ARTICLE - Bone Marrow Failure

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

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Received: September 13, 2021.
Accepted: February 23, 2022.
Prepublished: April 14, 2022.

https://doi.org/10.3324/haematol.2021.279981

Supplemental Table 1. Physical abnormalities seen in patients with FA and their distributions by Field or Clinic cohort.

Detailed characterization of physical abnormalities in patients with FA; number of patients (percentage). All types of vertebral abnormalities were more frequent in clinic cohort (CC). Other VACTERL-H features were similar in frequency between field cohort (FC) and CC. Most of the PHENOS features and other physical abnormalities were more common in CC than FC. Frequencies were compared by 2-sided Fisher's test and p values < 0.05 were considered significant. Cells with red frame represent phenotype significantly different between the FC and CC.

Microcephaly and macrocephaly were defined as head circumference (HC) below 3rd percentile (< -2 SD) and above 97th percentile (> +2 SD), respectively. We used HC curves described by Rollins et al[1] which assembles the normative data that can be used for the U.S. population. Short stature was defined as length or height measurement that is more than 2 SD below the mean for age and sex, and the calculations were made according to the CDC growth charts. Skin café-au-lait macules (CALM) were considered positive if the patients had 6 or more spots (based on diagnostic criterion established for Neurofibromatosis type 1)[2] when the number was available, or if the patients were reported to have multiple CALM when the number was not available. Small for gestational age (SGA) was defined as a birth weight below the 10th percentile for the gestational age. SGA calculations were made according to the CDC growth charts for the term newborns and 2013 Fenton growth charts for preterm infants.[3]

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Supplemental Table 2. Pathogenic/Likely pathogenic variants of patients with FA in the NCI cohort

P: Pathogenic, LP: Likely pathogenic, VUS: variant of unknown significance.
Variants were classified according to the 2015 ACMG/AMP variant interpretation guidelines[1] with the following modifications using VarSeq™ v2.2 (Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com) and VarSome[2] : 1) AutoPVS1 tool[3] was implemented for interpreting the loss of function variants; 2) PVS1_M was applied for variants positioned at the last base before the splice site; 3) PP3 score was applied based on HSF prediction for splice site variants. REVEL,[4] MetaSVM[5] CADD[6] and BayesDel[7] *in silico* tools were used for missense variants (REVEL >0.5, MetaSVM >0, CADD_phred >20 and BayesDel > 0.07 with MaxAF and > -0.057

without MaxAF predict deleterious effect). PP3 or BP4 scores were applied only if 3 or more of these tools predicted a deleterious or tolerated effect, respectively; 4) PP4 score was applied to patients with bone marrow failure and/or some VACTERL-H features, along with a positive chromosomal breakage test; 5) PP5 evidence level was increased to PP5_M and PP5_S based on ClinVar entries rated with 2 stars and 3 stars, respectively. We considered large insertions and multi-exon deletions as pathogenic; therefore, we did not perform ACMG/AMP interpretations on them.

GenBank accession numbers= *FANCA*, NM_000135.4; *FANCC*, NM_000136.2; *FANCD1/BRCA2*, NM_000059.3; *FAND2*, NM_033084.4*; FANCF*, NM_022725.4; *FANCG*, NM_004629.2; *FANCI*, NM_001113378.2; *FANCJ/BRIP1*, NM_032043.3; *FANCQ*, NM_005236.3; *FANCR*, NM_002875.5.
*gnomAD exome frequencies were accessed on February 25th, 2021.
Available at: http://www.rockefeller.edu/fanconi.
^ Available at: https://www.ncbi.nlm.nih.gov/clinvar/

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Supplemental Table 3. Regression analyses for BMF and MDS

Weighted multiple Cox regression models, that take the timing of the event into account, were created to explore the effects of variables included in the tables for BMF and MDS outcomes, and average hazard ratios were reported. Bold values: statistically significant results. (A) 133 patients with phenotype, genotype and available BMF data were included in the model. Male patients were more at risk for BMF compared to females. A higher risk of BMF was associated with having PHENOS (≥4 of 6 features). *FANCC, G, I* and *J* genes showed a higher hazard of BMF compared to *FANCA* genotype. The type of variant did not have a significant effect. (B) 138 patients with genotype, phenotype and available MDS data were included in the model. *FANCC* and *FANCD1* genotypes had a higher risk of MDS compared to *FANCA*. Among other genes, 1 *FANCF* patient developed MDS. Neither phenotypes nor the type of variant showed a significant effect on the risk of MDS.

Supplemental Table 4. Regression analyses for hypothyroidism and pregnancy

Weighted multiple Cox regression models, that take the timing of the event into account, were created to explore the effects of variables included in the tables for hypothyroidism and pregnancy outcomes, and average hazard ratios were reported. Bold values: statistically significant results. (A) 139 patients with available phenotype, genotype and hypothyroidism data were included in the model. Patients who underwent HCT were at 9 times more risk for hypothyroidism. VACTERL-H (≥ 3/8) was associated with an increased risk of hypothyroidism. Most genotypes except *FANCC* were at higher risk of hypothyroidism compared to *FANCA*. Patients with other genes were less likely to develop hypothyroidism; they included 2 *FANCF*, 1 *FANCE, FANCQ* and 1 *FANCR* patients. Only 1 *FANCE* patient developed hypothyroidism. (B) 60 female patients who were over 16 years of age at the time of last follow up and had available data were included in the model. Females who have neither VACTERL-H nor PHENOS, thus mild phenotype, were 7 times more likely to become pregnant. Prior HCT, having BMF or its severity did not show a significant effect.

Supplemental Table 5. Pregnancies in 14 patients with FA

One patient used an egg donor and other 13 patients conceived naturally. None of the patients had VACTERL-H associations and only 1/14 had PHENOS. Median age at first pregnancy was 25 years. BMF: bone marrow failure, HCT: hematopoietic cell transplant, MDS: myelodysplastic syndrome, G: gravida, P: parity, n/a: information not available.

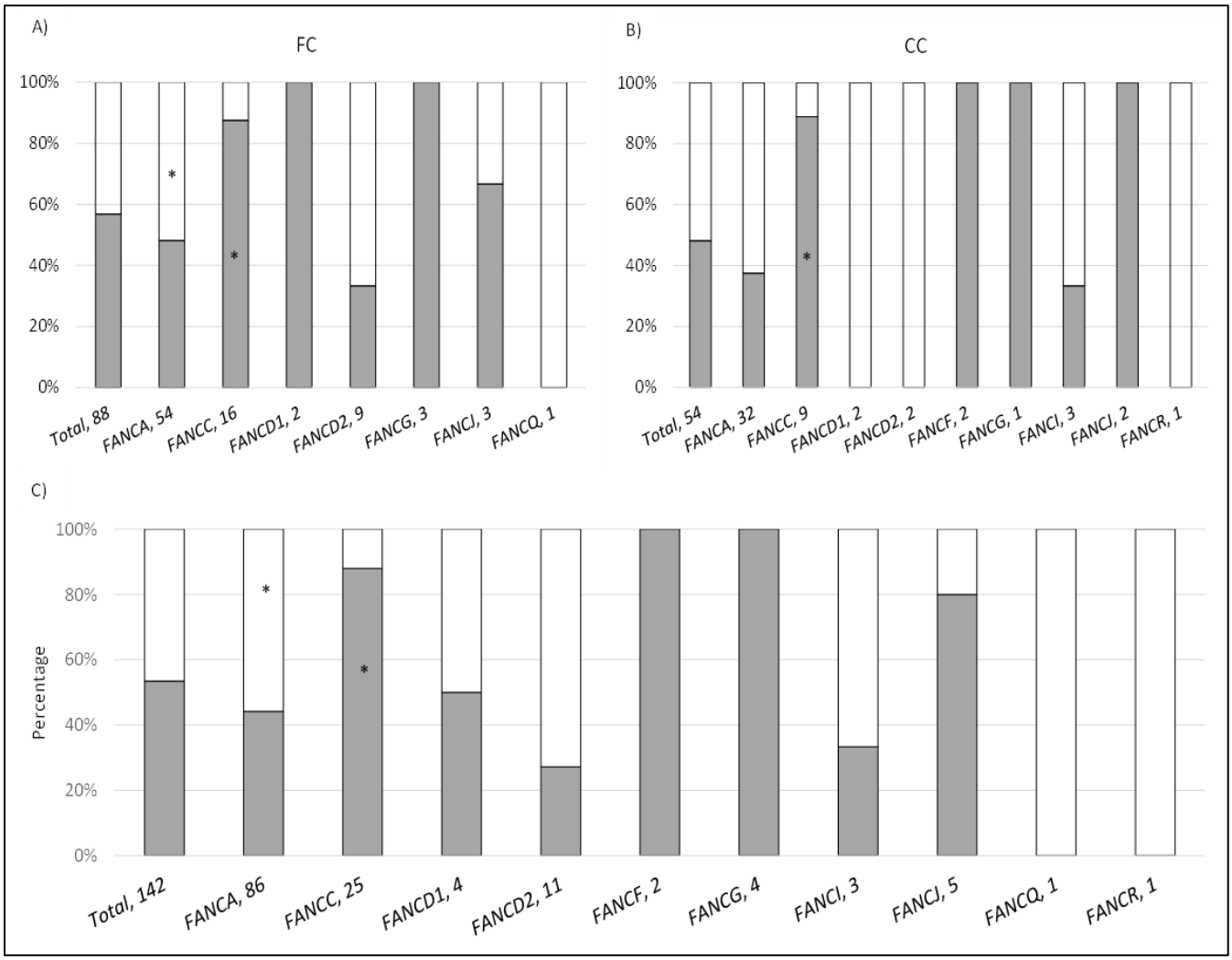
Supplemental Figure 1. Type of Pathogenic Variants according to Gene. Gray: null genotype; white: hypomorphic genotype. Horizontal axis: gene, number of patients with variants

available; vertical axis: percent of cases within each gene. *$p < 0.05$. (A) Distribution in the FC. Hypomorphic variants were more common *FANCA* ($p= 0.048$) and null variants were more common in *FANCC* ($p = 0.01$). (B) Distribution in the CC. Null variants were more common in *FANCC* ($p= 0.01$). (C) Distribution among all patients. The type of variant could be determined in 142 out of 203 patients (69.95%) based on available information.

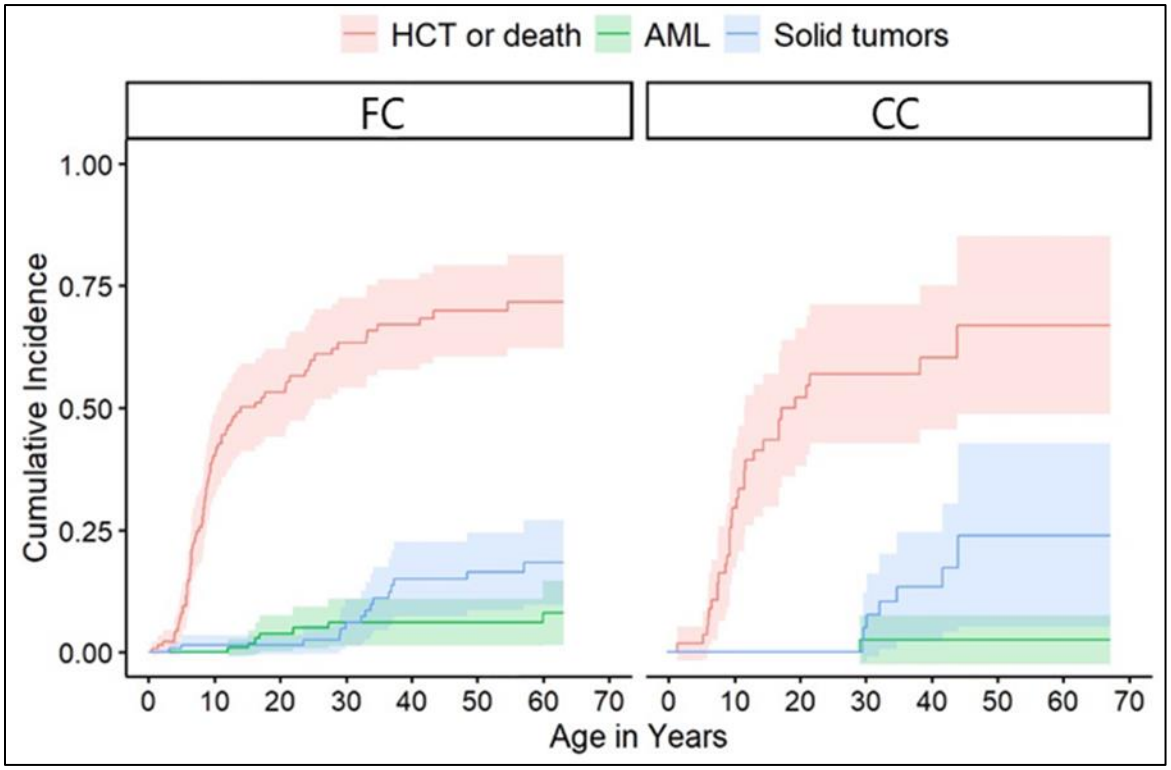
Supplemental Figure 2. Cumulative incidence of adverse events in field and clinic cohort. Cumulative incidence of BMF leading to HCT or death, AML and solid tumors (in the presence of competing risk) were similar in FC and CC; HCT or death in red, AML in green, solid tumors in blue. FC: field cohort, CC: clinic cohort.

Supplemental Figure 3. Characterization of patients with malignancies. Vertical axis: clinical, genotype and cancer type information; horizontal axis: patients with cancers. 36 patients developed solid tumors and 8 patients AML. Head and neck cancers were observed in *FANCA* and *FANCC* genotypes, in both sexes, at various ages and both in hypomorphic and null variant types. All 4 patients who developed esophageal cancer had loss of function variants and were in null category. All 3 patients with brain tumor had *FANCD1/BRCA2* genotype, 2 of which were null and 1 was hypomorphic.

Supplemental Figure 1. Type of Pathogenic Variants according to Gene



Supplemental Figure 2. Cumulative incidence of adverse events in field and clinic cohort



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