Genotype-phenotype and outcome associations in patients with Fanconi anemia: the National Cancer Institute cohort

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Abstract

Fanconi anemia (FA) is caused by pathogenic variants in the FA/BRCA DNA repair pathway genes, and is characterized by congenital abnormalities, bone marrow failure and an increased risk of cancer. We conducted a genotype-phenotype and outcomes study of 203 patients with FA in our cohort. We compared across the genes, FA/BRCA DNA repair pathways (upstream, ID complex and downstream), and type of pathogenic variants (hypomorphic or null). We explored differences between the patients evaluated in our clinic (clinic cohort) and those who provided data remotely (field cohort). Patients with variants in the upstream complex pathway had less severe phenotype, lacking VACTERL-H (**V**ertebral, **A**nal, **C**ardiac, **T**racheo-esophageal fistula, **E**sophageal/duodenal atresia, **R**enal, **L**imb, **H**ydrocephalus) association and/or PHENOS (**P**igmentation, small **H**ead, small **E**yes, **N**eurologic, **O**tologic, **S**hort stature) features. ID complex was associated with VACTERL-H. The clinic cohort had more PHENOS features than the field cohort. PHENOS was associated with increased risk of bone marrow failure, and VACTERL-H with hypothyroidism. The cumulative incidences of severe bone marrow failure, solid tumors and leukemia as the first event were 70%, 20% and 6.5%, respectively. Head and neck and gynecological cancers were the most common solid tumors, with a further increased risk after hematopoietic cell transplantation. Among patients with *FANCA*, variants in exons 27-30 were associated with a higher frequency of solid tumors. Overall the median survival was 37 years; patients with leukemia or *FANCD1/BRCA2* variants had the poorest survival. Patients with variants in the upstream complex had better survival than those with variants in ID or the downstream complex (*P*=0.001 and *P*=0.016, respectively). FA is phenotypically and genotypically heterogeneous; detailed characterization provides new insights towards understanding this complex syndrome and guiding its clinical management.

Introduction

Fanconi anemia (FA), a predominantly autosomal recessive genomic instability disorder, is the most common inherited bone marrow failure (BMF) syndrome. FA is characterized by hypersensitivity to DNA cross-linking agents, specific congenital abnormalities, progressive BMF and predisposition to cancer, particularly head and neck squamous cell carcinomas and acute myeloid leukemia (AML).[1,2] Pathogenic variants in at least 22 genes have been identified in the FA/BRCA DNA repair pathway which functions to remove DNA interstrand crosslinks. The involved genes are grouped according to their function in the pathway as upstream (*FANC-A*, *B*, *C*, *E*, *F*, *G*, *L*, *M* and *T*), ID (*FANC-I* and *D2*) and downstream complexes (*FANC-D1*, *J*, *N, O, P, Q, R, S, U, V* and *W*).[3-5]

Guido Fanconi reported the first cases of FA in 1927 in three brothers with microcephaly, short stature, skin hyperpigmentation, microorchidism and macrocytic anemia.[6] Many other abnormalities affecting multiple organ systems have since been defined. Approximately 40% of FA cases have no physical abnormalities.[7] Congenital anomalies commonly seen in FA are included in the VACTERL-H association (**V**ertebral abnormalities, **A**nal atresia, **C**ardiac abnormalities, **T**racheo-esophageal fistula, **E**sophageal or duodenal atresia, **R**enal abnormalities, upper **L**imb abnormalities and **H**ydrocephalus).[8] We recently grouped six other common FA features into the acronym PHENOS

(skin **P**igmentation abnormalities, small **H**ead, small **E**yes, structural central **N**ervous system abnormalities, **O**tologic abnormalities and **S**hort stature), and found that patients with three or more of the eight VACTERL-H features frequently had four or more PHENOS features.[9] BMF in FA usually develops during the first decade of life and varies from mild to severe cytopenias requiring hematopoietic cell transplantation (HCT), or progression to myelodysplastic syndrome (MDS) or AML.[10] Endocrine, metabolic and reproductive abnormalities have been reported in approximately 80% of patients[11] and may be due to the syndrome and/or the treatment.

Cohort studies investigating genotype-phenotype associations in FA have mostly been limited by patient numbers, and the findings across studies were not always consistent due to the rarity of the syndrome and population differences.[12-16] Specific early cancers were reported in patients with pathogenic variants in *FANCD1/BRCA2* and *FANCN/PALB2*.[17-19]

Our group previously reviewed the genotype-phenotype associations in FA from literature cases.[7] We now explore genotype-phenotype and outcome associations in patients with FA enrolled in the National Cancer Institute (NCI) inherited BMF syndrome cohort according to the FA genes, mutation pathways and the type of pathogenic variants. We also investigate differences between the patients followed in the field *versus* those seen in our clinic. We hypothesized that there would be differences in phenotypes, hematologic, oncological, and endocrine outcomes between patients enrolled in the field cohort and those seen in our clinic.

Methods

Patients with FA were enrolled in the NCI Institutional Review Board-approved inherited BMF syndrome cohort study (clinicaltrials.gov identifier: NCT00027274) from January 2002 through November 2020. Participants and/or their proxies signed written consent and medical record release forms. All individuals were initially enrolled in the "Field Cohort" (FC) and completed individual information questionnaires. A subset of patients was then evaluated at the National Institutes of Health Clinical Center and formed the "Clinic Cohort" (CC) (Figure 1A).[2] Data on all participants (FC and CC) were abstracted from the individual information questionnaires, biennial follow-up forms, medical records, and National Institutes of Health evaluations (for CC participants).

FA was diagnosed by an abnormal chromosomal breakage test and confirmed by genetic testing when possible. Physical abnormalities were grouped as VACTERL-H, PHENOS and Other. The VACTERL-H association was defined by the presence of three or more of the eight features, and PHENOS as four or more of the six features. Other physical findings are described in *Online Supplementary Table S1*. Phenotypes not stated as present were considered absent.

BMF was defined by the presence of cytopenia for age[20] and categorized as non-severe or severe (Table 1). Diagnoses of MDS, AML and solid tumors were based on the review of medical records, personal or proxy reports. Endocrine, metabolic, and gonadal problems including hormone deficiencies, low bone mineral density, diabetes

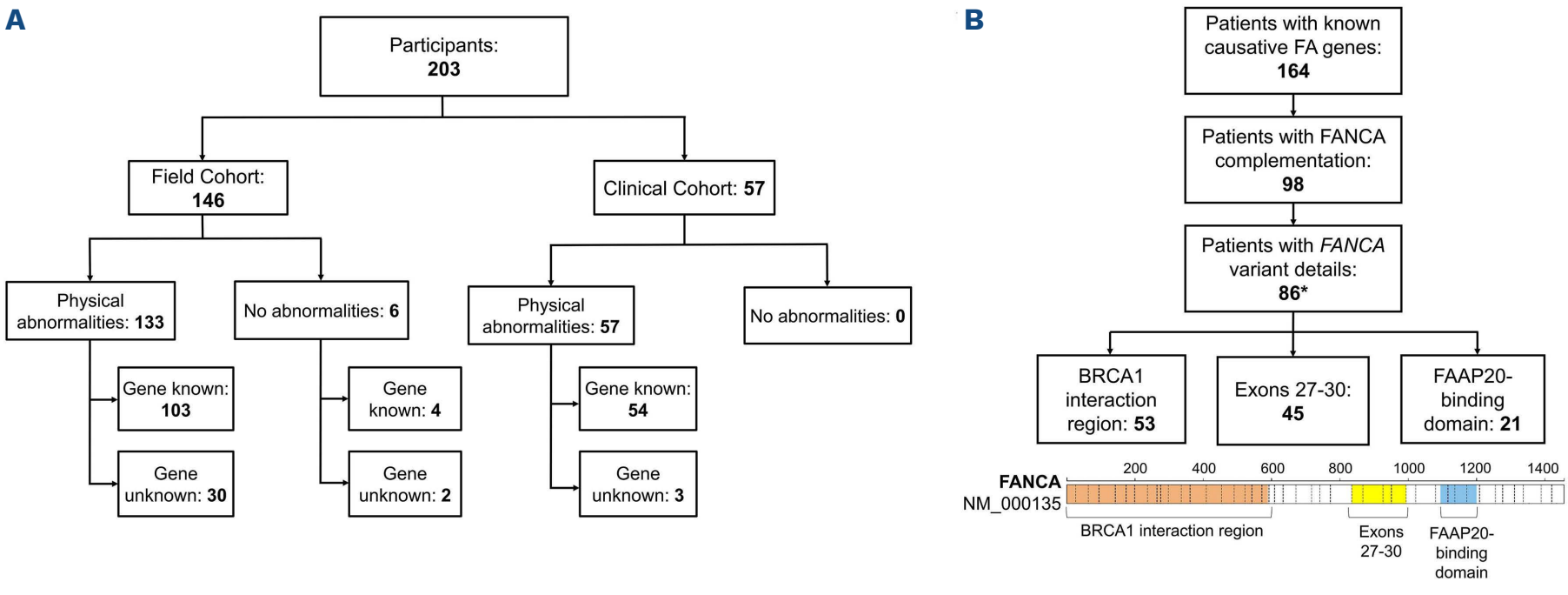


Figure 1. Study flow chart. (A) The distribution of patients with Fanconi anemia (FA) in the field cohort and clinic cohort. Phenotype information was not available for seven patients in the field cohort. (B) The distribution of patients with *FANCA* variants according to the location of the variants on the FANCA protein. *The sum of the numbers with BRCA1 interaction region, exons 27-30 and FAAP-20 binding domain variants exceeds 86 because some patients had variants in multiple regions of the FANCA protein.

mellitus, insulin resistance, dyslipidemia, abnormal body mass index, and infertility, were recorded.

The genetic variants were annotated using ANNOVAR,[21] BayesDel[22] and Human Splicing Finder.[23] Variants were classified according to the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines[24] with the modifications specified in the legend of *Online Supplementary Table S2*.

Frameshift, nonsense, start loss or deletion of four or more nucleotides and splice site variants that were predicted to cause mis-splicing were classified as null. Missense, in-frame insertion/deletion and splice site variants causing a protein change were classified as hypomorphic. Patients with bi-allelic null variants were grouped as having a "null" genotype, and those with one or both hypomorphic variants as having a "hypomorphic" genotype.

Since *FANCA* was the most frequently affected gene, we investigated phenotype and outcome associations in pa-

Table 1. Patients' demographics.

	Field cohort N (%)	Clinic cohort N (%)	*P*-value	Total N (%)
Number of patients	146	57		203
Sex (male : female)	66:80	22:35	0.4	88:115
Age at diagnosis, median (range)	5.4 (0-59.7)	5.3 (0-55.8)	0.5	5.4 (0-59.7)
Age at enrollment, median (range)	11.1 (0-59.8)	14.7 (0.4-55.4)	0.1	11.2 (0-59.8)
Age at last follow-up, median (range)	***15.7 (0.2-63.1)***	***25.2 (3.3-67.4)***	***<0.001***	***17.1 (0.2-67.4)***
Race, N (%)				
White	***99 (67.8)***	***51 (89.4)***	***0.001***	***150 (73.9)***
Asian	8 (5.5)	1 (1.8)	0.45	9 (4.4)
Black or African/American	4 (2.7)	1 (1.8)	1	5 (2.5)
American Indian/Alaska Native	1 (0.7)	0	1	1 (0.5)
Mixed	4 (2.7)	4 (7)	0.2	8 (3.9)
Unknown	***30 (20.6)***	***0***	***<0.001***	***30 (14.8)***
Hematology				
BMF (N=198)	122 (86.5)	48 (84.2)	0.7	170 (85.9)
Non-severe BMF	34 (27.9)	12 (25)	0.8	46 (27.1)
Severe BMF	88 (72.1)	36 (75)		124 (72.9)
Abnormal cytogenetics (N=177)	***22 (18.3)***	***23 (40.4)***	***0.003***	***45 (25.4)***
MDS (N=190)	17 (12.8)	10 (17.5)	0.4	27 (14.2)
HCT (N=202)	71 (49)	32 (56.1)	0.4	103 (51)
Median age at HCT (N=102)	8.6 (3.9-46.6)	10.2 (1.6-44.1)	0.1	9.1 (1.6-46.6)
Somatic mosaicism (N=192)	***1 (0.74)***	***4 (7.02)***	***0.03***	***5 (2.6)***
Endocrine abnormality				
Hypothyroidism (N=168)	33 (29.7)	24 (42.1)	0.1	57 (33.9)
GH deficiency (N=149)	18 (18)	14 (28.6)	0.2	32 (21.5)
Osteoporosis (N=132)	***8 (8.8)***	***9 (22)***	***0.049***	***17 (12.9)***
Metabolic abnormality				
Diabetes mellitus (N=158)	17 (16.8)	6 (10.5)	0.4	23 (14.6)
Dyslipidemia (N=80)	9 (34.6)	21 (38.9)	0.8	30 (37.5)
Abnormal BMI (N=163)	43 (40.2)	20 (35.7)	0.6	63 (38.7)
Reproductive abnormality				
Male reproductive (N=33)	13 (68.4)	5 (35.7)	0.09	18 (54.6)
Female reproductive (N=52)	18 (29.2)	20 (76.9)	0.8	38 (73.1)
Pregnancies (N=56)	10 (32.26)	4 (16)	0.2	14 (25)

Ages are reported in years with median and range. Denominators were adjusted based on data availability. Bold and italicized values: significant differences. Bone marrow failure (BMF) was defined as hemoglobin less than standard for age, absolute neutrophil count <1,500 cells/mm^3 and/or platelet count <150x10^9/L. Severe BMF was defined as hemoglobin level <8 g/dL, absolute neutrophil count <500 cells/mm^3 and platelet count <30x10^9/L or BMF requiring chronic red blood cell or platelet transfusions (≥10 transfusions), androgen use or hematopoietic cell transplant. Somatic mosaicism was considered when the chromosomal breakage test was normal in peripheral blood lymphocytes but increased in skin fibroblasts. Thyroid-stimulating hormone levels >4 mU/L were considered to indicate thyroid function abnormality. A diagnosis of growth hormone deficiency was included when an abnormal growth hormone stimulation test result was available. Osteoporosis was defined as an age-matched Z-score of less than -2 when a dual-energy X-ray absorptiometry (DEXA) report was available. The diagnosis of diabetes mellitus was based on medical records, and National Institutes of Health evaluations. Standard diagnostic criteria were used for defining insulin resistance, dyslipidemia and abnormal body mass index, as described earlier.[38] N=number of patients with available data, n=number of patients with the defined feature/complication; HCT: hematopoietic cell transplant; MDS: myelodysplastic syndrome; GH: growth hormone; BMI: body mass index.

tients with *FANCA* variants, focusing on the location of variants on the FANCA protein (Figure 1B). *FANCA* variants were plotted using ProteinPaint.[25] Patients with one or both variants in (i) the BRCA1 interaction region, (ii) the FAAP20-binding domain, and (iii) exons 27-30 (where we observed a clustering of variants), were compared with patients without variants in these regions.

Analyses were performed using Stata 16 (StataCorp. TX, USA) and RStudio (RStudio, Boston, MA, USA). A two-sided Fisher exact test was used for frequency comparisons, the Mann-Whitney U test was used for continuous variables; *P*-values less than 0.05 were considered statistically significant. The Bonferroni correction was applied for multiple comparisons. Cox regression models were used to estimate relative risks of clinical outcomes; weighted Cox models were used when the proportional hazards assumption was violated, and average hazard ratios (AHR) are reported. A competing risk analysis for cumulative incidences of first adverse events (BMF leading to HCT or death, leukemia or solid tumors) was performed as described previously.[26] Survival probabilities were calculated by the Kaplan-Meier method in the absence of competing risks with censoring at last follow-up; the log-rank test was used for comparisons.

Results

Patients

The FA cohort included 203 patients, 146 in the FC and 57 in the CC (Figure 1A). Phenotype information was not available for seven FC participants. The genotype was known for 54 patients in the CC and 110 patients in the FC; for 19 patients in the FC, only the complementation groups were known, but no gene variants were reported. Demographics and clinical characteristics are summarized in Table 1. The sex distribution was similar in the FC and CC (*P*=0.4) with more females than males in each cohort. The median age at diagnosis of FA was 5.4 years and the median age at study enrollment was 11.2 years with no significant difference between the cohorts. The FC was younger than the CC at last follow-up with median ages

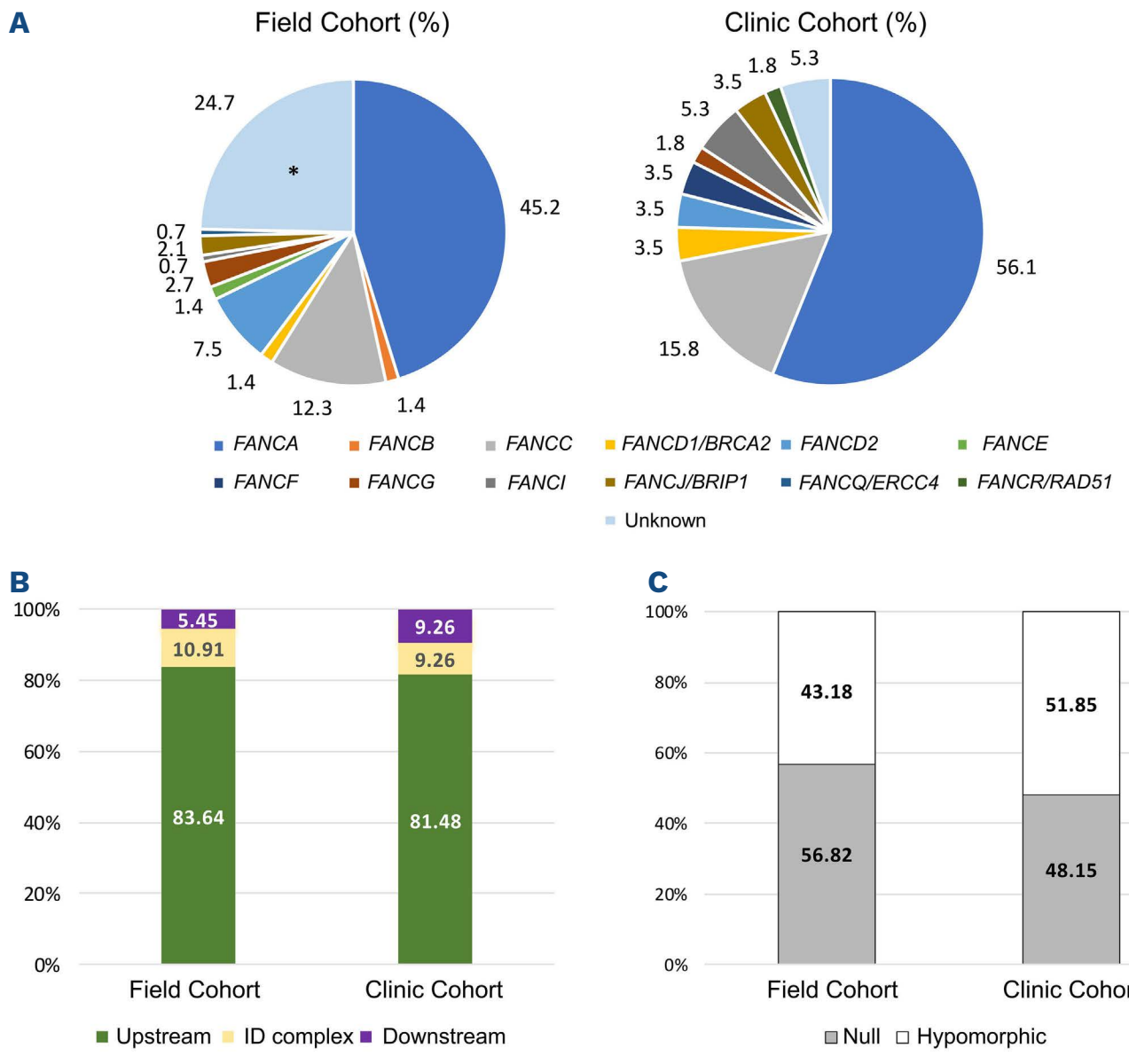


Figure 2. Distribution of patients according to genotypes, mutation pathways and type of variants in the field cohort and the clinic cohort. (A) Distribution of Fanconi anemia genes, including gene unknown patients. *Patients with unknown genotype were more common in the field cohort than in the clinic cohort (*P*=0.001); (B) Distribution by mutation pathways; (C) Distribution by hypomorphic and null genotypes. Panels (B) and (C) include only patients with available pathway and genotype data, respectively.

of 15.7 and 25.2 years, respectively (*P*<0.001). Most patients self-reported as white in both cohorts.

FANCA was the most frequently affected gene, observed in 98 patients. Fifty-three patients with *FANCA* had one or bi-allelic variants within or involving the BRCA1 interaction region of the FANCA protein, 21 had variants involving the FAAP20-binding domain and 45 had variants involving the region of exons 27-30 (Figure 1B). Patients with variants in *FANCA* accounted for 45% of the FC and 56% of the CC, followed by those with variants in *FANCC*, who accounted for 12% and 16%, respectively (Figure 2A). More than 80% of the variants were in the upstream complex in both cohorts (Figure 2B). Genotypes were similarly distributed as hypomorphic or null in both the FC and CC among the 142 patients with available molecular diagnoses (Figure 2C). The hypomorphic genotype was more common in *FANCA* (*P*=0.006) and the null genotype was more common in *FANCC* (*P*<0.001) (*Online Supplementary Figure S1*).

Physical abnormalities

Physical abnormalities were identified in 133 of 139 (95.7%) FC and all CC patients (Figure 1). The VACTERL-H association (≥3/8 features) was present in 23% of the FC and 35% of the CC (*P*=0.1). Vertebral abnormalities were more often recognized in CC patients (*P*<0.001). Other VACTERL-H features were identified at similar frequencies in both cohorts (Figure 3). The most common abnormalities were upper limb (60%) and renal (35-37%) and the least common (<10%) were tracheo-esophageal fistula, hydrocephalus, and anal atresia (Figure 3).

PHENOS features (≥4/6) were present in 56% of the CC and 33% of the FC (*P*=0.004). The most common findings were skin pigmentation abnormalities (68% in the FC, 82% in the CC; *P*=0.04), followed by small eyes (52% in the FC, 82% in the CC; *P*<0.001). Structural central nervous system abnormalities were detected more often in the CC than in the FC (*P*<0.001). Other physical findings not part of VACTERL-H or PHENOS were also more common in the CC (Figure 3). VACTERL-H plus PHENOS was more frequent in the CC (31.6% *vs*. 16.7%, *P*=0.02) and neither VACTERL-H nor PHENOS was more frequent in the FC (60.1% *vs*. 40.4%, *P*=0.03) (Figure 3).

Physical abnormalities according to the gene, mutation pathway, and type of pathogenic variant

Phenotype comparison across FA genes after Bonferroni correction revealed significant associations. All gene-spe-

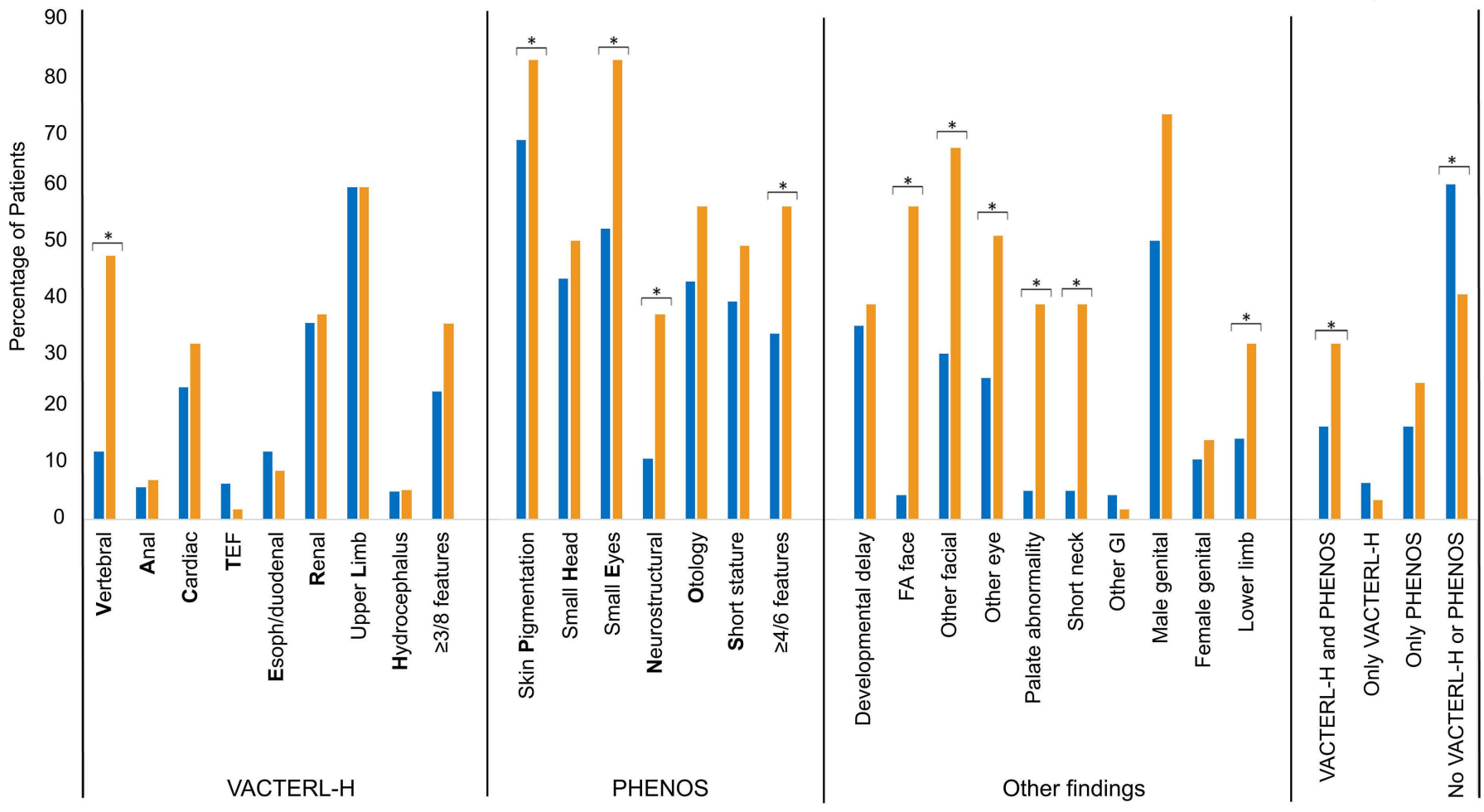


Figure 3. Physical abnormalities in field cohort patients *versus* clinic cohort patients. Blue: field cohort; orange: clinic cohort. Horizontal axis: types of abnormalities; vertical axis: percentage of patients with abnormalities. VACTERL-H: vertebral, anal, cardiac, tracheo-esophageal fistula, esophageal or duodenal atresia, renal, upper limb, hydrocephalus; PHENOS: skin pigmentation, small head, small eyes, structural nervous system, otology, short stature; TEF: tracheo-esophageal fistula; FA: Fanconi anemia; GI: gastrointestinal. Comparisons were performed by the Fisher exact test, *P<0.05.

Table 2. Association of specific abnormalities with gene, FA/BRCA DNA repair pathway and type of pathogenic variant.

Physical abnormality		Gene									Pathway			Genotype	
		A	*C*	*D1*	*D2*	*E*	*F*	*G*	*I*	*J*	US	ID	DS	Hypo	Null
VACTERL-H	Vertebral														
	Anal			+							-		+		
	Cardiac										-	+			
	TEF														
	Esophageal/duodenal atresia														
	Renal										-				
	Upper imb										-			+	
	Hydrocephalus			+							-				
PHENOS	Skin pigmentation														
	Small head		-		+						-	+	+		
	Small eyes										-				
	Nervous structural										-	+	+		
	Otology														
	Short stature										-				
Other abnormalities	Neurodevelopmental	-									-	-			
	FA facies														
	Other face														
	Other eye														
	Palate														
	Short neck														
	Other GI														
	Male genital														
	Female genital														
	Lower limb														
	VACTERL-H	-									-	-	+	+		
	PHENOS				+						-		+			
	VACTERL-H and PHENOS	-			+						-		+	+		
	VACTERL-H or PHENOS (≥1)				+						-	-	+			
	Neither VACTERL-H or PHENOS				-						+	+	-			
		*$P < 0.0063$									*$P < 0.02$			$P < 0.05$	

(+) Positive association and (-) inverse association between the abnormality and the gene, FA/BRCA DNA repair pathway or type of variant. Field cohort highlighted in blue, clinic cohort in orange. **P*-values after Bonferroni correction. VACTERL-H: vertebral, anal, cardiac, tracheo-esophageal fistula (TEF), esophageal or duodenal atresia, renal, upper limb and hydrocephalus. PHENOS: skin pigmentation, small head, small eyes, structural nervous system, otology and short stature. GI: gastrointestinal. US: upstream complex, ID: ID complex, DS: downstream complex. Hypo: hypomorphic genotype.

cific associations were isolated to either the FC or the CC, as the number of patients in each cohort varied (Table 2A). Patients with variants in the upstream complex pathway were least likely to have neurodevelopmental abnormalities, VACTERL-H association, or the presence of at least one of VACTERL-H or PHENOS in both the FC and the CC (P=0.01, P<0.001 and P<0.001, respectively). Variants in the ID complex were associated with VACTERL-H and most physical abnormalities in FC patients (Table 2B). The hypomorphic genotype was associated with upper limb ab-

normalities in the FC (Table 2C).

Hematologic outcomes

BMF developed in 170/198 patients (86%) at a median age of 6.6 years in the combined FC and CC (Table 1). Twenty-eight percent of patients were dependent on transfusions and 24% received androgen therapy in either cohort. None of the four patients with *FANCD1/BRCA2* had cytopenia. Cytogenetic abnormalities were reported in 40.4% of the CC and 18.3% of the FC patients (P=0.03). MDS was reported in 27 patients at a median age of 15 years (range, 9.9-24.2) and AML in eight patients at a median age of 19 years (range, 12-60). A total of 103 (51%) patients received HCT with the frequency being similar in the FC and CC; 75.3% for severe BMF, 22.7% for cytogenetic abnormalities or MDS and 2% for AML.

A weighted multiple Cox regression model for BMF included 133 patients with available data and excluded the four patients with *FANCD1/BRCA2* due to lack of BMF. The analysis identified a higher risk of BMF in males than in females (AHR=1.65, 95% confidence interval [95% CI]: 1.1-2.6). The presence of PHENOS predicted a higher risk of BMF than absence (AHR=2.9, 95% CI: 1.7-4.95). Compared with patients with *FANCA*, patients with *FANCC*, *G*, *I* and *J* had a higher risk of BMF by the same age (*Online Supplementary Table S3A*). A weighted Cox regression model for MDS that included 138 patients with available data showed that patients with *FANCC* (AHR=4.7, 95% CI: 1.55-14.4) and *FANCD1/BRCA2* gene variants (AHR=61, 95% CI: 4.4-848.9) were at higher risk of MDS than patients with *FANCA* (*Online Supplementary Table S3B*). The increased risk of MDS in *FANCD1/BRCA2* was based on one out of the four patients with *FANCD1/BRCA2* who received HCT for a diagnosis of hypocellular MDS associated with cytogenetic changes without antecedent pancytopenia.

Endocrine, metabolic, and reproductive outcomes

Approximately 30% of the FC and 42% of the CC developed hypothyroidism at a median age of 7.2 and 13.9 years (P=0.005), respectively. Hypothyroidism predated HCT in 64% of cases. A weighted multiple Cox regression model for 139 patients with available data showed an increased risk of hypothyroidism in patients with prior HCT (AHR=11.1, 95% CI: 4.3-28.4), VACTERL-H association (AHR=4.7, 95% CI: 1.92-11.2), and variants in *FANCD1*, *D2*, *G*, *I* and *J* (*Online Supplementary Table S4A*).

Osteoporosis was more often reported in the CC than in the FC (P=0.049); median age was 23 years in both cohorts. Growth hormone deficiency was reported in 28.6% of the CC and 18% of the FC patients and it was associated with HCT in 10% of cases. Diabetes mellitus developed in 17% of the FC and 10.5% of the CC; 11/23 of these patients had treatment-induced diabetes, 5/23 had type 1 diabetes and 7/23 had type 2 diabetes. Overall, 57% of patients with available data had insulin resistance, 38.7% had abnormal body mass index (19.6% were underweight,

Table 3. Types of malignancies seen in patients with Fanconi anemia.

Cancers	Field cohort (N= 139*)	Clinic cohort (N=57)	Median age at first cancer, years (range)	Total observed (= 196*)
All sites	59 (32)	31 (16)		90 (48)
Solid tumors	35 (22)	25 (14)	31 (2-58)	60 (36)
Head and neck	16 (10)	15 (8)	34.4 (15-44)	31 (18)
Vulva/vagina/cervix	5 (4)	6 (5)	29.3 (23-36)	11 (9)
Esophagus	3 (3)	1 (1)	34.7 (33-38)	4 (4)
Brain	2 (2)	1 (1)	3.1 (2-5)	3 (3)
Breast	1 (1)	1 (1)	32 (30-34)	2 (2)
Anus	0	1 (1)	32.5	1 (1)
Thyroid	2 (2)	0	27 (23.5-30)	2 (2)
Lung	2 (2)	0	53 (48.5-57.5)	2 (2)
Liver	1 (1)	0	36.5	1 (1)
Lymphoma	1 (1)	0	30	1 (1)
Bladder	1 (1)	0	37.6	1 (1)
Unknown type	1 (1)	0	29.5	1 (1)
Leukemia	7 (7)	1 (1)	19 (12-60)	8 (8)
Skin cancers	17 (8)	5 (3)	31 (11-41)	22 (11)
Squamous cell carcinoma	1 (1)	4 (3)		5 (4)
Basal cell carcinoma	12 (6)	1 (1)		13 (7)
Melanoma	2 (2)	0		2 (2)
Unknown type	1 (1)	0		1 (1)
Patients with multiple cancers	35 (9)	22 (7)		57 (16)

Data are presented as number of cancers (number of patients). *Cancer data were not available for seven patients in the field cohort.

11.7% overweight and 7.4% obese), and 14% had metabolic syndrome.

We previously described obstetric outcomes in seven women with FA.[27] We now report 26 pregnancies in 14 women resulting in 20 live births (*Online Supplementary Table S5*). The median age at first pregnancy was 25 years (range, 19-32 years). Ten patients were *FANCA* cases; notably, four of these patients had hypomorphic c.3624C>T (p.Ser1208=) synonymous variants and five had a null genotype. The FA gene was unknown for four patients. A Cox regression analysis showed that patients with milder phenotype (no VACTERL-H or PHENOS) were more likely to become pregnant (HR=7.3, 95% CI: 1.003-52.9); prior HCT and the presence or severity of BMF were not significant predictors (*Online Supplementary Table S4B*).

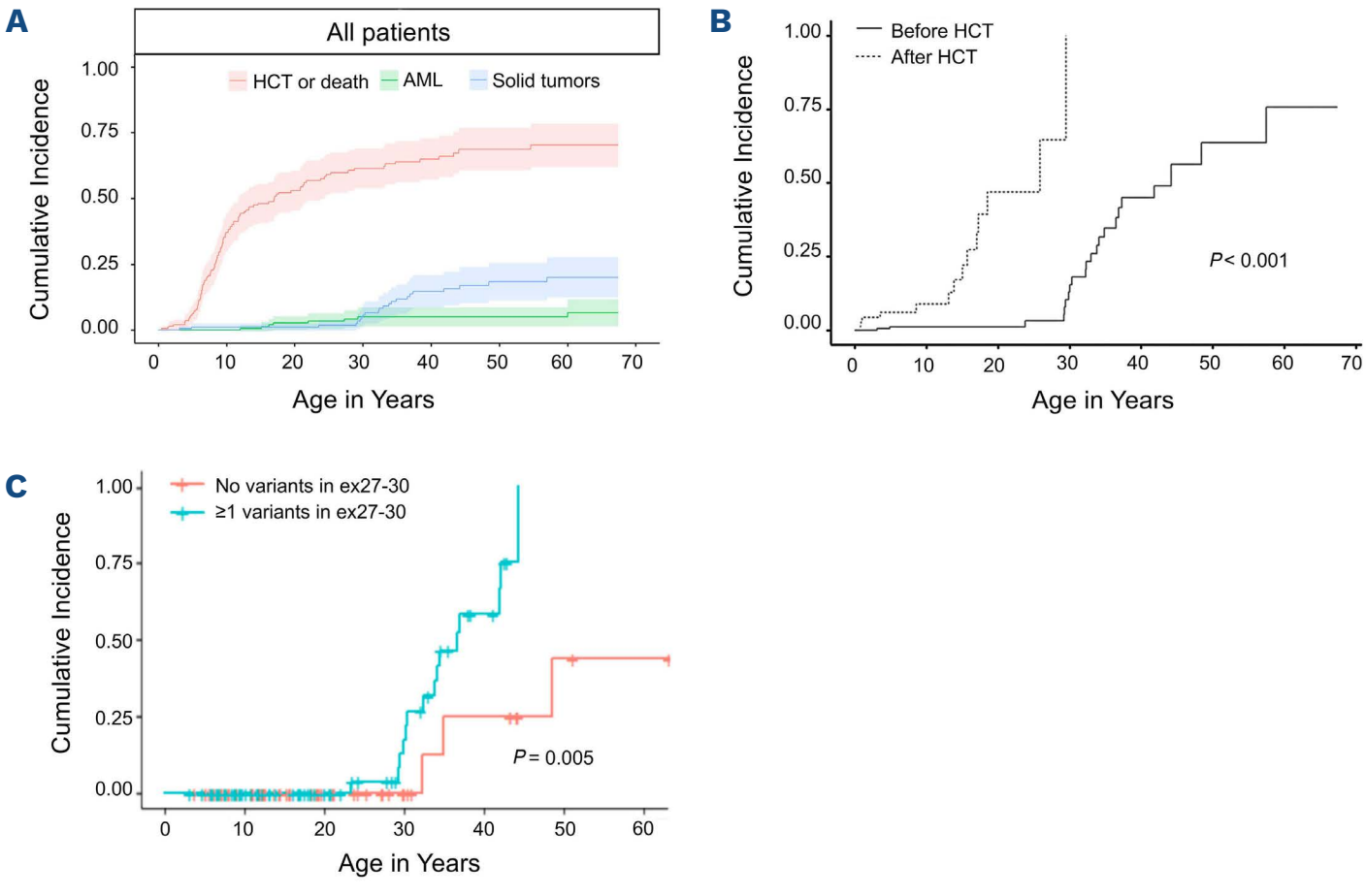
Cancer outcomes

Forty-eight of 196 patients (24.4%) developed at least one malignancy; eight had leukemia, 36 had solid tumors and 11 had skin cancers. Head and neck squamous cell carcinoma was the most common solid tumor and developed at a median age of 34.4 years. The second most common was vulvar cancer, which developed at a median age of 29.3 years (Table 3).

Competing risk analyses of first adverse events showed a similar cumulative incidence of severe BMF leading to HCT or death in the FC (72%) and CC (67%) by the age of 60 years. Solid tumors developed in 18% of the FC and 24% of the CC by age 60 (P=0.7). The cumulative incidence of leukemia was 6% in the FC and 2.5% in the CC by age 30; one patient in the FC developed AML in his 60s (*Online Supplementary Figure S2*). By the age of 70, the cumulative incidences of severe BMF, solid tumors and leukemia were 70.4%, 19.8% and 6.5%, respectively, in the combined cohort (Figure 4A). Patients who underwent HCT had an increased risk of solid tumors with a hazard ratio of 4.6 (95% CI: 2.2-9.8) for transplanted *versus* non-transplanted patients (Figure 4B).

Solid tumors were seen in patients with *FANCA*, *FANCC* and *FANCD1* variants. Brain tumors were exclusive to *FANCD1/BRCA2* and developed at a median age of 3.1 years. Head and neck squamous cell carcinomas and vulvar/cervical cancers in patients with *FANCA* and *FANCC* variants were seen with both hypomorphic and null genotypes. All four patients with esophageal cancer had *FANCA* null genotype. FA genes for four of the eight leukemia patients were unknown; one had *FANCA* and three had *FANCC* (*Online Supplementary Figure S3*).

Further analysis of data from 86 patients with *FANCA* pathogenic variants showed a significant association of solid tumors with variants within/involving exons 27-30. Fifteen of 45 patients (33.3%) with one or both single nucleotide variants/small insertion-deletions within exons 27-30 or multi-exon deletions covering this region developed solid tumors compared to three of 41 patients (7.3%) without a variant in this region (P=0.003). A Cox model adjusting for HCT status showed that patients with any pathogenic variant within/involving exons 27-30 appeared to have a higher risk of solid tumors (HR=6.2, 95% CI: 1.36-28.2) than patients without variants in this region, with a cumulative



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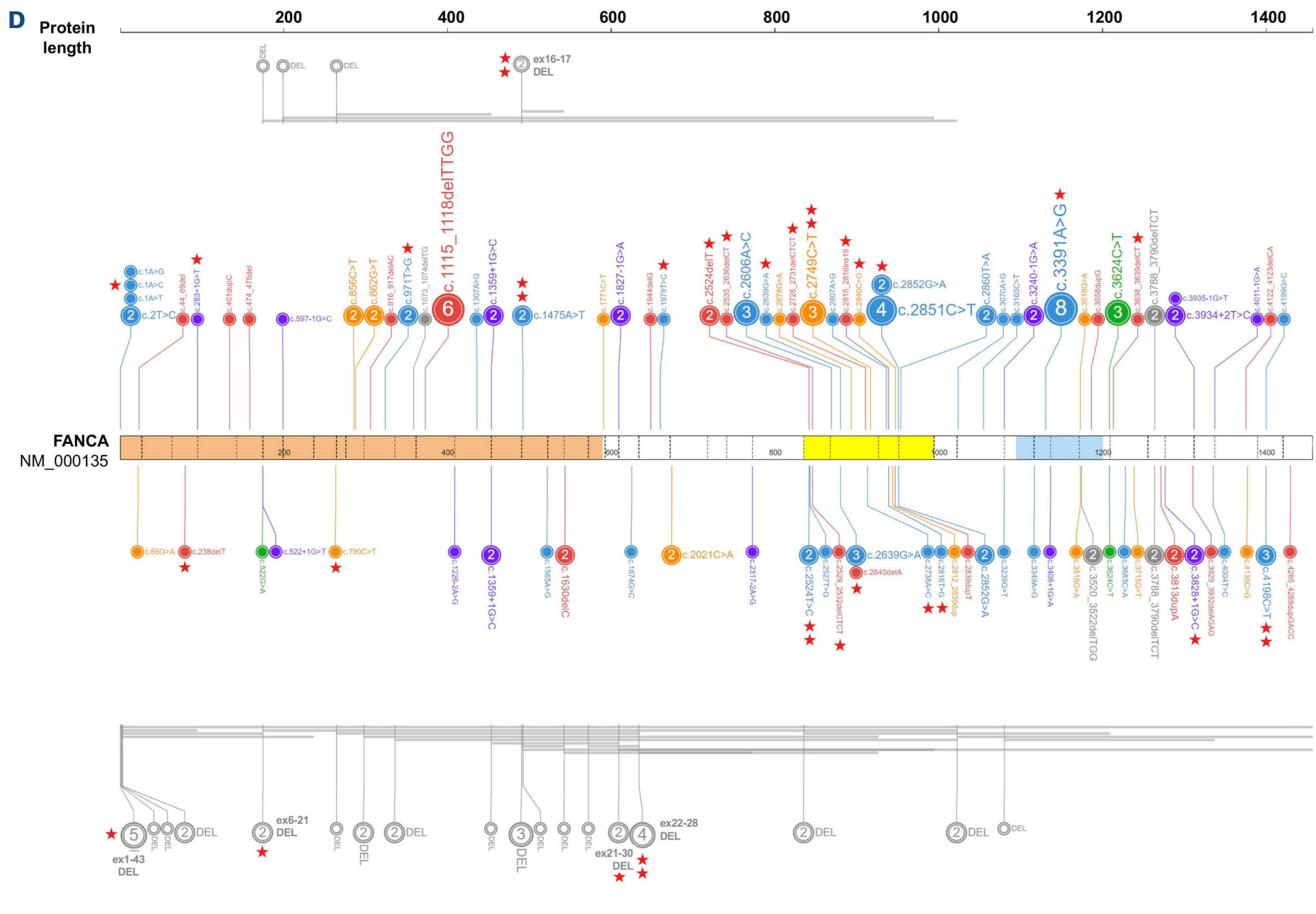

Figure 4. Cumulative incidence of adverse events and increased cancer risk in patients with variants within/involving exons 27-30 of FANCA. (A) Cumulative incidence of bone marrow failure leading to hematopoietic cell transplantation or death (red), acute myeloid leukemia (green) and solid tumors (blue) in the presence of competing risks. (B) Cumulative incidence of solid tumors by age in non-transplanted (solid line) and transplanted patients (dotted line) in the absence of competing risks. HCT: hematopoietic cell transplantation; AML: acute myeloid leukemia. (C) Cumulative incidence of solid tumors in patients with (green) and without (orange) variants within/involving exons 27-30 of FANCA protein. (D) Distribution of variants in the *FANCA*. Shaded areas; orange: BRCA1 interaction region;[50] yellow: exons 27-30; blue: FAAP20-binding domain.[51] Numbers in circles represent the number of alleles for each variant. Horizontal gray bars represent multi-exon deletions and colored circles represent single nucleotide variants/small indels. Red stars highlight the variants observed in patients who developed solid tumors.

incidence of 60% *versus* 25% by age 40 (Figure 4C). Phenotypes, BMF, endocrine outcomes and age at first cancer were not different. Cancer outcomes were similar among patients with either a single variant or both variants within exons 27-30. Individual variants are shown in Figure 4D.

Survival estimates

Overall survival data were available for 200 patients. The median survival age was 37 years (95% CI: 34.7-43.1); 81% of patients were older than 18 years at last follow-up (Figure 5A). Patients with leukemia had a poorer survival (median age 17.7 years; range, 12.1-27.3) than patients with no leukemia (after exclusion of the patient who developed AML in his 60s) (Figure 5B).

The median survival of patients with *FANCA* variants was 43.4 years (95% CI: 36.5-49.5) and that of patients with *FANCC* variants was 32.6 years (95% CI: 24.4-not available). Patients with *FANCD1/BRCA2* variants had the poorest survival (median 4.3 years) (Figure 5C). Patients with variants in upstream complex genes had a better survival (median survival age 39 years) than patients with variants in ID or downstream complex genes (P=0.001 and P=0.016, respectively) (Figure 5D). Survival among patients with hypomorphic or null genotype was similar (Figure 5E).

Discussion

This study of a large cohort of patients with FA provides a detailed assessment of physical abnormalities and clinical outcomes in relation to FA genes, mutation pathways and type of pathogenic variants.

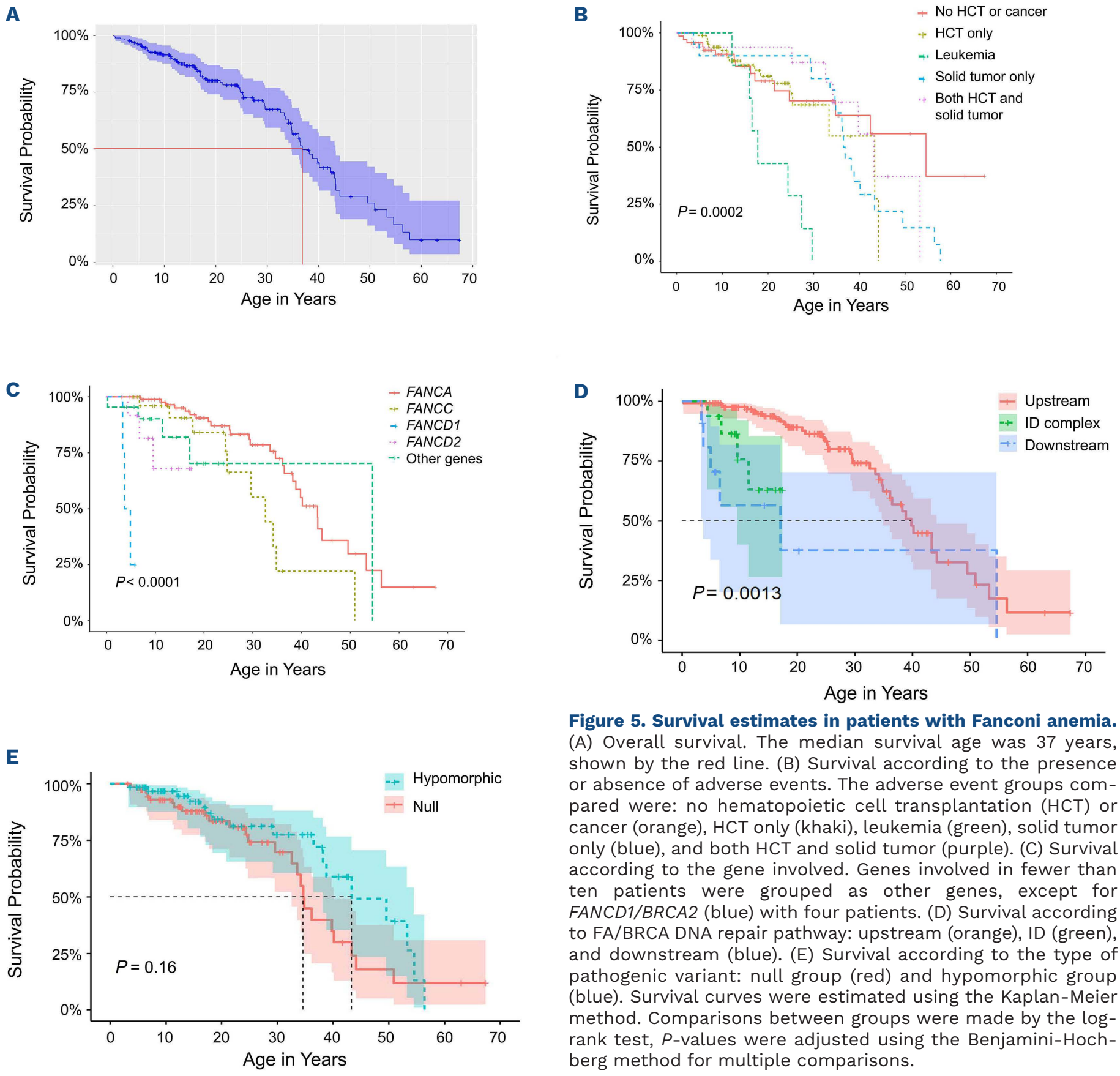

Figure 5. Survival estimates in patients with Fanconi anemia. (A) Overall survival. The median survival age was 37 years, shown by the red line. (B) Survival according to the presence or absence of adverse events. The adverse event groups compared were: no hematopoietic cell transplantation (HCT) or cancer (orange), HCT only (khaki), leukemia (green), solid tumor only (blue), and both HCT and solid tumor (purple). (C) Survival according to the gene involved. Genes involved in fewer than ten patients were grouped as other genes, except for *FANCD1/BRCA2* (blue) with four patients. (D) Survival according to FA/BRCA DNA repair pathway: upstream (orange), ID (green), and downstream (blue). (E) Survival according to the type of pathogenic variant: null group (red) and hypomorphic group (blue). Survival curves were estimated using the Kaplan-Meier method. Comparisons between groups were made by the log-rank test, *P*-values were adjusted using the Benjamini-Hochberg method for multiple comparisons.

Almost all patients in our cohort (96.9%) had at least one physical abnormality; this is a higher percentage than in previous reports (60-90%).[7,28,29] We corroborated our earlier findings of a higher frequency of VACTERL-H association in FA than that described in the literature cases.[7,9] VACTERL-H features that cause functional compromise (cardiac anomaly, tracheo-esophageal fistula, esophageal, duodenal or anal atresia) or those that are generally recognized in early life (upper limb and renal abnormalities) were present at similar rates in the CC and FC participants. Vertebral anomalies and structural central nervous system abnormalities whose detection requires imaging procedures were more frequently identified in the CC because baseline skeletal survey and brain magnetic resonance imaging are part of the systematic evaluations of CC participants. Other PHENOS features such as skin pigmentary changes and small eyes, which may escape recognition if not specifically looked for, were more often identified in the CC. Likewise, PHENOS (≥4/6 features) and PHENOS plus VACTERL-H (≥3/8 features) were more common in the CC and proportions in both cohorts were higher than in cases reported in the literature.[7] This underscores the need for all patients with FA to have a full dysmorphology evaluation upon diagnosis.

Our findings of milder phenotype in patients with upstream complex pathway variants, with less likelihood of VACTERL-H or PHENOS, is mainly a reflection of inclusion of patients with *FANCA* and *FANCC* variants who had fewer physical abnormalities than patients with variants in other gene groups. Consistent with the review of the literature cases, variants in the ID complex pathway were associated with a more severe phenotype (VACTERL-H) in both cohorts and the presence of at least one of VACTERL-H or PHENOS in the FC.[7] However, unlike the literature cases, we found no significant association of VACTERL-H and severe phenotype with null genotype.[7] The results from the FC were more comparable with the literature cases, but data from the CC provided a more complete picture of the FA phenotype. The discrepancies between the literature cases and the NCI cohort are likely due to lack of variant details and phenotypes in patients within these groups and may be overcome by the development of a standardized approach for systematic evaluation of all individuals with FA. This will facilitate international and trans-institutional comparisons, risk stratification, and genotype-phenotype and outcome assessment of patients with this rare syndrome.

We identified a higher risk of BMF in patients with PHENOS (≥4/6) whereas VACTERL-H (≥3/8) was not a significant predictor of BMF. We previously noted abnormal radii as the strongest predictor of early BMF and developed a congenital abnormality score (CABS; between 0-5 based on the number of abnormalities in five categories: developmental delay, heart or lung, kidney, hearing and small head) to distinguish prognostic groups in patients with normal radii.[30] The data from the German FA registry[31] and the Israeli FA cohort[29] indicated that CABS was a strong risk factor for BMF. Notably, two of the features of PHENOS (hearing and small head) are included in CABS. Studies incorporating PHENOS (≥4/6) or individual PHENOS features in prediction models may help in estimating BMF risk stratification and pre-emptive transplant decisions in FA.[32]

The risk of MDS was increased in our patients with *FANCC* and *FANCD1/BRCA2* compared with *FANCA* (*Online Supplementary Table S3*). The increased risk of MDS in *FANCD1/BRCA2* should be interpreted with caution as it was based on a single patient with hypocellular MDS. The cumulative incidence of AML as the first event as well as the median age at AML were similar to prior estimates.[1,2,14] Clonal cytogenetic abnormalities and MDS were previously reported in *FANCA*, *C*, *D1/BRCA2* and *G* groups.[33,34] Frequent bone marrow evaluations in these groups may detect clonal changes and dysplasia earlier. Chromosomal aberrations and other somatic events in the genome may have prognostic importance and be clues to clonal hematopoiesis.[33,35,36] Longitudinal studies characterizing somatic variants are needed to understand how variants drive clonal hematopoiesis in FA and their association with leukemogenesis, as identified in Shwachman Diamond syndrome.[37]

The frequencies of endocrine, metabolic and reproductive abnormalities were similar to those in earlier reports.[11,27,38,39] We confirmed endocrine and metabolic disturbances as long-term complications following HCT in FA patients, as previously described.[40,41] The relationship between VACTERL-H, FA genes and hypothyroidism needs further study to be fully elucidated. Recently, dysregulated tryptophan metabolism and hyperserotonemia were proposed as mechanisms of metabolic disturbances in FA.[42] Larger studies with broader representation of various FA gene groups may provide mechanistic insights and identify gene-specific associations in metabolism. We noted that four of the ten women with *FANCA* variants who became pregnant had the c.3624C>T synonymous variant which causes aberrant splicing.[43] It is possible that some residual protein function may be present with this variant and is sufficient for patients to conceive. Functional analysis of this variant will be important to understand its *in vivo* effect on FANCA protein.

Here we updated cumulative incidence estimates of cancer.[2] The cumulative incidence of solid tumors as the first event was similar to our earlier estimates, and confirmed the increased risk of solid tumors in transplanted patients.[2,26] The most common solid tumors were head and neck squamous cell carcinomas and gynecological cancers followed by esophageal cancers (all esophageal cancers were in patients with null *FANCA* variants) (*Online Supplementary Figure S3*). Brain tumors remained exclusive to the *FANCD1/BRCA2* group. Despite the severe phenotype observed in the *FANCD2* group, these patients were not at an increased risk of BMF compared with those in the *FANCA* group and did not develop cytogenetic abnormalities, MDS or cancer. In our cohort, among five patients with the c.2444G>A *FANCD2* variant, two had early BMF and four had VACTERL-H plus PHENOS, in contrast with the report by Kalb *et al.*[44] in which mild phenotype and adult-onset BMF were described with this variant. This may be due to differences in the second allele affecting outcomes. Further study of patients with this recurrent variant may uncover a unique phenotype in this group.

An interesting and novel finding from our study was the high frequency of solid tumors in patients with one or both *FANCA* variants within/involving the region of exons 27-30 when compared with that in patients who had no *FANCA* variant within/involving this region. The exon 27-30 region is within the C-terminal domain of the protein. FANCA forms a homodimer interacting through the C-terminal domain to function and the C-terminal domain is crucial for nuclear localization of FANCA.[45,46] After our data were locked in November 2020, we became aware of an

additional patient in our cohort who has developed a head and neck squamous cell carcinoma; this patient has a *FANCA* variant within the exon 27-30 region. Collaborative studies with more patients are needed to further investigate the relevance of this finding. Functional analysis of variants in this region and other variants in *trans* would also provide insights into carcinogenesis in FA and may have implications for personalized cancer screening.

We observed a significant variant heterogeneity in *FANCA*, whereas many variants were recurrent in *FANCC* and *FANCD2*. This could explain the complexity of establishing genotype-phenotype correlations in FA but may not be the only reason. Phenotypes and outcomes varied greatly even between affected siblings. With regard to this, we observed three FA patients with bi-allelic variants in one FA gene and a third variant in another FA gene. Investigating how additional variants contribute to disease may explain differences between patients with the same disease-causing FA variants. Tomaszowski *et al.* hypothesized that FA is a polygenic disease demonstrating a FA phenotype with different affected FA genes.[47] Studies on the combined effect of variants in FA and/or non-FA genes, as well as epigenetic regulations are needed.

Most patients reached adulthood, with the median survival being 37 years, similar to that in our prior report.[2] Patients with *FANCA* and *FANCC* variants had better survival than those with variants in other genes. Variants in the upstream complex pathway were associated with better survival. Patients with leukemia had the poorest survival. The outcomes of FA patients with AML has improved with improvements in HCT regimens and clinical care, although the overall survival remains around 40-50% and treatment toxicities and relapse rates are high.[48,49]

In summary, we have reported detailed phenotypes and outcomes in a large, prospectively followed FA cohort, consisting of both children and adults. We found significant differences between the FC and the CC. We were limited by patient and family reports on some outcomes and genotype data. Medical records for the FC were not as complete as those for the CC. There may be recent complications of which we were not aware, especially for FC patients as some had old records and may be less engaged with the study. Variant classification was based on the existing literature and *in silico* predictions. More data and functional studies of variants may reveal further information.

Comprehensive investigations of physical phenotypes and clinical outcomes in centers experienced with FA will enhance our knowledge of FA, and increase the possibility of using phenotypes and genotypes to stratify risks of outcomes. Follow-up studies investigating finer molecular associations will provide opportunities for tailored risk estimates, surveillance strategies and treatment plans.

Disclosures

No conflicts of interest to disclose.

Contributions

BA, NG, LJM and BPA designed the study, and established and curated the dataset; BA, NG, LJM, AB and BPA contributed to the methodology; NG, LJM and BPA provided supervision; BA, NG and BPA analyzed and interpreted the data and drafted the manuscript; all authors contributed to revising the manuscript and approved the submitted version.

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Data-sharing statement

De-identified clinical and genetic data are available, upon reasonable request, from Neelam Giri (girin@mail.nih.gov).

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