from the gnomAD data that the FANCA: c.479T>A variant is absent in the population. In silico tools showed conflicting data for this variant. On phenotype assessment, no additional data were found in ClinVar or the literature regarding this variant. Given the fact that this variant was homozygous in our patient, and carrier status was identified in the parents through segregation analysis, as well as its absence in the general population, it was considered that this variant might be associated with the disease. Similar findings applied to the FANCI: c.2108A>G variant as well, where gnomAD data showed that it is not observed in the general population, and conflicting in silico assessments were noted. Again, no data were found in ClinVar or the literature regarding this variant. The only difference from the previous variant was its location in a region with high evolutionary conservation (PhyloP100way). Our patient was homozygous for the variant and the parents were heterozygous. When evaluated together with the clinical findings of the patient, it was considered that this variant might also be clinically relevant.

Two other variants, NM_032444.4 (SLX4): c.643C>T (p.Gln215Ter) and NM_000059.4 (BRCA2):c.426-1G>C p.(?), were classified as "likely pathogenic" according to the ACMG guidelines. The SLX: c.643C>T variant was found to be absent in the population based on the gnomAD data. In silico assessments mostly indicated that the variant is "damaging". It was noted that this variant is located in a relatively conserved region across species. ClinVar and literature data were not available for this variant either. Segregation analysis showed that the patient was homozygous for the variant, and the parents were carriers. The fact that it is a nonsense variant also suggested that it is a loss-of-function mutation. Taken together with the patient's clinical picture, we considered that this variant could be associated with the phenotype. Likewise, the BRCA2: c.426-1G>C variant was not observed in the population according to the gnomAD data. In silico tools mostly showed that the variant is "damaging". As with the aforementioned variants, ClinVar and literature data were not available for this variant. The patient was homozygous for the variant, and the parents were heterozygous, as shown by segregation analysis. This variant was also located in a region relatively conserved across species. Its location in the splicing region supported the consideration that it is a loss-of-function mutation. This variant, together with the patient's phenotypic characteristics, was considered to cause the clinical picture. In light of the aforementioned data as well as the fact that these variants were not previously reported in the literature, all four variants were considered as novel mutations associated with phenotypic changes.

Understanding the impact of these mutations is important for improving the diagnosis and treatment of patients with FA. By identifying specific mutations, healthcare professionals can better predict the course of the disease and tailor treatment plans accordingly. Distinguishing heterozygous carriers from non-carriers has genetic implications because the former may be at an increased risk of cancer. Knowledge of the pathogenic variants that run in a family is essential to identify carriers or other affected members.[15]

Cancer is a major complication of FA, and patients with FA are at a high risk of developing various types of cancer, particularly acute myeloid leukemia (AML), head and neck squamous cell carcinoma (HNSCC), and gynecologic cancers. Patients with FA have a 500-fold increased risk of AML than the general population. In these patiens, the cumulative incidence of AML is 13% by the age of 50 years, and the incidence of solid tumors is higher than that of general population. Solid tumors, particularly HNSCC, most commonly develop in patients with FA, occurring with a 500-700-fold higher incidence compared to the general population.[4] In the current study, none of our patients had solid tumors but 2 (5%) of the patients had leukemia. We think that this low malignancy rate can be attributed to the young age of our patients. The frequency of cancer in our patients is expected to increase as they get older during follow-up.

In conclusion, FA is a highly heterogeneous disorder manifesting with a wide range of clinical features. In our study, the most common presenting symptom was cytopenia, and more than half of the patients in our cohort had at least one major malformation, with skeletal anomalies being the most common. FA should be considered in the

differential diagnosis of patients presenting with cytopenias, and congenital malformations, especially if there is a family history of hematological abnormalities or other congenital anomalies. In our study, the most common mutation was associated with FANCA (89.5%) and four new variants were identified, highlighting the ongoing need for comprehensive genetic analyses to fully understand the genetic landscape of FA. Recognition of the pathogenic variants in a family is crucial to identify carriers or other affected members. In our study, surprisingly, none of our patients had solid tumors but two patients had leukemia. Cancer is a major problem among patients, and the risk increases by the age. Early diagnosis of FA is essential for timely management of complications and improvement of patient outcomes. The progression of FA is variable and patients require life-long monitoring. Treatment strategies depend on the stage of the disease and the extent of physical anomalies and complications. Therefore, it is important to raise awareness of FA among healthcare professionals and the general public to ensure early diagnosis and appropriate management of this rare disorder.

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