Characterization and genotype-phenotype correlation of patients with Fanconi anemia in a multi-ethnic population

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ABSTRACT

Fanconi anemia (FA), an inherited bone marrow failure (BMF) syndrome, caused by mutations in DNA repair genes, is characterized by congenital anomalies, aplastic anemia, high risk of malignancies and extreme sensitivity to alkylating agents. We aimed to study the clinical presentation, molecular diagnosis and genotype-phenotype correlation among patients with FA from the Israeli inherited BMF registry. Overall, 111 patients of Arab (57%) and Jewish (43%) descent were followed for a median of 15 years (range: 0.1-49); 68% were offspring of consanguineous parents. One-hundred patients (90%) had at least one congenital anomaly; over 80% of the patients developed bone marrow failure; 53% underwent hematopoietic stem-cell transplantation; 33% of the patients developed cancer; no significant association was found between hematopoietic stem-cell transplant and solid tumor development. Nearly 95% of the patients tested had confirmed mutations in the Fanconi genes FANCA (67%), FANCC (13%), FANCG (14%), FANCI (3%) and FANCD1 (2%), including twenty novel mutations. Patients with FANCA mutations developed cancer at a significantly older age compared to patients with mutations in other Fanconi genes (mean 18.5 and 5.2 years, respectively, \(P=0.001\)); however, the overall survival did not depend on the causative gene. We hereby describe a large national cohort of patients with FA, the vast majority genetically diagnosed. Our results suggest an older age for cancer development in patients with FANCA mutations and no increased incidence of solid tumors following hematopoietic stem-cell transplant. Further studies are needed to guide individual treatment and follow-up programs.

Introduction

Fanconi Anemia (FA), an inherited bone marrow failure (BMF) syndrome, results from defects in the DNA repair pathway, leading to chromosomal instability. The disease is rare with an estimated prevalence of 1:130,0001 but tends to be higher in certain communities due to founder mutations, especially those with a high rate of...
consanguinity. The clinical phenotype includes congenital anomalies, aplastic anemia, a high risk of malignancies, and extreme sensitivity to cross-linking agents. Patients with FA classically present with multiple congenital anomalies and cytopenias, although several patients have no physical defects and normal blood counts, complicating the diagnosis, which is based on the chromosomal breakage test. Genotyping confirms the diagnosis and allows for proper genetic counseling. Some patients exhibit mosaicism, requiring testing by chromosomal breakage and genetic analysis in non-hematopoietic tissue.

FA is usually inherited in an autosomal recessive fashion, with the exception of the X-linked FANCB and the dominantly inherited FANCR. To date, mutations in more than 20 genes have been detected. Detecting genotype-phenotype correlations is important for prognostic predictions, treatment decisions and establishment of directed follow-up programs. However, to date, a clear correlation between the affected gene and the patient’s phenotype has not been found. One exception is the more pronounced cancer predisposition in patients with FANCD1/BRCA2 and FANCN/PALB2 mutations, in which early onset of malignancy is almost invariably present. Some evidence suggests that the type of mutation correlates better with the phenotype than the specific gene. For example, one report found that patients with null mutations in FANCA present with a more severe phenotype than those with mutations leading to altered FANCA protein production. Conversely, in a separate report, no functional or clinical difference was found between patients with absent FANCA protein or those with altered FANCA protein. It is possible that specific mutations, as opposed to affected gene or type of mutation, best correlate with the phenotype. However, even for a specific mutation, there is a variable phenotypic severity among different ethnicities and among siblings, even twins, suggesting a role for genetic or epigenetic modifiers and/or environmental factors.

Here, we present data regarding 111 patients with FA in Israel. This large cohort is unique due to Israel’s ethnic diversity, a high degree of consanguinity and a very high percentage of genetically diagnosed patients.

Methods

FA was defined by an abnormal chromosomal breakage test and/or a genetic diagnosis of biallelic mutations in one of the known FA genes. Patients with FA were registered by their treating hematologist as part of the Israeli inherited bone marrow failure registry (I-IBMFR). The I-IBMFR is approved by each local Institutional Review Board. Data was collected at entry to the registry and annually. Data were extracted by the treating physician or by the research team from the patients’ charts including demographics, clinical characteristics, laboratory data (including chromosomal breakage test results), molecular diagnosis, and data regarding treatment.

BMFM was defined by one of these criteria: a patient who underwent hematopoietic stem cell transplantation (HSCT) for a non-malignant indication, transfusion dependence or at least one cytopenia defined as: absolute neutrophil count (ANC) <1000/μL, platelet count <100,000/μL or hemoglobin <10 g/dL. Severe BMFM was further defined as ANC <500/μL and platelet count <20,000/μL.

A five-item congenital abnormality score (CABS) was calculated for each patient by adding up the total number of phenotypic abnormalities in a set consisting of developmental delay, heart or lung abnormalities, renal abnormalities, hearing loss and head abnormalities. Whenever possible, genetic analysis was performed as part of the routine work-up. Patients were included in this study if they were clinically suspected as having FA and had either a confirmed genetic diagnosis of FA or an abnormal chromosomal breakage test or both.

Genetic analysis was performed by Sanger sequencing, as previously described. Sequencing was performed on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Chromatograms were visualized with CHROMAS (v.2.6.4; www.thechmelnyzium.com.au). Variant pathogenicity was determined based on the American College of Medical Genetics and Genomics (ACMG) criteria. Multiplex ligation dependent probe amplification (MLPA) was performed using the commercially available kit SALSA MLPA probemix P031-B2/P032-B2 FANCA (MRC-Holland) following the manufacturer’s instruction.

Data organization was performed using Microsoft Excel (Windows, Version 16.11.1). The data were analyzed using BMDF software (1993, University of California Press, USA). Pearson’s chi-square test or Fisher’s exact test (two-tailed) was used for analysis of between-group differences in discrete variables, and analysis of variance (ANOVA) was used for continuous variables. Those variables which did not have Gaussian distributions or when the sample size was very small were compared using the non-parametric Mann-Whitney test. The Kaplan-Meier estimate was used to show survival for the cohort and various sub-groups. Calculations and graphic representation of survival curves were performed on Prism 7 (GraphPad Software) and on MedCalc software (Belgium). A P-value of ≤0.05 was considered significant.

Results

Patient demographics

One hundred and eleven patients (53% male) with FA diagnosed between 1980 and 2016 were registered in the I-IBMFR and followed for a median of 15 (range: 0.1-49) years (Table 1). The median survival time was 27.9 years Brookmayer-Crowley 95% Confidence interval [CI]: 24-35 (Figure 1A).

Ethnic Origin

Over half of the patients with FA in our cohort were of Arab descent, while the rest were Jewish, mostly of Sephardic origin (Table 1). 65% of the patients were offspring of consanguineous parents. Consanguinity was reported in 93% of Arab patients and in 21% of Jewish patients. Notably all of the Druze patients were offspring of consanguineous parents.

Table 1. Characteristics of Fanconi anemia patients in Israel.

| N  | Ethnicities |
|----|-------------|
| 46 | Sephardic   |
| 10 | Ashkenazi   |
| 9  | Jewish      |
| 54 | Mixed       |
| 6  | Ethiopian   |
| 2  | Muslim      |
| 62 | Druze       |
| 1  | Arab        |
| 1  | Christian   |
Clinical Features
The median age at the time of diagnosis of FA was 6 years (range: 0-26.5); 20% were born small for the gestational age, and 57% fit criteria for short stature. The majority of patients (90%) had at least one congenital anomaly. More than half had café-au-lait spots (52.3%) followed by renal anomalies (39.6%). Of the 18% of the patients with hearing loss, all had a conductive component (Table 2).

Previous publications have reported an association between the presence or absence of radial ray anomalies and a five-item CABS on disease prognosis.10,12 Five-item CABS were calculated for each patient,10 resulting in 41 patients with CABS 0, an additional 41 patients with CABS 1, 22 patients with CABS 2, five patients with CABS 3 and one patient each with CABS 4 and CABS 5.

There was no correlation between the CAB score and survival (Figure 1B). In our cohort, 82% of the patients developed BMF, of which 18% fit criteria for severe BMF. All patients with higher CABS (3-5) exhibited BMF with the exception of one patient who developed infant AML and was transplanted at the age of five months. No association was found between radial ray anomalies and BMF development.

Malignancy
During the follow-up period of this patient cohort, 30% developed myelodysplastic syndrome (MDS), leukemia and/or solid tumors (Table 3). The mean age for the first event of MDS was 13.3 years (standard deviation [SD] 8.6), for leukemia 10.8 years (SD 6.2) and for solid tumors 26.6 years (SD 4.9). No significant difference was detected

Figure 1. Survival curves for patients with Fanconi anemia (FA) in Israel, calculating the proportion of live patients by age using the Kaplan-Meier methods. (A) Survival for the whole cohort. (B) Survival divided by cab score 0 (blue), 1 (red) or greater than 1 (green). No significant difference was found between the groups.
between the age of the first event of MDS and the age of the first event of leukemia; however, both MDS and leukemia were diagnosed significantly earlier relative to solid tumors \((P=0.001\) and \(P<0.001\), respectively) (Figure 2A). Seven patients developed two cancers, three patients developed three cancers, and two patients developed four cancers.

**Hematopoietic stem cell transplantation**

Approximately half of the patients with FA in the registry (n=59) underwent a HSCT; seven of the patients underwent a second transplant. The median age for the first transplant was 9.3 years (range: 0.45-30.8) (Figure 2B). Indication for HSCT was MDS/leukemia in 15 patients; the rest (n=44) were transplanted due to BMF. 25% were transplanted in the 1990s, 39% in the years 2000-2009, and 36% between 2010-2017. No association was found between a history of HSCT and incidence of solid tumor or age of first cancer (Figure 2C). Solid tumors were reported in six non-transplanted patients and in four transplanted patients, of which two were transplanted due to MDS/leukemia and the other two due to BMF. Among the four transplanted patients, the median time from transplant until solid tumor diagnosis was 10 years (range: 2.5-18). The five patients with at least three events of cancer had no previous HSCT.

**Survival**

Currently 65 of the 111 patients (59%) in the cohort are alive with a median age of 16.5 years (range: 0.8-37). Forty-three patients have died, and three were lost to follow-up. The median age of death was 11 years (range: 0.1-49). Causes of death were mostly cancer related (23 patients; 53%) or transplant-related (11 patients; 25%). 64% of transplanted patients are alive, and 56% of non-transplanted patients are alive and no significant (NS) difference was found in the age of death between transplanted and non-transplanted patients. The 5-year survival of the post-HSCT cohort was 44%, and 10-year post-HSCT survival was 27%. Among the transplanted patients, 78% of those transplanted due to BMF are alive, while only 27% of those transplanted for MDS/leukemia are alive.

**Genotype**

Genetic analysis was performed on 94 patients (85% of the cohort), of which 88 patients (94% tested) reached a genetic diagnosis. The majority were found to have mutations in the \(FANCA\) gene, followed by mutations in \(FANCG\), \(FANCC\), \(FANCJ\), and \(FANCD1\) (Figure 3). The six undiagnosed patients had an incomplete genetic analysis performed. Of these, two siblings were found to have a heterozygous mutation in \(FANCC\) (Table 4).

Table 2. Congenital anomalies of patients with Fanconi anemia in Israel.

| Anomaly                        | N=111 |
|--------------------------------|-------|
| Any anomaly                    | 100 (90.1%) |
| Café-au-lait spots             | 58 (52.3%) |
| Renal structure                | 44 (39.6%) |
| CNS structure                  | 21 (18.9%) |
| Hearing loss                   | 20 (18%) |
| Congenital heart disease       | 18 (16.2%) |
| Male genitourinary             | 18 (16.2%) |
| Radial ray                     | 18 (16.2%) |
| Gastrointestinal structure     | 9 (8.1%) |
| Spine                          | 6 (5.4%) |
| Cleft lip/palate               | 2 (1.8%) |

Anomalies as reported in patient charts. CNS: central nervous system.
34 different mutations were found, including 21 in FANCA and three each in FANCC, FANCD1, FANCG and FANCJ (Table 4); 76 patients were homozygous for mutations in Fanconi genes, and 12 patients were compound heterozygous; 33 different combinations of mutations were found (Table 4); 20 novel mutations were detected in the cohort, as detailed in the Online Supplementary Table S1; 7 of these were previously reported by our group.13-15 The type of mutation varied, as detailed in Table 4. 26 patients had frameshift mutations, 19 patients had splice site mutations, 15 patients had deletions, 13 patients had nonsense mutations, nine patients had missense muta-

| Cancer type          | Total | FANCA | FANCC | FANCD1 | FANCG | FANCJ | Undiagnosed |
|----------------------|-------|-------|-------|--------|-------|-------|-------------|
| MDS                  | 19    | 8     | 3     | 0      | 1     | 1     | 1           | 6           |
| Leukemia             |       |       |       |        |       |       |             |
| AML                  | 14    | 3     | 1     | 1      | 2     | 1     | 6           |
| ALL                  | 1     | 1     | 0     | 0      | 0     | 0     | 0           |
| Head and Neck        | 6     | 3     | 0     | 0      | 0     | 0     | 3           |
| GU                   | 3     | 3     | 0     | 0      | 0     | 0     | 0           |
| GI                   | 3     | 1     | 0     | 0      | 0     | 0     | 0           |
| Skin                 | 3     | 2     | 0     | 0      | 0     | 0     | 1           |
| Breast               | 1     | 0     | 0     | 0      | 0     | 0     | 1           |
| MB                   | 1     | 0     | 0     | 1      | 0     | 0     | 0           |

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; GI: gastrointestinal; GU: genitourinary; MB: medulloblastoma; MDS: myelodysplastic syndrome.

Figure 3. The distribution of the Fanconi anemia (FA) genes in Israel: (A) including siblings and (B) excluding siblings.
Table 4. Genetics of Fanconi anemia in Israel.

| #  | Gene  | Mutation 1                           | Mutation 2                           | Effect 1       | Effect 2       | Ethnicities   |
|----|-------|--------------------------------------|--------------------------------------|----------------|----------------|---------------|
| 11 | FANCA | c.2172-2173 insG (p.575Vfs*69) (13)   | c.2172-2173 insG (p.575Vfs*69) (13)   | frame          | shift          | Sephardic     |
| 8  | FANCA | c.4261-2A>C (IVS4-2a>c) (14)         | c.4261-2A>C (IVS4-2a>c) (14)          | splice site    | splice site    | Arab Muslim   |
| 3  | FANCA | ex6-31 del (14)                      | ex6-31 del(14)                       | large deletion | large deletion | Arab Muslim   |
| 5  | FANCA | ex21 del(26, 27)                     | ex21 del(26, 27)                     | large deletion | large deletion | Arab Muslim   |
| 3  | FANCA | c.4069-4070insT*                      | c.4069-4070insT*                     | frame          | frame          | Arab Muslim   |
| 2  | FANCA | ex31-37del(15)                       | 4275delT (p.Asp142Thr fsX6) (13)     | large deletion | deletion       | Sephardic     |
| 1  | FANCA | p.Pro1164Ser^                        | p.Pro1164Ser^                        | missense       | missense       | Arab Muslim   |
| 1  | FANCA | c.2172-2173 insG (p.575Vfs*69) (13)  | deletion exons                       | frame          | shift          | mixed         |
| 1  | FANCA | c.4275delT (p.Asp142Thr fsX6) (13)   | c.2172-2173 insG (p.575Vfs*69) (13)  | frame          | shift          | Sephardic     |
| 1  | FANCA | c.4275delT (p.Asp142Thr fsX6) (13)   | 4275delT (p.Asp142Thr fsX6) (13)     | frame          | shift          | Sephardic     |
| 1  | FANCA | c.65G>A (p.Trp22Ter) (25)            | c.65G>A (p.Trp22Ter) (25)            | missense       | missense       | Jewish        |
| 1  | FANCA | c.65G>A (p.Trp22Ter) (25)            | ex1-24del^                           | missense       | large deletion | mixed         |
| 1  | FANCA | c.891-893 delIGCTG (13)              | c.2172-2173 insG (p.575Vfs*69) (13)  | frame          | shift          | Sephardic     |
| 1  | FANCA | c.2172-2173 insG (p.575Vfs*69) (13)  | ex12,4,5del^                         | frame          | large deletion | Sephardic     |
| 1  | FANCA | c.891-893 delIGCTG (13)              | c.65G>A (p.Trp22Ter) (25)            | frame          | shift          | mixed         |
| 1  | FANCA | ex15-21 del(28)                      | ex15-21 del(28)                      | large deletion | large deletion | Arab          |
| 1  | FANCA | ex1-6 del (29)                       | ex1-6 del (29)                       | large deletion | large deletion | Arab          |
| 1  | FANCA | c.189+1G>A (IVS2+1g>a) ^             | c.2778+2T>C (IVS28+2 T>C) ^         | splice site    | splice site    | Arab Muslim   |
| 1  | FANCA | p.Arg880Ter (30)                     | p.Arg880Ter (30)                     | nonsense       | nonsense       | Ashkenazi     |
| 1  | FANCA | c.3520-3522 delTG (25)               | c.1471-401_1626+395del ^             | deletion       | mixed         |
| 7  | FANCC | c.456+4a>t (IVS4+4 a т) (5)          | c.456+4a>t (IVS4+4 a т) (5)          | splice site    | splice site    | Ashkenazi     |
| 2  | FANCC | p. Gln3Ter ^                         | p. Gln3Ter ^                         | nonsense       | nonsense       | Arab Muslim   |
| 1  | FANCC | c.456+4a>t (IVS4+4 a т) (5)          | del 97116249-97124749 (31)           | deletion       | mixed         |
| 1  | FANCD1 | c.6174delT(24)                      | c.6174delT(24)                       | frame          | shift          | mixed         |
| 1  | FANCD1 | c.7579delG (p.V2527X)^               | c.9693delA (p.S3321fs16)^            | nonsense       | frame shift    | Ethiopian     |
| 6  | FANCG | c.1742C>G (p.Ser581Ter)^             | c.1742C>G (p.Ser581Ter)^             | nonsense       | frame shift    | Arab Muslim   |
| 4  | FANCG | c.212T>C (p.Leu71Pro) (32)           | c.212T>C (p.Leu71Pro) (32)           | nonsense       | nonsense       | Arab Muslim   |
| 3  | FANCG | c.510+3A>G (IVS4+3 A=G) ^           | c.510+3A>G (IVS4+3 A=G) ^            | splice site    | splice site    | Arab Muslim   |
| 1  | FANCI | p.Arg515Gly (33)                     | p.Arg515Gly (33)                     | nonsense       | nonsense       | Arab Muslim   |
| 1  | FANCI | p.Glu376Ter^                         | p.Glu376Ter^                         | nonsense       | nonsense       | Arab Muslim   |
| 2  | FANCC | c.456+4a>t (IVS4+4 a т) (5)          | Unknown                               |splice site    | mixed         |
| 4  | @     | Unknown                               | Unknown                               |Unknown         | Unknown        | mixed         |

* number of patients; ^ mutations not previously described; @ not included in the analyses.
tions and six patients had a combination of mutation types.

In our cohort, the most common mutations in \textit{FANCA} were c.2172-2173 insG (p.S725Vfs*69), most frequent in the Sephardic Jewish population, c.4261-2A>C (IVS43-2a>c) in Arab Muslims and Christians and c.3788-3790delTCT, detected in both Arab Druze and Sephardic Jews. The most common mutation in \textit{FANCC} was c.456+4a>t (IVS4+4 a>t) in the Ashkenazi Jewish population. The mutation most commonly found in \textit{FANCG} was the novel mutation c.1742C<G (p.Ser581Ter) in the Arab Muslim population.

**Genotype-phenotype correlations**

Genotype-phenotype correlations were first analyzed by the specific affected gene. In addition, patients were grouped by function of altered genes into those encoding proteins of the core complex (\textit{FANCA}, \textit{FANCC}, \textit{FANCG}) versus those downstream (\textit{FANCD1}, \textit{FANCJ}). Finally, analysis was done according to the type of mutation (deletion, frame shift, missense, nonsense, splice site).

No association was found between the affected gene and survival (Figure 4). Survival was not significantly different between patients with core complex mutations and those with mutations in downstream genes. Neither the specific FA gene nor function were associated with the development of BMF. Of note, neither of the patients with \textit{FANCD1} mutations developed BMF. One underwent HSCT for AML before the age of six months, while the other had no complications by the age of 17 years.

Looking at congenital anomalies, no association was found between the CAB score and the affected FA gene. Rib abnormalities were observed only in patients with \textit{FANCC} mutations. Cleft lip was more common in patients with \textit{FANCC} mutations, compared with other FA genes ($P<0.001$). Patients with mutations in the downstream genes \textit{FANCD1} and \textit{FANCJ} were significantly shorter compared with the others ($P=0.003$). Patients with downstream mutations were found to have significantly more skull anomalies ($P<0.001$), central nervous system (CNS) abnormalities ($P=0.005$) and genitourinary anomalies ($P=0.03$), compared with patients with core complex mutations.

All the solid tumors in our cohort were reported in patients with \textit{FANCA} mutations or in undiagnosed patients, except for one case of medulloblastoma in a patient with \textit{FANCD1} mutation (Table 3). Due to the relatively small numbers of reported cancers in patients with non-\textit{FANCA} mutations (Table 3), we compared the age of the first cancer (including MDS) between patients with...
FANCA mutations and patients with non-FANCA mutations. The mean age of the first cancer was 18.5 years (SD 6.3 years) for patients with FANCA, relative to 5.2 years (SD 3.7 years) for patients with FANCC, FANCD1, FANCG and FANCJ mutations, with a statistically significant difference (P=0.001). This difference remains statistically significant upon exclusion of solid tumors; patients with FANCA mutations developed MDS/leukemia at a significantly older age as compared to patients with FANCA mutations (P=0.002). All patients with mutations in FANCA developed cancer after the age of 10 years, while all other genetically diagnosed patients developed their first cancer by the age of 10 years. There was a trend towards more MDS in patients with FANCC mutations and less MDS and cancer in patients with FANCG mutations, compared with patients with mutations in other genes (NS).

No association was found between the mutation type and survival. In addition, no association was found between the mutation type and the development of MDS, leukemia or solid tumors, although patients with nonsense and splice site mutations developed the first cancer at a significantly lower age than patients with deletions (P=0.011 and P=0.012, respectively). No association was found between the CAB score and mutation type. However, some significant correlations were found between the mutation type and specific congenital anomalies. Patients with deletions were shorter than patients with nonsense mutations (P=0.018). Patients with splice site mutations had significantly more CNS anomalies and developmental delay, compared with the other patients (P=0.03 and P=0.038, respectively). Patients with missense mutations had significantly less congenital heart disease (P=0.022).

Discussion

We hereby present a large cohort of 111 patients with FA in Israel. In a previous report of Israeli patients with BMF syndromes, 66 of these patients were included. Our cohort is unique in a few aspects. First, the vast majority of the patients included in this cohort are genetically diagnosed. Second, the ethnic diversity in this population is distinct with a larger representation of patients from Arab descent compared to those of Jewish descent; this is in contrast to the general population of Israel comprised of 74% Jews and 21% Arabs. In addition, the patient population exhibited a high degree of consanguinity, especially in the Arab population, most likely the cause of their skewed representation in this cohort.

The large majority of our patients (90%) had at least one congenital malformation. In an Italian registry, including 97 patients, only 76% had at least one somatic malformation, although abnormal facial features were not included. We calculated a CAB score for all the patients in our cohort as described in a previous publication. In the German cohort, including 181 patients, this score predicted BMF. In agreement, in our cohort, all patients with higher CAB scores (CABS 3-5) developed BMF. In addition, the two patients in our cohort with the highest CAB scores (CABS 4-5) did not develop cancer. This low number of patients does not allow statistical analysis; however, it is consistent with previous publications finding an inverse correlation between congenital anomalies and malignancy in patients with FA. Of the patients in this FA cohort, 82% developed BMF. This is similar to the 80% described by the International Fanconi Anemia Registry. In contrast, in the German cohort, only 36% developed BMF. Neither of the patients with FANCD1 mutations in our cohort developed BMF, in agreement with previous publications. However, it should be noted that one of the patients with a FANCD1 mutation was transplanted at a very young age for the treatment of leukemia, essentially eliminating the risk of BMF development.

Nearly one third of this cohort of patients with FA in Israel developed MDS, leukemia and/or solid tumors. Twelve of the 111 patients had more than one cancer event. The median age at initial diagnosis of cancer was 16 years in our cohort. Hematological malignancies appeared at a significantly earlier age relative to solid tumors. Of note, one patient with a FANCD1 mutation developed medulloblastoma at the age of 3 years. Patients with FANCD1 mutations have been previously described as uniquely developing solid tumors early in life, requiring screening for childhood cancer from a very young age. Excluding this particular patient with FANCD1 mutation, initial diagnosis of solid tumors in this cohort ranged from 21-32 years of age. These data support the need to start early cancer surveillance for patients with FA.

Approximately half of the patients in this cohort underwent HSCT, similar to the Italian FA registry report. There was no difference in survival between patients who did or did not undergo a HSCT. In the International Fanconi Anemia Registry, HSCT was found to be a predictor of poor prognosis. The patients from our cohort were transplanted over a three-decade time frame. Therefore, differences in donor selection and conditioning treatment plus patient selection bias may explain the discrepancy. The indication for HSCT had a large impact on survival in our cohort, with patients transplanted due to BMF having a much better survival relative to those transplanted due to MDS/leukemia. These results, if confirmed in future studies, may influence the decision on choosing the right timing for HSCT.

In this cohort, HSCT did not appear to hasten the onset of solid tumors. Similar findings were reported in the International Fanconi Anemia Registry as well as in the Italian Fanconi Anemia Registry, including 754 and 180 patients, respectively. In contrast, the German registry reported a hazard ratio of 3.8 for developing solid tumors in patients with FA post-transplant, compared to those not transplanted. The National Cancer Institute also detected an increased incidence of cancer in FA patients and a younger age at cancer detection post-transplant in their cohorts of patients with FA. Indeed, reconciling this discrepancy holds paramount importance in clinical decision-making regarding optimal timing for initiation of cancer surveillance. More up-to-date studies will be needed to identify any association exists.

We aimed to perform a genetic diagnosis for all Israeli patients with FA for whom DNA was available. By conventional Sanger sequencing and MLPA, we arrived at a genetic diagnosis in almost 95% of those tested. Of the six patients for whom genetic diagnosis was not achieved, only a partial work-up was performed due to the lack of remaining DNA samples. Two of these were found to be heterozygous for a FANCC mutation. In our cohort, 34 different mutations were found, with 20 of
them either not previously published or reported only by our group (Table 4).

In this Israeli cohort of FA patients, two-thirds of genetically diagnosed patients had biallelic FANCA mutations. These numbers are similar to the International Fanconi Anemia Registry, in which 60% of the diagnosed patients had FANCA mutations,17 and the European cohort, in which 70% had FANCA mutations2 but in contrast to the Italian cohort, in which 90% of the patients diagnosed were found to have FANCA mutations.18 The other FA genes were represented in our cohort similar to previous publications.2,25 We did not find a significant correlation between survival and the affected gene. This is in contrast to the International Fanconi Anemia Registry, in which patients with FANCC mutations had a poorer survival.17

Some mutations were exclusively or more commonly found in specific ethnic populations in our cohort. For example, in the Ashkenazi Jewish population, the most common mutation was c. 456+4a>t (IVS4+4 a>t) in FANCC, as previously described.24 In contrast, in the Sephardic Jewish population, the most common mutations were c.1172-2173 insG (p.S725Vfs*69) in FANCA. Both these mutations were exclusive to Ashkenazi Jewish and Sephardic Jewish patients with FA, respectively. Patients with FANCG and FANCJ mutations were all from Arab Muslim descent.

A number of correlations were found between the genotype and development of cancer in our cohort. Patients with FANCA mutations developed cancer at a significantly older age, compared with patients with non-FANCA mutations. In addition, there was a trend towards a higher prevalence of MDS in patients with FANCC mutations and less MDS and cancer in patients with FANCG mutations in our cohort. In contrast, in a larger cohort recently published from the National Cancer Institute,20 there was no clear association between the genotype and malignancy. Patients with FANCG mutations were even reported previously to have a higher incidence of leukemia.3 These data may reflect the unique population in our cohort, as most of the patients with FANCG mutations were of Arab Muslim origin, and all 13 patients were homozygous for 1 of 3 mutations: c.1742C>G (p.Ser581Ter), c.212T>C (p.Leu71Pro) and c.510+3A>G (IVS4+3 A>G). Further studies will be needed to elucidate the specific characteristics of these mutations.

Regarding the type of mutation in our cohort, no association was found between the mutation type and survival, the risk of development of cancer or the CAB score. However, we found a few correlations between specific congenital anomalies and the type of mutation. A few previous publications looked at correlations between specific mutation types of FANCA and phenotype. One study reported a higher incidence of leukemia in patients with null mutations of FANCA, compared to those with other types of mutations.2 In contrast, in the Spanish cohort, no association was found between the type of FANCA mutations and hematologic disease or somatic malformations.8 The discrepancies between these studies may reflect specific population characteristics, making it difficult to rely on the Fanconi group or the type of mutation in defining the risk for disease complications.

This cohort includes patients treated in various medical centers in Israel. The biggest limitation of this report is that not all patients were seen by the same medical team. We overcame this by using a standardized and elaborate medical form for each patient included in the I-IBMFR, followed by an annual update. In addition, genetic analysis was uniformly performed in our centralized hematology molecular laboratory.

This study includes a relatively large cohort of patients with FA in a nation with a unique ethnic diversity and a high degree of consanguinity. Our high success rate of genetic diagnosis has enabled the detection of several novel mutations and unreported genotype-phenotype correlations. We found that patients with FANCA mutations developed cancer at a later age; however the causative gene was not found to affect the overall survival of patients. In our cohort, HSCT did not increase the risk of solid tumor development. Continuation of this registry and establishment of similar registries worldwide are paramount for further advancement of our understanding of this rare disease.

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