Population frequency of Fanconi pathway gene variants and their association with survival after hematopoietic cell transplant for severe aplastic anemia

Lisa J. McReynolds1, Youjin Wang1, Ashley S. Thompson1, Bari J. Ballew2,3, Jung Kim1, Blanche P. Alter1, Belynda Hicks2,3, Bin Zhu2,3, Kristine Jones2,3, Stephen R. Spellman4,5, Tao Wang4,5, Stephanie J. Lee4,6, Sharon A. Savage1, Shahinaz M. Gadalla1

1Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA
2Cancer Genomics Research Laboratory, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA
3Leidos Biomedical Research, Inc. Frederick National Laboratory for Cancer Research, Frederick, MD, USA
4Center for International Blood and Marrow Transplant Research, Medical College of Wisconsin, Milwaukee, WI, USA
5Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI, USA
6Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Abstract

Severe aplastic anemia (SAA) is most frequently immune mediated, however, rare inherited bone marrow failure syndromes, such as Fanconi anemia (FA), may be causal, and can present as aplastic anemia (AA). FA is primarily an autosomal recessive disorder caused by having two pathogenic variants in a single FA/BRCA DNA repair pathway gene. Patients with SAA often undergo genetic testing during clinical evaluation that may identify single deleterious alleles in FA pathway genes. We quantified the rate of germline single deleterious alleles in 22 FA genes using both a general population database (3234 variants/125,748 exomes) and in a cohort of patients with SAA undergoing hematopoietic cell transplant (HCT) (21 variants/730 patients). The variants were classified as deleterious using in silico tools (REVEL, MetaSVM, VEP) and database resources (ClinVar, LOVD-FA). We found similar rates of single deleterious alleles in an FA genes in both groups (2.6 and 2.9%). The presence of a single deleterious variant in a gene for FA in SAA HCT recipients did not affect the overall survival after HCT (HR=0.85, 95% CI=0.37–1.95, p=0.71), or post-HCT cancer risk (p=0.52). Our results show that the identification of a germline

Corresponding Author: Lisa J. McReynolds MD, PhD, Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive 6E434, Bethesda, MD 20892, 240-276-5047.

Publisher’s Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have no financial disclosures or conflicts of interest.
A monoallelic deleterious variant in an FA gene, in a patient with idiopathic SAA does not influence the outcome of HCT. Our findings suggest that there is no need for special treatment considerations for patients with SAA and a single deleterious FA allele identified on genetic testing.

**Keywords**
Fanconi; population; mutation; aplastic anemia; genes; outcomes; blood and marrow transplant

**Introduction**

Aplastic anemia (AA) is a rare disorder characterized by pancytopenia and bone marrow failure. The majority of severe aplastic anemia (SAA) cases are acquired immune-mediated, or sometimes triggered by known environmental or toxic exposures (Miano and Dufour 2015). Inherited forms of bone marrow failure do exist, such as Fanconi anemia (FA) which is the most common. FA is due to pathogenic germline variants in one of 22 FA/BRCA DNA repair pathway genes (so far 20 autosomal recessive, one X-linked, one autosomal dominant), resulting in defects in DNA damage repair, specifically the inability to repair inter-strand cross links (Nalepa and Clapp 2018). The estimated FA birth rate is 1 in 130,000 and carrier frequency ranges between 1 in 90 to 1 in 200 (Rosenberg, et al 2011). It is important that all patients with SAA are screened for inherited bone marrow failure syndromes (IBMFS) as treatment recommendations differ for the inherited versus immune mediated group (Miano and Dufour 2015). Patients with FA are often identified because of characteristic birth defects such as short stature, microcephaly, abnormal thumbs and café-au-lait spots; however, some patients have no physical findings (Fiesco-Roa, et al 2019).

FA patients are at high risk of AA, myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), as well as head and neck squamous cell carcinoma and other cancers (Alter, et al 2018, Nalepa and Clapp 2018). Hematopoietic cell transplantation (HCT) remains the only curative option for the bone marrow failure associated with FA and mitigates the risk of leukemia development (Dufour 2017). There has been significant HCT survival improvement observed for patients with FA after the introduction of fludarabine-based reduced intensity conditioning regimen (Tan, et al 2006). However, HCT is associated with an apparent increased risk of developing head and neck and other cancers that are associated with poor survival outcomes (Alter, et al 2018, Rosenberg, et al 2011).

While autosomal recessive inheritance of two pathogenic germline FA gene alleles clearly cause the disorder, the clinical effect of a single deleterious FA gene variant remains an open question. Variants from patients with FA have been cataloged at LOVD (https://databases.lovd.nl/shared/genes/FANCA), but this database is not comprehensive; as not all laboratories contribute, and not all variants in the database have been functionally analyzed. A 2014 study examined two publicly available sequencing databases (1000 Genomes and the Exome Sequencing Project) and found a surprisingly high number of deleterious FA gene variants within the population (78%), in 16 FA genes (Rogers, et al 2014). Here, we calculated the frequency of single deleterious FA gene variants in a large publicly available
database and in patients with SAA undergoing HCT, and examined the effect of those germline variants on patient post-transplant survival and cancer risk.

**Methods**

**Data Sources**

We used whole exome sequencing data from 125,748 individuals publicly available in the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org and gs://gnomadpublic/release/2.1; accessed on April 9, 2019) as a general population database. For patients with SAA, we used whole exome sequencing data generated in the Transplant Outcomes in Aplastic Anemia (TOAA) project cohort. TOAA is a large collaborative project between the National Cancer Institute (NCI) and the Center for International Blood and Marrow Transplant Research (CIBMTR) aiming at improving HCT outcomes for patients with SAA (Gadalla, et al 2016, Gadalla, et al 2015, Wang, et al 2019). This study uses samples and clinical information from the CIBMTR database and biorepository; available samples are mainly from unrelated donor transplants with recent collection from related donors started in 2007. The diagnosis of acquired SAA was the clinical diagnosis at the time of transplant.

Patient pre-HCT blood samples, clinical data and HCT outcome data were provided by the CIBMTR. The current study included 732 patients with acquired SAA (diagnosis provided by transplant center) who received an unrelated donor (n=636) or matched related donor (n=96) blood/marrow transplant with high-resolution HLA typing between 1989 and 2015 and had available DNA for whole exome sequencing. The study was approved by the National Marrow Donor Program institutional review board. All patients or their parents/guardians provided informed consent.

**TOAA Whole Exome Sequencing (WES) and Variant Validation**

We extracted DNA from pre-HCT peripheral blood mononuclear cells or whole blood from the recipients using the QIAamp Maxi Kit procedure (Qiagen Inc., Valencia, CA). Whole exome sequencing data were generated at the NCI Cancer Genomics Research Laboratory as described (Shi, et al 2014). Briefly, exon-enriched libraries were generated via NimbleGen v3 or v3+UTR capture kit. Samples were sequenced (Illumina MiSeq or HiSeq) to an average depth of ~55x and minimum coverage of >80% at 15x. Reads were aligned to the hg19 reference genome (Novoalign, Picard). GATK UnifiedGenotyper, GATK HaplotypeCaller, and Freebayes were used to call germline variants in an ensemble pipeline developed and optimized by the NCI’s Cancer Genomics Research Laboratory for germline familial exome analysis, as described elsewhere (Shi, et al 2014). All variants had a variant allele frequency of 40–60%. Variant calling was limited to only the regions in the intersection of both the v3 and v3+UTR capture kit.

All deleterious variants (see pathogenicity criteria below) from patients with SAA were reviewed using Integrative Genomics Viewer (IGV) software (http://software.broadinstitute.org/software/igv/), and 85% of the variants were also confirmed by an AmpliSeq targeted panel sequencing. A targeted, multiplex PCR primer panel was
designed using the custom Ion AmpliSeq Designer v7.06 (ThermoFisher, Pittsburgh, PA, USA) for the FA genes. Sample DNA (30ng) was amplified using this custom AmpliSeq panel, and libraries were prepared following the manufacturer’s Ion AmpliSeq Library Preparation protocol. Individual sample libraries were barcoded, pooled, templated, and subsequently sequenced on the Ion S5 XL Sequencer per manufacturer’s instructions. All variants were present at an allelic balance consistent with germline origin. One variant was not confirmed and was removed from the analysis. Two patients with SAA were found to have homozygous/biallelic deleterious mutations in an FA gene (FANCA and FANCP) and were excluded from our HCT outcome analysis because they were likely patients with FA not previously identified as such. Hence total number of patients included in this study is 730 (related donor n=95, and unrelated donor n=635).

**Variant filtering and pathogenicity criteria**

TOAA variants were initially filtered to remove: 1) synonymous variants 2) those with >1% minor allele frequency (MAF) in 1000 Genomes (http://www.internationalgenome.org/home), or ESP (http://evs.gs.washington.edu/EVS/) databases, 3) those with Fisher strand bias > 80, 4) those present in a run of homozygosity (>6) or in a segmental duplication, or 5) had positive C-score filter (https://bcbio-nextgen.readthedocs.io/en/latest/contents/citations.html). TOAA variants were limited to 22 genes in the FA/BRCA pathway using a bed file generated from the UCSC genome browser and bedtools (https://bedtools.readthedocs.io/en/latest/, http://genome.ucsc.edu). For both TOAA and gnomAD variants, variant annotation was done using ANNOVAR, Snpeff and ClinVar (Cingolani, et al 2012, Wang, et al 2010) (https://www.ncbi.nlm.nih.gov/clinvar/). Only rare variants, <1% MAF in ExAC non-TCGA, were evaluated. Intronic, UTR, in-frame deletions and start/stop loss variants were removed. Variants were considered deleterious if they were pathogenic or likely pathogenic by ClinVar based on clinical laboratories meeting minimum requirements for data sharing to support quality assurance by ClinGen (“badged lab” (http://www.clinicalgenome.org/lablist); if they were loss of function (frameshift, splice acceptor/donor site or stop gain); or if a missense variant had a positive in silico score (2 of 3 CADD>20, MetaSVM=D, REVEL>0.5) (Ioannidis, et al 2016, Kim, et al 2017, Rentzsch, et al 2019). Splice site variants were analyzed using the Variant Effect Predictor (VEP) (https://useast.ensembl.org/Tools/VEP) to determine rf and ada scores (Jian, et al/2014). Variants from the TOAA cohort were also reviewed for their presence in the FA variant database (https://databases.lovd.nl/shared/genes/FANCA). Variants were not scored using the ACMG/AMP guidelines due to the lack of information on patient and family history. The variant annotations noted here are predicted deleterious, and not clinically defined as pathogenic, unless noted by ClinVar.

**Statistical analysis**

Statistical analysis was done with the Kaplan-Meier estimator to calculate the probability of overall survival (OS) and 95% confidence intervals (CIs). The log-rank test was used to compare the survival distribution between patients with and without a variant in an FA gene. Follow-up time started at the date of HCT and ended at death or censoring at date of last follow-up or end of study on August 30, 2017. For multivariable analysis, we used Cox proportional hazard models adjusted for clinical variables. The selection of clinical factors
Results

Frequency of monoallelic FA deleterious variants in patients with SAA and the general population

Patients with SAA in the study ranged in age from 0.2–77.4 years (median=19.9), were mostly Caucasian, and received HCT primarily from an unrelated donor. Median follow-up was 31.7 months (range <1–294 months). No difference in conditioning regimen was noted between patients with and without an FA variant. Table 1 summarizes patient demographics and transplant-related factors by the presence or absence of a deleterious variant in an FA gene. We identified 21 single deleterious variants in 21 patients with SAA (2.9% of the total cohort; n=21/730). Identified deleterious variants were most frequently noted in FANCM (5/21) (Supplemental Table 1 provides a full list of identified deleterious variants and scoring details). Eleven of the 21 variants were previously reported in the FA-LOVD database. Ten of the 11 were classified as affecting function and one was inconclusive by FA-LOVD, but was predicted loss of function and hence was classified as deleterious. Of the identified variants, four were in BRCA2 and one is in BRCA1. BRCA1 and BRCA2 are DNA repair genes, and deleterious variants in the biallelic/homozygous form do cause Fanconi anemia, however, they are more often associated with heterozygous/autosomal dominant hereditary breast and ovarian cancer risk. Excluding these patients show a frequency of 2.2% (16/730) of single deleterious variants in FA non-BRCA gene variants in patients with SAA.

In gnomAD, we identified 3234 deleterious FA gene variants from 125,748 exomes. FANCA was the most common FA gene with a variant identified and is the most commonly mutated gene in patients with FA (495/3234, 2.6%, Table 2) (D’Andrea 2003). Five hundred thirteen of these variants were in the ClinVar database as pathogenic or likely pathogenic, and the remaining were deemed deleterious due to being a loss of function or predicted deleterious by in silico tools. Overall, combining all 22 genes, 2.6% of the individuals in gnomAD have a single deleterious variant in an FA gene, assuming one variant per exome (Table 2). This rate is similar to what we identified in patients with severe aplastic anemia (2.9%, Table 3). Excluding BRCA1 and BRCA2 variants from the analysis leaves 2344 non-BRCA1/2 FA gene variants in 125,748 exomes (1.9%).

Monoallelic FA deleterious variants and survival after HCT

The overall survival (OS) probabilities in patients with and without a deleterious variant in an FA gene were not different (p-log rank=0.68) (Figure 1). The probabilities of 3-year OS with and without a variant are 68% and 67%, respectively. This was also similar in analysis stratified by donor type (in unrelated donor HCT: 3-year OS=63% vs. 64%, respectively,
p=0.88; and in related donor HCT=100% vs. 90%, p=0.56). Multivariable analysis restricted to patients who received an unrelated donor HCT confirmed this observation (HR=0.86, 95% CI=0.38–1.98, p=0.73). The small event numbers and short follow-up for the related donor HCT subgroup did not allow for well powered adjusted analysis.

Given the known cancer predisposition of heterozygous \textit{BRCA1} and \textit{BRCA2} pathogenic variants we performed subset analyses with a separate category for patients with single deleterious \textit{BRCA1} and 2 variants, or after excluding them from the main analysis. No statistically significant differences in post-HCT overall survival between compared categories were noted in either analysis (Supplemental Figure 1&2).

We then evaluated whether monoallelic FA deleterious variants affected the patient’s post-HCT risk of cancer in the subset of SAA patients with unrelated HCT where information was available. No statistically significant difference in the frequency of post-HCT cancers was observed when comparing SAA patients with or without a single FA deleterious variant; post-HCT cancer was reported in 2/17 (11.8%) (prostate cancer (\textit{BRCA2}) and unknown cancer (\textit{FANCE})), and 47/606 (7.8%) in those with or without FA variant, respectively (p=0.54).

\section*{Discussion}

It is not uncommon to identify a single deleterious germline variant in an FA gene in patients with SAA, particularly due to the increased use of gene panels and whole exome sequencing in clinical testing. These variants can be a challenge to clinicians, as they may be identified in patients without suspicion or stigmata of FA. It is unknown whether these single variants may influence disease progression or change transplant outcome. In this study, we showed a comparable frequency of those variants in patients with SAA and in a large publicly available database, gnomAD. Our outcome analysis showed no association between the presence of those variants and patient survival or risk of cancer after HCT.

We show that deleterious variants in FA genes are not rare in the general population (2.6%); however, they are not nearly as common as previously reported. Rogers et al. reported a population frequency of 78% in the databases used (Rogers, \textit{et al} 2014). It is likely that the overestimation was due to a significantly smaller sample size (N=7578, Exome Sequencing Project and 1000Genomes), and/or aggregation of both healthy and diseased populations. Recent improvements in bioinformatics and in silico prediction tools, and the increased annotation of variants in ClinVar and other variant repositories have also improved our ability to classify variant pathogenicity.

Our results show a similar frequency of single deleterious FA variants in patients with SAA and the general population. In agreement with our findings, Przychodzen and colleagues found similar frequency of FA gene variants in 151 patients with AA and that in the ExAC database (5.9% vs. 6%, respectively); in contrast a higher frequency was noted in patients with myelodysplastic syndrome (9%) (Przychodzen, \textit{et al} 2018). This suggests that there is no relationship between risk of bone marrow failure in individuals with a single deleterious variant in an FA gene. We also show that patients with SAA with a single deleterious variant
in an FA gene do not have an increased risk of post-HCT mortality. These data suggest that finding a single deleterious FA variant on genetic testing done in the evaluation for bone marrow failure, is not likely to be of significant concern when considering HCT for presumed acquired SAA, in the absence of other relevant pathogenic variants, since no difference in OS has been noted. However, possible differences in patient profile of treatment related toxicity is possible and warrants further investigation. Caution should be exercised when interpreting our results due to the small sample size of patients with a FA variant, however this is the largest-to-date SAA cohort. A similar frequency of post-HCT cancer in SAA patients with and without single deleterious FA variants was also seen. However, it is possible that the relatively short follow-up time for some of the patients might have affected these results. Our study does not address the clinical implication of the presence of such variants in HCT donors, and this remains an open question for further investigation.

The study strengths include the availability of pre-transplant blood samples and post-transplant follow-up outcome data on a large population of patients with SAA. Limitations include our use of gnomAD database to represent the general population. The gnomAD database is a resource of genomic information gathered from various cohorts around the world, and thus contains data from both healthy and diseased individuals including those with cancer in proportion that may or may not reflect their frequencies in the general population. It is possible that this resulted in an overestimation of the rate of FA gene variants in the population. In addition, sequencing and calling methodologies differ between the two groups which may affect our reported comparisons. The variants were classified as deleterious using the same criteria for both groups. The small number of patients with monoallelic variants in FA genes may limit the study’s power to detect a statistically significant difference if present.

In conclusion, we examined the frequency of deleterious variants in FA genes a cohort of SAA patients, and found it to be similar to a general population database. Our study showed no association of such variants on HCT overall survival or post-transplant cancer risk. Thus, the identification of a single deleterious FA gene variant in SAA patients should not alter HCT planning, particularly in light of the current use of reduced intensity regimens for all patients with SAA.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgements**

The study was supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute; by a U.S. Public Health Service grant (U24-CA76518) from the National Cancer Institute, the National Heart Lung and Blood Institute and the National Institute of Allergy and Infectious Diseases, Heath Resources, and Services Administration (HHSH234200637015C); and by two grants N00014–17-1–2388 and N00014–18-1–2888 from the Office of Naval Research.
References

Alter BP, Giri N, Savage SA & Rosenberg PS. (2018) Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up. Haematologica, 103, 30–39. [PubMed: 29051281]

Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X & Ruden DM. (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin), 6, 80–92. [PubMed: 22728672]

D’Andrea A. (2003) Fanconi anemia. Curr Biol, 13, R546.

Dufour C. (2017) How I manage patients with Fanconi anaemia. Br J Haematol, 178, 32–47. [PubMed: 28474441]

Fiesco-Roa MO, Giri N, McReynolds LJ, Best AF & Alter BP. (2019) Genotype-phenotype associations in Fanconi anemia: A literature review. Blood Rev, 37, 100589.

Gadalla SM, Wang T, Dagnall C, Haagenson M, Spellman SR, Hicks B, Jones K, Katki HA, Lee SJ & Savage SA. (2016) Effect of Recipient Age and Stem Cell Source on the Association between Donor Telomere Length and Survival after Allogeneic Unrelated Hematopoietic Cell Transplantation for Severe Aplastic Anemia. Biol Blood Marrow Transplant, 22, 2276–2282. [PubMed: 27641680]

Gadalla SM, Wang T, Haagenson M, Spellman SR, Lee SJ, Williams KM, Wong YJ, De Vivo I & Savage SA. (2015) Association between donor leukocyte telomere length and survival after unrelated allogeneic hematopoietic cell transplantation for severe aplastic anemia. Jama, 313, 594–602. [PubMed: 25668263]

Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, Cannon-Albright LA, Teerlink CC, Stanford JL, Isaacs WB, Xu J, Cooney KA, Lange EM, Schleutker J, Carpten JD, Powell JJ, Cussenot O, Cancel-Tassin G, Giles GG, MacInnis RJ, Maier C, Hsieh CL, Wiklund F, Catalona WJ, Foulkes WD, Mandard D, Eeles RA, Kote-Jarai Z, Bustamante CD, Schaid DJ, Hastie T, Ostrander EA, Bailey-Wilson JE, Radivojac P, Thibodeau SN, Whittemore AS & Sieh W. (2016) REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. Am J Hum Genet, 99, 877–885. [PubMed: 27666373]

Jian X, Boerwinkle E & Liu X. (2014) In silico prediction of splice-altering single nucleotide variants in the human genome. Nucleic Acids Res, 42, 13534–13544. [PubMed: 25416802]

Kim S, Jhong JH, Lee J & Koo JY. (2017) Meta-analytic support vector machine for integrating multiple omics data. BioData Min, 10, 2. [PubMed: 28149325]

Miano M & Dufour C. (2015) The diagnosis and treatment of aplastic anemia: a review. Int J Hematol, 101, 527–535. [PubMed: 25837779]

Nalepa G & Clapp DW. (2018) Fanconi anaemia and cancer: an intricate relationship. Nat Rev Cancer, 18, 168–185. [PubMed: 29376519]

Przychodzen B, Makishima H, Sekeres MA, Balasubramanian SK, Thota S, Patel BJ, Clemente M, Hirsch C, Dienes B & Maciejewski JP. (2018) Fanconi Anemia germline variants as susceptibility factors in aplastic anemia, MDS and AML. Oncotarget, 9, 2050–2057. [PubMed: 29416752]

Rentzsch P, Witten D, Cooper GM, Shendure J & Kircher M. (2019) CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res, 47, D886–D894. [PubMed: 30371827]

Rogers KJ, Fu W, Akey JM & Monnat RJ Jr. (2014) Global and disease-associated genetic variation in the human Fanconi anemia gene family. Hum Mol Genet, 23, 68156825.

Rosenberg PS, Tamary H & Alter BP. (2011) How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for Fanconi Anemia in the United States and Israel. Am J Med Genet A, 155a, 1877–1883. [PubMed: 21739583]

Shi J, Yang XR, Ballev B, Rotunno M, Calista D, Fargnoli MC, Ghiorzo P, Bressacde Paillerets B, Nagore E, Avril MF, Caporaso NE, McMaster ML, Cullen M, Wang Z, Zhang X, Bruno W, Pastorino L, Queirolo P, Banuls-Roca J, Garcia-Casado Z, Vaysse A, Mohamdi H, Riazalhosseini Y, Foglio M, Jouenne F, Hua X, Hyland PL, Yin J, Vallabhaneni H, Chai W, Minghetti P, Pellegrini C, Ravichandran S, Eggennert A, Lathrop M, Peris K, Scarr GB, Landi G, Savage SA, Sampson B. Biol Blood Marrow Transplant. Author manuscript; available in PMC 2021 May 01.
JN, He J, Yeager M, Goldin LR, Demenais F, Chanock SJ, Tucker MA, Goldstein AM, Liu Y & Landi MT. (2014) Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. Nat Genet, 46, 482–486. [PubMed: 24686846]

Tan PL, Wagner JE, Auerbach AD, Defor TE, Slungaard A & Macmillan ML. (2006) Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation. Pediatr Blood Cancer, 46, 630–636. [PubMed: 16078221]

Wang K, Li M & Hakonarson H. (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res, 38, e164.

Wang Y, McReynolds LJ, Dagnall C, Katki HA, Spellman SR, Wang T, Hicks B, Freedman ND, Jones K, Lee SJ, Savage SA & Gadalla SM. (2019) Pre-transplant short telomeres are associated with high mortality risk after unrelated donor haematopoietic cell transplant for severe aplastic anaemia. Br J Haematol.
Highlights

Similar rate of single deleterious FA gene variants in SAA patients and the population.

Having an FA variant in an SAA patient does not affect overall survival after HCT.

No increased risk of cancer in patients with SAA and an FA gene variant after HCT.
Figure 1.
FA variants and survival after HCT in patients with SAA. Kaplan-Meier analysis comparing SAA patients with (dashed) and without (solid) a single deleterious FA gene variant.
### Table 1.

Patient characteristic by FA deleterious variant status

| Variable                                                      | Present (N=21) | Absent (N=709) | \( p \)  |
|---------------------------------------------------------------|---------------|----------------|---------|
| Recipient age at transplant, n (%)                           |               |                |         |
| <10                                                           | 5 (24%)       | 145 (20%)      | 0.97    |
| >10, <20                                                      | 6 (29%)       | 212 (30%)      |         |
| >20, <40                                                      | 7 (33%)       | 224 (32%)      |         |
| >40                                                           | 3 (14%)       | 128 (18%)      |         |
| Recipient sex, male, n (%)                                   | 13 (62%)      | 396 (56%)      | 0.58    |
| Recipient race<sup>3</sup>, Caucasian, n (%)                 | 16 (80%)      | 557 (79%)      | >0.99   |
| Karnofsky Performance Score<sup>3</sup>, n (%)               |               |                |         |
| <90                                                           | 4 (29%)       | 146 (25%)      | 0.76    |
| 90–100                                                        | 10 (71%)      | 429 (75%)      |         |
| HCT source, unrelated donor, n (%)                           | 17 (81%)      | 618 (87%)      | 0.34    |
| Stem cell source<sup>3</sup>, bone marrow, n (%)             | 19 (90%)      | 587 (83%)      | 0.56    |
| GVHD Prophylaxis<sup>3</sup>, n (%)                          |               |                |         |
| Tacrolimus based                                             | 6 (29%)       | 298 (42%)      | 0.51    |
| CSA based                                                    | 12 (57%)      | 334 (47%)      |         |
| Other                                                        | 3 (14%)       | 70 (10%)       |         |
| No GVHD prophylaxis                                          | 0 (0%)        | 6 (1%)         |         |
| Conditioning regimen<sup>3</sup>, n (%)                      |               |                |         |
| Myeloablative                                                | 7 (33%)       | 271 (38%)      | 0.59    |
| RIC/Nonmyeloablative                                         | 9 (43%)       | 327 (46%)      |         |
| Other                                                        | 5 (24%)       | 110 (16%)      | 0.71    |
| Donor age at transplant<sup>3</sup>, n (%)                   |               |                |         |
| <30                                                          | 6 (35%)       | 282 (43%)      |         |
| >30, <40                                                     | 5 (29%)       | 189 (29%)      |         |
| >40                                                          | 6 (35%)       | 181 (28%)      |         |
| Donor/recipient CMV serostatus, n (%)                        | 6 (29%)       | 192 (27%)      | 0.90    |
| Donor Negative/Recipient Negative                            | 5 (24%)       | 212 (30%)      |         |
| Donor Positive/Recipient Negative                            | 2 (10%)       | 83 (12%)       |         |
| Donor Positive/Recipient Positive                            | 7 (33%)       | 176 (25%)      |         |
| Unknown                                                      | 1 (5%)        | 46 (6%)        |         |
| Number HLA allele matching<sup>3,4</sup>, n (%)              |               |                |         |
| 8/8                                                          | 10 (59%)      | 385 (64%)      | 0.63    |
| <7/8                                                         | 7 (41%)       | 212 (36%)      |         |
| Year of transplant<sup>3</sup>, n (%)                        | 12 (57%)      | 242 (34%)      | 0.04    |

<sup>1</sup>\( p \) values indicate statistical significance.
| Variable   | Present (N=21) | Absent (N=709) | P<sup>1</sup> |
|------------|---------------|----------------|--------------|
| 2006–2010  | 2 (10%)       | 238 (34%)      |              |
| 2011–2015  | 7 (33%)       | 228 (32%)      |              |

<sup>1</sup> Chi-squared test unless otherwise specified

<sup>2</sup> Fisher’s exact test.

<sup>3</sup> Column totals may not add to total due to missing data; % presented are column percent

<sup>4</sup> Among recipients of unrelated donor HCT
### Table 2.

Number of Deleterious FA Gene Variants Identified in gnomAD

| Gene               | Total | LoF | ClinVar | In Silico Predicted | Rate  | Gene Size (kB) |
|--------------------|-------|-----|---------|---------------------|-------|----------------|
| **FANCA**          | 495   | 192 | 27      | 276                 | 0.39  | 79             |
| **FANCB**          | 1     | 1   | 0       | 0                   | 0.00  | 77             |
| **FANCC**          | 74    | 23  | 28      | 23                  | 0.06  | 328            |
| **FANCD1 (BRCA2)** | 482   | 63  | 170     | 249                 | 0.38  | 85             |
| **FANCD2**         | 164   | 103 | 3       | 58                  | 0.13  | 75             |
| **FANCE**          | 39    | 29  | 1       | 9                   | 0.03  | 15             |
| **FANCF**          | 33    | 27  | 4       | 2                   | 0.03  | 4.3            |
| **FANCG**          | 80    | 50  | 2       | 28                  | 0.06  | 6.2            |
| **FANC1**          | 278   | 112 | 2       | 164                 | 0.22  | 73             |
| **FANCJ (BRIP1)**  | 250   | 48  | 50      | 152                 | 0.20  | 185            |
| **FANCL**          | 86    | 54  | 2       | 30                  | 0.07  | 82             |
| **FANCM**          | 164   | 96  | 0       | 68                  | 0.13  | 65             |
| **FANCN (PALB2)**  | 102   | 37  | 60      | 5                   | 0.08  | 32             |
| **FANCO (RAD51C)** | 80    | 20  | 29      | 31                  | 0.06  | 43             |
| **FANCP (SLX4)**   | 112   | 98  | 3       | 11                  | 0.09  | 45             |
| **FANCQ (ERCC4)**  | 183   | 56  | 2       | 125                 | 0.15  | 30             |
| **FANCR (RAD51)**  | 26    | 12  | 0       | 14                  | 0.02  | 38             |
| **FANCS (BRCA1)**  | 408   | 40  | 123     | 245                 | 0.32  | 126            |
| **FANC (UBE2T)**   | 30    | 17  | 0       | 13                  | 0.02  | 10             |
| **FANCU (XRCC2)**  | 50    | 23  | 7       | 20                  | 0.04  | 31             |
| **FANCV (MAD2L2)** | 22    | 15  | 0       | 7                   | 0.02  | 17             |
| **FANCW (RFWD3)**  | .75   | 43  | 0       | 32                  | 0.06  | 38             |
| **Total**          | 3234  | 1159| 513     | 1562                |       |               |
| Rate % (#variants/#exomes) | 2.6  | 0.9 | 0.4     | 1.2                 |       |               |

* Loss of function
### Table 3.
Number of Deleterious FA Gene Variants Identified in the SAA Cohort

| Gene             | Total | LoF | ClinVar | In Silico Predicted |
|------------------|-------|-----|---------|---------------------|
| FANCA            | 3     | 1   | 2       | 0                   |
| FANCD1 (BRCA2)   | 4     | 0   | 3       | 1                   |
| FANCE            | 2     | 2   | 0       | 0                   |
| FANCl (BRIP1)    | 1     | 0   | 0       | 1                   |
| FANCM            | 5     | 4   | 0       | 1                   |
| FANCO (RAD51C)   | 1     | 0   | 1       | 0                   |
| FANCO (ERCC4)    | 2     | 0   | 1       | 1                   |
| FANCS (BRCA1)    | 1     | 0   | 0       | 1                   |
| FANCU (XRCC2)    | 2     | 1   | 1       | 0                   |
| Total            | 21    | 8   | 8       | 5                   |
| Rate % (#variants/patients) | 2.9 | 1.1 | 1.1 | 0.7 |

* Loss of function