ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine
Open Access WILEY

The causes of Fanconi anemia in South Asia and the Middle East: A case series and review of the literature

Ashley S. Thompson[1] | Nusrat Saba[2] | Lisa J. McReynolds[1] | Saeeda Munir[2] | Parvez Ahmed[3] | Sumaira Sajjad[2] | Kristine Jones[4] | Meredith Yeager[4] | Frank X. Donovan[5] | Settara C. Chandrasekharappa[5] | Blanche P. Alter[1] | Sharon A. Savage[1] 🄳 | Sadia Rehman[2]

[1]Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA

[2]Institute of Biomedical and Genetic Engineering, Islamabad, Pakistan

[3]Quaid-i-Azam International Hospital, Islamabad, Pakistan

[4]Cancer Genomics Research Laboratory, Leidos Biomedical Research, Frederick National Laboratory for Cancer Research, Frederick, MD, 20850, USA

[5]Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

Correspondence
Sharon A. Savage, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Rm. 6E456, MSC 9772, Bethesda, MD 20892-9772, USA.
Email: savagesh@mail.nih.gov

Sadia Rehman, Institute of Biomedical and Genetic Engineering, Islamabad G9/1 Mauve area, Pakistan 44000.
Email: sadiawasiq@gmail.com

Abstract

Background: Fanconi anemia (FA) is an inherited bone marrow failure syndrome associated with characteristic dysmorphology primarily caused by biallelic pathogenic germline variants in any of 22 different DNA repair genes. There are limited data on the specific molecular causes of FA in different ethnic groups.

Methods: We performed exome sequencing and copy number variant analyses on 19 patients with FA from 17 families undergoing hematopoietic cell transplantation evaluation in Pakistan. The scientific literature was reviewed, and we curated germline variants reported in patients with FA from South Asia and the Middle East.

Results: The genetic causes of FA were identified in 14 of the 17 families: seven *FANCA*, two *FANCC*, one *FANCF*, two *FANCG*, and two *FANCL*. Homozygous and compound heterozygous variants were present in 12 and two families, respectively. Nine families carried variants previously reported as pathogenic, including two families with the South Asian *FANCL* founder variant. We also identified five novel likely deleterious variants in *FANCA*, *FANCF*, and *FANCG* in affected patients.

Conclusions: Our study supports the importance of determining the genomic landscape of FA in diverse populations, in order to improve understanding of FA etiology and assist in the counseling of families.

KEYWORDS
Fanconi anemia, genetic testing, hematopoietic cell transplantation, inherited bone marrow failure syndrome, population genetics

1 | INTRODUCTION

Fanconi anemia (FA, MIM:227650) is a cancer-prone inherited bone marrow failure syndrome associated with radial ray abnormalities, characteristic facies, and other medical problems (Fiesco-Roa et al., 2019; Kottemann & Smogorzewska, 2013). Approximately 5% of patients with FA have the VACTERL-H phenotype (Vertebral anomalies, Anal atresia, Cardiac anomalies, Tracheoesophageal fistula, Esophageal atresia, Renal structural anomalies, Limb anomalies [primarily radii and/or thumbs], and Hydrocephalus; Alter & Rosenberg, 2013). Additional FA phenotypic findings are associated with Pigmentation of the skin, small Head, small Eyes, central Nervous system anomalies (excluding hydrocephalus), Otologic anomalies, and Short stature (PHENOS; Alter & Giri, 2016). Patients with FA have exceedingly high risks of head and neck squamous cell carcinoma (HNSCC) and leukemia compared with the general population (Alter et al., 2018). FA-associated bone marrow failure (BMF) frequently requires hematopoietic cell transplantation (HCT).

The laboratory diagnosis of FA is based on increased chromosome breakage in lymphocytes or fibroblasts after culture with clastogens such as mitomycin C (MMC) or diepoxybutane (DEB) and can be confirmed by germline genetic testing (Fargo et al., 2014). The majority of individuals with FA have autosomal recessive inheritance of pathogenic germline variants in specific DNA repair genes (Fiesco-Roa et al., 2019; Knies et al., 2017). *FANCB* and *FANCO/RAD51C* are the exceptions, inherited in an X-linked and autosomal dominant pattern, respectively. *FANCA* accounts for approximately 65% of cases, while *FANCC* and *FANCG* account for an additional 20% of cases in individuals of European ancestry (Wang & Smogorzewska, 2015). FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM comprise the core FA complex (upstream) in conjunction with FANCT/UBE2T, which is responsible for recognizing interstrand crosslinks and activating the FA DNA repair pathway through interaction with FANCD2 and FANCI (the ID complex). The downstream pathway facilitates DNA repair by homologous recombination performed by FANCD1/BRCA2, FANCJ/BRIP1, FANCN/PALB2, FANCO/RAD51C, FANCP/SLX4, FANCQ/ERCC4, FANCR/RAD51, FANCS/BRCA1, FANCU/XRCC1, FANCV/REV7, and FANCW/RFWD3 (Inano et al., 2017; Kitao & Takata, 2011; Rodriguez & D'Andrea, 2017).

There have been a limited number of reports on the genetic etiology of FA in populations from South Asia and the Middle East. Such studies have identified several novel disease-causing germline genetic variants, including the first reports of FA caused by pathogenic variants in *FANCO/RAD51C* or *FANCE* (Aftab et al., 2017; Aslan et al., 2015; Aymun et al., 2017; Balta et al., 2000; Castella et al., 2011; de Winter et al., 2000; Donovan et al., 2019; Dorsman et al., 2007; Esmail Nia et al., 2016; Ghazwani et al., 2016; Gille et al., 2012;

Kalb et al., 2007; Koc et al., 1999; Levran et al., 1997; Moghadam et al., 2016; Salem et al., 2014; Shahid et al., 2019; Shamseldin et al., 2012; Shukla et al., 2013; Solanki et al., ,2016, 2017; Tamary et al., ,2000, 2004; Vaz et al., 2010; Vundinti, 2014; Waisfisz et al., 1999; Wegner et al., 1996; Wijker et al., 1999; Zareifar et al., 2019) as well as discovery of a founder mutation in *FANCL* (Donovan et al., 2019), highlighting the importance of germline genetic studies of FA in underrepresented regions. In this report, we evaluated the genetic causes of FA in 19 patients from 17 unrelated families being considered for HCT in Pakistan and conducted a detail review of the causes of FA in South Asia and the Middle East.

2 | METHODS

2.1 | Editorial policies and ethical compliance

This project was approved by the ethical review committee of the Institute of Biomedical and Genetic Engineering (IBGE, Islamabad, Pakistan). Written informed consent was obtained from all study participants or the parent or guardian of participants who were under the age of 18 years old. Consent for the publication of identifying images or other personal or clinical details of participants that have the potential to compromise anonymity was obtained from all study participants or the parent or guardian of participants who were under the age of 18 years old.

2.2 | Study subjects

Individuals with FA and their first-degree relatives were evaluated by their referring physicians. Chromosome breakage using MMC on primary lymphocytes was performed at the Armed Forces Institute of Bone Marrow Transplant Center (ABMTC, Rawalpindi, Pakistan; Cervenka et al., 1981). All affected individuals had chromosome breakage results consistent with FA and severe BMF necessitating evaluation for HCT. De-identified blood-derived DNA samples from 17 unrelated families, including 19 individuals with FA and 33 relatives, were sent to the National Cancer Institute's Cancer Genomics Research Laboratory (NCI CGR, Gaithersburg, MD, USA) for sequencing and genotyping; a list of all study participants and their disease status is in Table S1.

2.3 | Sequence analysis

Exome sequencing was performed at NCI CGR as previously described (Ballew et al., 2013). Variants were filtered based

on their presence as homozygous or biallelic in previously identified FA-associated genes. Data from family members was evaluated when available. Variant annotation was performed using ANNOVAR (Wang et al., 2010) and the computational resources of the National Institutes of Health (NIH) High Performance Computing (HPC) Biowulf cluster (http://hpc.nih.gov). Additional filters applied include a Genome Aggregation Database (gnomAD) minor allele frequency (MAF) <1% in all populations, bioinformatic prediction tools (criteria for deleterious variants: MetaSVM >0, REVEL ≥0.5, and CADD phred >20), and clinical significance indicators (ClinVar and InterVar; Ioannidis et al., 2016; Karczewski et al., 2019; Kim et al., 2017; Kircher et al., 2014; Landrum et al., 2018; Li & Wang, 2017). Potential splice site variants were assessed using Human Splicing Finder (Desmet et al., 2009). We used BAM-matcher and vcftools to assess potential consanguinity as described (Manichaikul et al., 2010; Wang et al., 2016; Yang et al., 2010).

Copy number variations (CNV) were detected using VarSeq™ v2.1 (VS-CNV), which analyzes changes in WES coverage between the sample and controls (Fortier et al., 2018), and the detected CNVs were visually evaluated using GenomeBrowse® (Golden Helix, Inc.; Golden Helix, 2018a, 2018b). Homozygous or heterozygous deletions were filtered based on p-values (< 0.001) and annotated using ClinVar (Landrum et al., 2018). Z-scores and ratio values were assessed when validating genotypes. Suspected CNV events underwent validation using targeted whole gene sequencing as described (Chandrasekharappa et al., 2013).

DNA from families 3-FA, 8-FA, 9-FA, 12-FA, 14-FA, and 16-FA was also sequenced using a targeted custom capture design (Roche, Inc.) for next generation sequencing (NGS) designed to include all known FA genes and FA candidate genes, including all intronic regions and 5 kb upstream and downstream of each gene, as previously described (Chandrasekharappa et al., 2013). Sequence reads were aligned to human genome build 19 (GRCh37) using Burrows-Wheeler Alignment tool and variants were called using HaplotypeCaller and the GATK best practices pipeline for germline variants (DePristo et al., 2011; H. Li & Durbin, 2009; Van der Auwera et al., 2013). SNVs and indels were annotated using ANNOVAR (K. Wang et al., 2010) CNVs were detected from the targeted NGS reads and annotated using Nexus Copy Number version 10.0 (BioDiscovery, Inc.).

Additionally, DNA from family 17-FA was sequenced using PacBio® long-range sequencing technology with custom IDT xGen® Lockdown® Probes designed to capture all intronic and exonic regions of *FANCA*. The manufacturer's PacBio® protocols were followed for shearing genomic DNA, end repair, ligation of linear barcoded adapters, amplification, sample pooling, and capturing using IDT xGen® Lockdown® Probes. Libraries were prepared using SMRTbell® protocol for primer annealing, polymerase binding, and sequencing on the Sequel system. Circular consensus reads were generated using default parameters (3 passes, 0.99 accuracy) and demultiplexed according to parameters for symmetrical barcodes. Sequence reads were aligned to human genome build 19 (GRCh37) and structural variants were called using pbsv default parameters.

2.4 | Compilation of FA gene variants reported in South Asia and the Middle East

A comprehensive literature review was performed using the The National Library of Medicine's PubMed database using the following search terms combined with countries in South Asia and the Middle East, (e.g. "Fanconi anemia and Pakistan" or "Fanconi and India") to curate previously published FA gene variants reported in patients from the following regions (accessed July 10, 2020): Afghanistan, Bangladesh, Egypt, India, Iran, Iraq, Israel, Jordan, Lebanon, Nepal, Oman, Pakistan, Saudi Arabia, Syria, Turkey, and Yemen. Large cohort studies and case reports which only consisted of phenotypic data and did not report patients' specific genotypes were excluded. Studies which only reported FA subtypes by complementation testing were also excluded unless further sequencing efforts revealed the specific variant(s) in the patient(s).

3 | RESULTS

3.1 | Participant characteristics

There were 19 patients with FA (16 males and 3 females) from 17 unrelated families evaluated in this study (Figure 1). The majority of families (12/17, 70%) were from Northern or Central Punjab. Other families were from Southern Punjab, Islamabad, Khyber Pakhtunkhwa, and Azad Kashmir. The median age at FA diagnosis was 7 years (range 4–12), and while all were evaluated for HCT, only 5 patients underwent matched sibling HCT. Eight of the 19 participants were deceased at the time of this study. The median age at death was 8.5 years (range 4–13). Pathogenic variants relevant to FA were identified in 14 families with *FANCA* being the most common (7/14, 50%). Homozygous variants in FA-associated genes were identified in 12 of the 14 solved families (86%) and 2 probands had compound heterozygous variants. Physical and genetic findings for all participants are listed in Tables 1 and 2, respectively.

3.2 | *FANCA* variants and phenotypes

Individual 1-FA presented at age 7 years with aplastic anemia which progressed to severe BMF. He had ectopic kidneys,
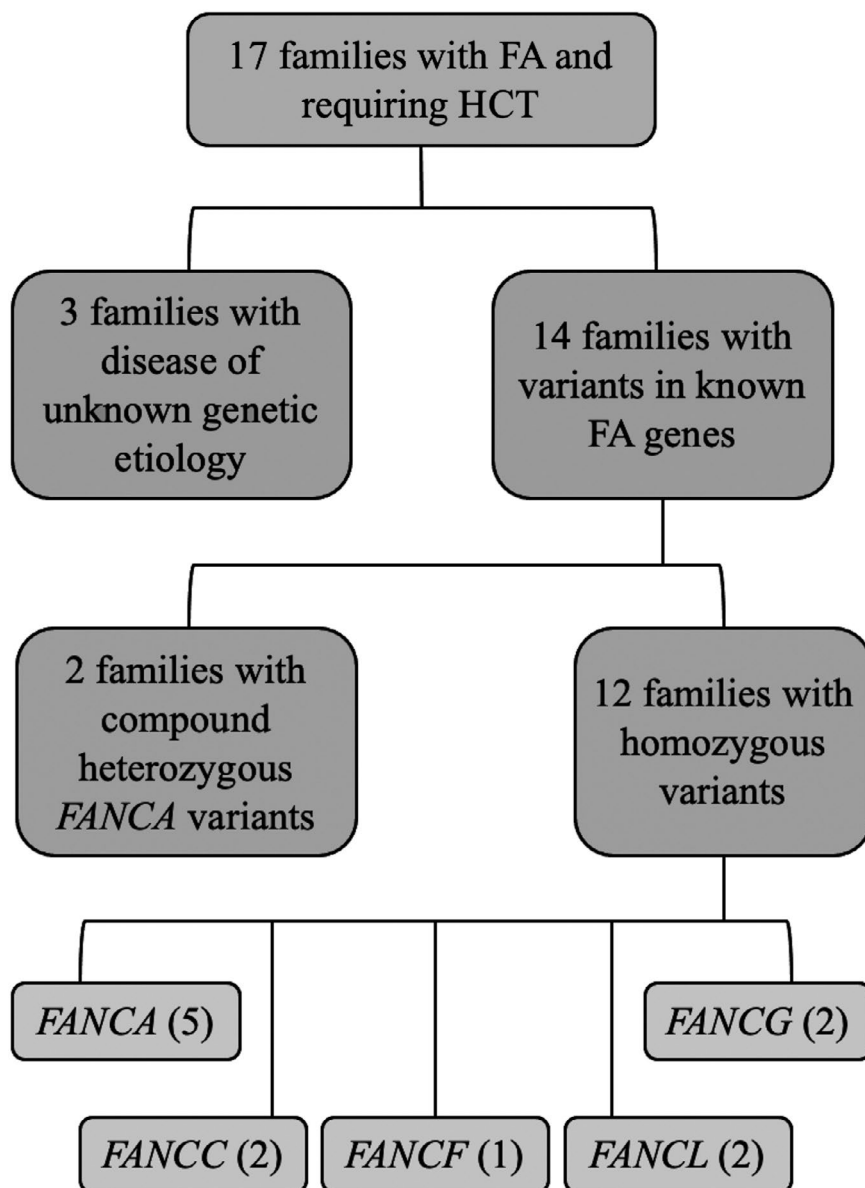
FIGURE 1 A flowchart of the genetic findings in 19 patients with FA from 17 unrelated families evaluated in this study

but no other phenotypic features were reported. We identified a homozygous in-frame deletion in exon 38 of *FANCA* (c.3788_3790delTCT, p.Phe1263del, NC_000016.9:g.89807 250_89807252delAGA, rs397507553, ClinVar:41003). Both parents were heterozygous carriers and his sibling was wild-type. The c.3788_3790delTCT variant is the most frequently reported *FANCA* variant and has been observed in multiple populations throughout the world, including FA patients from Pakistan (Castella et al., 2011; Kimble et al., 2018; Shahid et al., 2019). This in-frame deletion variant has particularly high prevalence in Spain and Brazil at an allele frequency of 0.01696% and 0.01633% in Latino/Admixed American and non-Finnish European populations, respectively (Castella et al., 2011; Levran et al., 1997).

Individual 3-FA had an abnormal thumb (Figure 2a) and café au lait spots noted at birth, as well as short stature and low gonadotrophin hormone levels. FA was diagnosed by

chromosome breakage on primary lymphocytes after he presented at 5 years of age with neutropenia that progressed to severe BMF. He underwent successful HLA-matched sibling donor HCT at the age of 6 years. 3-FA had two deletions in *FANCA* (NC_000016.9:g.89871674_89880557del, and NC_000016.9:89861527_89863726del) affecting exons 4–7 and 11, respectively. These two deletions have been previously reported in an Indian FA patient (Solanki et al., 2016). The exon 11 deletion was paternally inherited, while the deletion of exons 4–7 was maternally inherited. Validation by targeted sequencing methods determined that one unaffected sibling did not carry either deletion. Another unaffected sibling was predicted to be a carrier of the exon 4–7 deletion by VS-CNV but there was insufficient DNA for sequencing validation.

Severe BMF developed at 7 years of age in individual 4-FA. Hemophagocytosis was reported on his bone marrow biopsy but no other phenotypic information was available.

TABLE 1   Clinical features of study participants with Fanconi anemia.

Patient ID[a]	Sex	Age at Diagnosis (Years), Vital Status	Age at and Cause of Death	Skin	Hand	Renal	HCT (Yes/No)[b]	Other
1-FA	M	7, deceased	9, NR			Ectopic kidneys	No	
3-FA	M	5, alive	—	Café au lait macules	Thumb malformation		Yes	Short stature
4-FA	M	7, alive	—				Yes	
5-FA	M	11, deceased	12, NR				No	Abnormal left leg growth, short stature
6-FA	M	9, deceased	10, hemorrhage NOS	Hyperpig mentation	Polydactyly		No[c]	
7-FA	M	7, deceased	8, NR				Yes	
8-FA	M	4, deceased	4, brain hemorrhage		Absent radii and metacarpal bones		No[c]	High arched palate, skeletal malformations NOS
9-FA	F	10, alive	—				Yes	
10-FA	M	5, deceased	6, hemorrhagic stroke		Polydactyly		No	
12-FA	M	11, alive	—				Yes	
14-FA	M	12, deceased	13, NR				No	
16-FA	F	9, alive	—		Bilateral thumb malformations		Yes	
17_01-FA	M	6, alive	—		Thumb malformation		No	Immunodeficiency
17_02-FA	M	10, alive	—		Thumb malformation		No	Immunodeficiency
18-FA	M	5, alive	—		Polydactyly and small right hand	Ectopic kidneys	Yes	Short stature
19-FA	F	8, unknown	—				No	
20-FA	M	5, alive	—				No	
21_01-FA	M	8, deceased	13, NR				Yes	Numerous rosettes with central eosinophilic material on bone marrow biopsy
21_02-FA	M	5, alive	—					Numerous rosettes with central eosinophilic material on bone marrow biopsy

Abbreviations: F, Female; HCT, hematopoietic cell transplantation; M, Male; NOS, not otherwise specified; NR, Not reported.

[a]All participants had bone marrow failure and were being considered or underwent hematopoietic cell transplantation. Empty boxes indicate where clinical information was not available.

[b]All participants who successfully underwent HCT received material from an HLA-matched sibling. Participants who did not undergo transplant did not have a matched donor, unless indicated otherwise.

[c]Participant died prior to HLA-matched sibling HCT.

TABLE 2 Germline genetic variants identified in study participants with Fanconi anemia

Gene	Patient ID	Genotype	Variant	Previously reported	ClinVar	gnomAD % MAF[a] All; South Asian ancestry (Karczewski et al., 2019)	Consequence of Variant[b] (Ioannidis et al., 2016; Kim et al., 2017; Kircher et al., 2014)
FANCA	1-FA	Homozygous	c.3788_3790delTCT, p.Phe1263del	Castella et al. (2011)	Pathogenic	0.009929; 0	In-frame deletion
	3-FA	Compound Heterozygous	g.89871674-89880557del	(Solanki et al., 2016)	NR		Exons 4-7 Deletion
			g.89861527-89863726del		NR		Exon 11 Deletion
	4-FA	Homozygous	c.37dupC, p.Gln13Profs*24		NR		Frameshift
	9-FA	Homozygous	g.89856782-89874222del	Esmail Nia et al. (2016)	NR		Exons 7-14 Deletion
	17_01-FA and 17_02-FA	Compound Heterozygous	c.2749C>T, p.Arg917*	Solanki et al. (2016)	Pathogenic		Stop-gain
			g.89847600-89853759del	Gille et al. (2012)	NR		Exons 15-17 Deletion
	19-FA	Homozygous	c.4070C>A, p.Ala1357Asp		NR		Missense, MetaSVM 0.9149, REVEL 0.702, CADD phred 24.1
	20-FA	Homozygous	c.1541C>A, p.Ala514Asp		NR	**0.0000398; 0.003266**	Missense, MetaSVM 0.876, REVEL 0.851, CADD phred 27.1
FANCC	5-FA	Homozygous	c.1642C>T, p.Arg548*	Aftab et al. (2017)	Pathogenic	0.004954; 0.01307	Stop-gain
	8-FA	Homozygous	c.1642C>T, p.Arg548*				Stop-gain
FANCF	10-FA	Homozygous	c.785T>G, p.Leu262*		NR	0.0007955; 0.003266	Stop-gain
FANCG	18-FA	Homozygous	c.710C>G, p.Ser237*		NR		Stop-gain
	21_01-FA and 21_02-FA	Homozygous	c.1471_1473delAAAinsG, p.Lys491Glyfs*9	Gille et al. (2012)	NR	**0.0003976; 0.003266**	Frameshift
FANCL	6-FA	Homozygous	c.1092G>A, p.Trp341_Lys364del	Donovan et al. (2019)	NR	**0.001994; 0.01634**	Exon 13 Skipping
	7-FA	Homozygous	c.1092G>A, p.Trp341_Lys364del				

Abbreviations: MAF, minor allele frequency; NR, Not reported.

in silico tools were used to predict the pathogenicity of missense variants.

[a]% MAF in bold indicate these variants were only reported in South Asian populations. Blank cells indicate the variant was not present in gnomAD.

FIGURE 2 (a) An abnormal right thumb noted at birth in 3-FA, (b) an absent left thumb and abnormal right thumb in patient 17_01-FA, and (c) a small ear canal seen in Patient 17_01-FA

He underwent successful HLA-matched sibling HCT. A homozygous *FANCA* frameshift variant in exon 1 (c.37dupC, p.Gln13Profs*24, NC_000016.9:g.89882986dupG) was identified by exome sequencing. One unaffected sibling was wild-type and the other was a carrier, but parental DNA was not available for analysis.

Individual 9-FA presented with neutropenia which progressed to severe BMF and was diagnosed with FA at 10 years of age. Exome sequencing identified and targeted whole gene sequencing validated a large homozygous deletion of exons 7–14 (NC_000016.9:g.89856782_89874222 del). Her unaffected sister was a heterozygous carrier. This specific deletion has not been previously reported, but similar large deletions in *FANCA* have been reported (Ameziane et al., 2012).

Affected brothers 17_01-FA and 17_02-FA both presented with abnormal thumbs at birth (Figure 2b). Small ear canals were also noted in 17_01-FA (Figure 2c). At the ages of 6 and 10 years, respectively, they presented with severe BMF and a non-specific immunodeficiency leading to an FA diagnosis. Biallelic variants in *FANCA* were identified in both siblings (c.2749C>T, p.Arg917*, NC_000016.9:g.89831327G>A, and NC_000016.9:g.89847600-89853759del). Exome sequencing also revealed a maternally inherited nonsense variant in exon 28 which has been previously identified in an Indian patient with FA and other populations (rs1060501880, ClinVar:408188; Kimble et al., 2018; Solanki et al., 2016). A large deletion of exons 15–17 (NC_000016.9:g.89847600-89853759del) was detected by targeted PacBio® long-range

sequencing in both affected siblings and has been previously reported in other patients with FA (Gille et al., 2012). This deletion was not detected in DNA from father's peripheral blood, but relatedness analyses confirmed paternity with large regions of homozygosity being consistent with offspring from a consanguineous relationship between third-degree relatives. Additionally, analyses of single nucleotide polymorphisms (SNP) in the *FANCA* locus provided evidence for a possible genotypic reversion in the paternal hematopoietic stem cells or paternal inheritance as a result of gonadal mosaicism. Both such occurrences have been previously reported in patients with FA (Fargo et al., 2014; Gregory et al., 2001; Gross et al., 2002; Krausz et al., 2019).

Neutropenia developed at 8 years of age and progressed over the next year to severe BMF in individual 19-FA. A homozygous *FANCA* missense variant in exon 41 (c.4070C>A, p.Ala1357Asp, NC_000016.9:g.89805638G>T) was identified by exome sequencing. Her unaffected brother is a heterozygous carrier, but parental DNA was not available. *FANCA* p Ala1357Asp is not present in gnomAD and is predicted deleterious by *in silico* tools (MetaSVM score = 0.915, REVEL = 0.702, CADD phred = 24.1).

Individual 20-FA presented with moderate aplastic anemia that progressed to severe BMF by 5 years of age. We identified a homozygous missense variant in *FANCA* (c.1541C>A, p.Ala514Asp, NC_000016.9:g.89849440G>T, rs1432656621). His unaffected sibling is a heterozygous carrier. Although not previously reported, this missense variant is rare in gnomAD

at a MAF of 0.0003977% and is predicted deleterious by *in silico* tools (MetaSVM score = 0.876, REVEL = 0.851, CADD phred = 27.1).

3.3 | *FANCC* homozygous variant and phenotypes

Two unrelated probands, 5-FA and 8-FA, were homozygous for the same *FANCC* variant (c.1642C>T, p.Arg548*, NC_000009.11:g.97864024G>A). Relatedness analyses determined that these probands were from distinct families. *FANCC* p.Arg548* (rs104886457, ClinVar:12047) has been previously reported in two FA patients from Pakistan (Aftab et al., 2017).

The clinical features of 5-FA included moderate aplastic anemia progressing to severe BMF at age11 years. He also had short stature and abnormal left leg growth. He had two brothers and one sister who died due to similar complications but without a diagnosis. His two surviving unaffected siblings and parents are all heterozygous carriers.

Individual 8-FA presented with moderate aplastic anemia that also progressed to severe BMF by 4 years of age. Skin hyperpigmentation, bone deformities including the absence of metacarpals, thumbs, and radii, and a high arched palate were also reported. He died at the age of 4 years due to a brain hemorrhage before HLA-matched sibling HCT could be performed. One unaffected sibling is a carrier, but parental DNA was not available.

3.4 | *FANCF* homozygous variant and phenotype

Individual 10-FA was homozygous for non-sense variant in *FANCF* (c.785 T>G, p.Leu262*, NC_000011.9:g.22646572A>C, rs368067979). He was diagnosed with FA at 6 years of age when aplastic anemia progressed to severe BMF. He also had polydactyly and died from a hemorrhagic stroke shortly after his FA diagnosis. Parental DNA was not available and the sibling available for testing was not a carrier.

3.5 | *FANCG* variants and phenotypes

Individual 18-FA had an extra digit, a small right hand, short stature, and ectopic kidneys. He was diagnosed with FA at 5 years of age and underwent successful HCT from his HLA-matched sibling for severe BMF. A homozygous non-sense variant in exon 6 of *FANCG* was identified by WES (c.710C>G, p.Ser237*, NC_000009.11:g.35077035G>C). His sibling is wild-type at this locus. The only parent who

was available for testing was heterozygous for this loss of function variant.

Proband 21_01-FA and his brother 21_02-FA were diagnosed with FA at the ages of 8 and 5 years, respectively. 21_01-FA had pancytopenia that progressed rapidly following his diagnosis with FA and he died due to a brain hemorrhage. Currently, 21_02-FA does not have cytopenias. The bone marrow of both FA-affected brothers was reported to have numerous rosettes with central eosinophilic material surrounded by small cells seen in a background of fibrosis. The affected brothers have a homozygous frameshift variant in exon 11 of *FANCG* (c.1471_1473delAAAinsG, p.Lys-491Glyfs*9, NC_000009.11:g.35075283_35075285delTTT insC, rs1018027137). One of their unaffected siblings was heterozygous for this variant. This variant has been previously reported in a heterozygous patient with FA (Gille et al., 2012).
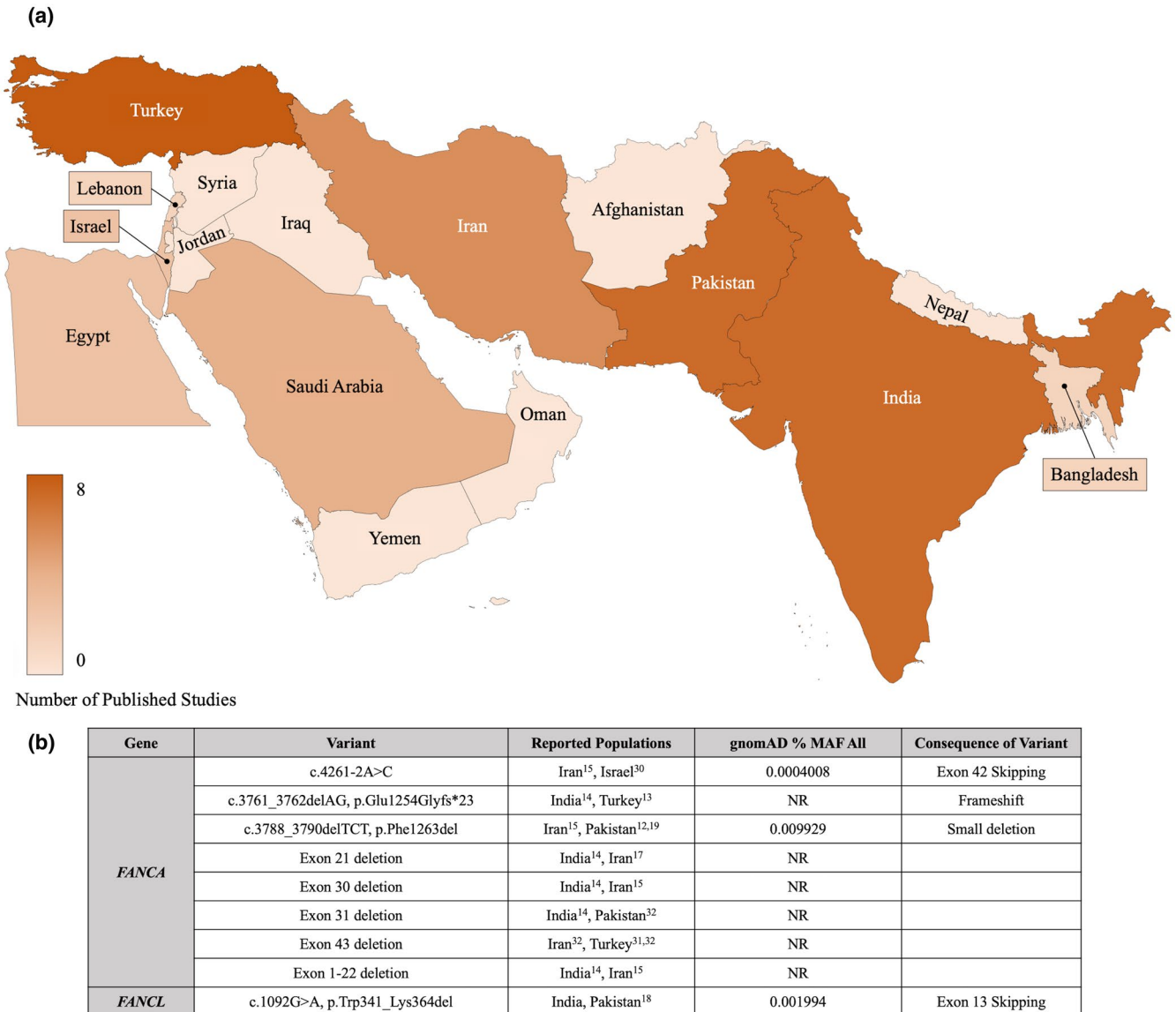
3.6 | *FANCL* variants and phenotypes

Unrelated probands 6-FA and 7-FA were both homozygous for a recently identified *FANCL* South Asian founder variant. Donovan *et al*. established this single nucleotide variation (NC_000002.11:g.58387243C>T) induces aberrant mRNA splicing to skip exon 13 (c.1021_1092del, p.Trp341_Lys364del, rs577063114), resulting in a 24 amino acid deletion from the RING domain of FANCL (Donovan et al., 2019). The gnomAD MAF of this variant is 0.001994% in all populations and 0.01634% in South Asian populations. A pairwise comparison between cases 6-FA and 7-FA was performed to assess potential relationships. A genotype comparison on approximately 7300 common SNPs between the probands and their siblings showed no indication of relatedness between families 6-FA and 7-FA. Parental sequencing data was not available.

Individual 6-FA had an extra thumb and areas of skin hyperpigmentation. He presented with aplastic anemia progressing to severe BMF at the age of 9 years. Although he was treated with androgens while awaiting an HLA sibling matched HCT, he died at 10 years of age due to an unspecified hemorrhage. Individual 7-FA was diagnosed with FA after presenting with severe BMF at 7 years of age. He died shortly after his diagnosis at the age of 8 years due to an unreported cause.

3.7 | Unsolved probands

Rare heterozygous variants in the 22 FA pathway genes were not identified in individual 12-FA, who was diagnosed with FA by chromosome breakage at the age of 11 years after presenting with BMF. He underwent a successful HLA-matched

(a)

Number of Published Studies

(b)

Gene	Variant	Reported Populations	gnomAD % MAF All	Consequence of Variant
FANCA	c.4261-2A>C	Iran[15], Israel[30]	0.0004008	Exon 42 Skipping
	c.3761_3762delAG, p.Glu1254Glyfs*23	India[14], Turkey[13]	NR	Frameshift
	c.3788_3790delTCT, p.Phe1263del	Iran[15], Pakistan[12,19]	0.009929	Small deletion
	Exon 21 deletion	India[14], Iran[17]	NR	
	Exon 30 deletion	India[14], Iran[15]	NR	
	Exon 31 deletion	India[14], Pakistan[32]	NR	
	Exon 43 deletion	Iran[32], Turkey[31,32]	NR	
	Exon 1-22 deletion	India[14], Iran[15]	NR	
FANCL	c.1092G>A, p.Trp341_Lys364del	India, Pakistan[18]	0.001994	Exon 13 Skipping

FIGURE 3 (a) A heat map showing the distribution of studies published from South Asia and the Middle East on the genetic etiology of FA. *This heat map was generated by the primary author using a tool available in Microsoft PowerPoint(v16.40)*. (b) The variants reported to cause FA in multiple populations in South Asia and the Middle East. MAF, minor allele frequency; NR, not reported

sibling HCT and had no reported dysmorphology. Family member DNA was not available to aid in further study.

Variants of potential interest but of uncertain significance were identified in individuals 14-FA and 16-FA and are reported in Table S2. FA was diagnosed in proband 14-FA at 12 year of age and he died 1 year after diagnosis due to an unreported cause. He was a heterozygous carrier for a variant of uncertain significance (VUS) c.583A>G, p.Ile-195Val in *FANCN* and a likely benign *FANCO* variant c.-48C>A. Bilateral thumb malformations were noted at birth in proband 16-FA who was diagnosed with aplastic anemia at age 9 and underwent a successful HLA-matched sibling HCT. Heterozygous VUS were present in *FANCA*, *FANCD2*, *FANCI*, and *FANCP*. The *FANCP* variant (c.2209C>T, p.Ar-g737Cys, NC_000016.9:g.3642818G>A, rs140706384) may

be deleterious as it has a REVEL score of 0.449 and a CADD score of 26.2, but the MetaSVM score was predicted as tolerated; additional functional studies are required to determine potential pathogenicity. There were no other deleterious variants or large CNV events detected in *FANCP*.

3.8 | Variants reported in individuals with FA from South Asia and the Middle East

We identified 29 published reports with data on gene variants in individuals with FA from South Asia and the Middle East. Reports from Turkey, India, and Pakistan were the most common, followed by Iran and Saudi Arabia (Figure 3a). Tables S3–S5 list the specific large deletions, single nucleotide variants,

and small insertions and deletions, respectively, along with the relevant references. Ten of the 29 publications reported on targeted sequencing of only the *FANCA* gene. The other publications performed a combination of hotspot variant analysis, targeted gene sequencing, Sanger sequencing, RNA sequencing, other next generation sequencing methods, and multiplex ligation-dependent probe amplification analysis. Variants reported in more than one population are noted in Figure 3b.

The majority of reported variants occurred in *FANCA (78%)* and were private to their respective populations. Variants reported in *FANCG*, *FANCC*, and *FANCE* accounted for 7.8%, 2.8%, and 2.8% of the total variants reported. The only large deletions reported were in *FANCA*, similar to our findings and consistent with others. Donovan et al recently identified a *FANCL* founder variant in South Asian populations (c.1092G>A, p.Trp341_Lys364del; Donovan et al., 2019). We identified this same founder variant in two, unrelated families from Pakistan in our cohort.

4 | DISCUSSION

The identification of the genetic causes of rare diseases such as FA is important to verify diagnoses, improve clinical management, allow for appropriate genetic counseling, and understand their underlying pathobiology. The genetic cause of FA was identified in 16 patients from 14 families in this study; three families remain molecularly undiagnosed. *FANCA* was the most commonly affected gene with pathogenic *FANCA* variants present in 50% of the families. The other pathogenic variants were present in *FANCC*, *FANCG*, or *FANCL* (2 families each), and *FANCF* (1 family). Only 6 of the 14 variants identified in our study were present in any gnomAD population and 3 of these were solely in South Asian populations (Table 2).

Homozygosity for pathogenic variants was present in 12 of the 14 families. Only two families had compound heterozygous inheritance (both in *FANCA*). Two families from the Central Punjab region of Pakistan were homozygous for the *FANCL* founder variant (Donovan et al., 2019). We were unable to evaluate consanguinity in 14 of the families in this study due to lack of parental DNA samples. However, the presence of homozygous pathogenic variants in our data is consistent with prior studies reporting an approximately 70% rate of consanguineous marriages in Pakistan (Hussain & Bittles, 1998; Mobarak et al., 2019; National Institute of Population, 2013). Nine of the 14 pathogenic variants identified in this study have been previously reported (Table 2; Aftab et al., 2017; Castella et al., 2011; Donovan et al., 2019; Esmail Nia et al., 2016; Gille et al., 2012; Solanki et al., 2016).

While our study is limited to individuals with FA and BMF severe enough to warrant HCT evaluation, it is one of a very few studies seeking to understand the type and frequencies of FA-associated germline variants in the Pakistani population. We expect that the individuals included in this study represent a small subset of FA cases in Pakistan. It is possible that in the absence of overt dysmorphology, the diagnosis of FA may be delayed or missed because chronic malnutrition could confound diagnoses such as anemia and even overt BMF (National Institute of Population, 2013).

The majority of studies published, to date, on the genetics of FA in South Asia and the Middle East focused primarily on targeted sequencing of *FANCA*, with *FANCC*, *FANCG*, and *FANCE* being evaluated but less frequently. *FANCA*, *FANCC*, and *FANCG* account for upwards of 85% of FA cases in individuals of northern European ancestry but the relatively small number of studies from other ethnic groups limits our understanding of the population genetics of FA-associated genes and the full scope of FA around the world. A limited number of FA genotype-phenotype studies suggest there may be phenotypic differences based on the associated genetic etiology (Fiesco-Roa et al., 2019). The presence of founder mutations in *FANCA*, *FANCG*, and *FANCL* warrants additional study in the context of biology and clinical manifestations.

Large genotype-phenotype studies of patients with FA around the world as well as population-based sequencing efforts are required to better understand the genetic variation of the FA-associated DNA repair genes in diverse populations in order to uncover disease etiology, improve diagnostics and patient management, and to provide appropriate genetic counseling.

AUTHORS' CONTRIBUTIONS

Data acquisition: NS, SM, PA, SS, SR; data analysis and interpretation: AST, LJM, KJ, MY, FXD, SCC, BPA, SAS, SR; drafted manuscript: AST, SAS; and all authors approved of the final manuscript.

DATA AVAILABILITY STATEMENT

The deidentified genomic data generated in this study are available upon reasonable request from Dr. Sadia Rehman (sadiawasiq@gmail.com, Institute of Biomedical and Genetic Engineering, Islamabad, Pakistan) through collaboration agreements.

ORCID

Sharon A. Savage https://orcid.org/0000-0001-6006-0740

REFERENCES

Aftab, I., Iram, S., Khaliq, S., Israr, M., Ali, N., Jahan, S., & Mohsin, S. (2017). Analysis of FANCC gene mutations (IVS4+4A>T, del322G, and R548X)in patients with Fanconi anemia in Pakistan. *The Turkish Journal of Medical Sciences*, *47*(2), 391–398. https://doi.org/10.3906/sag-1506-53

Alter, B. P., & Giri, N. (2016). Thinking of VACTERL-H? Rule out Fanconi Anemia according to PHENOS. *American Journal of Medical Genetics Part A*, *170*(6), 1520–1524. https://doi.org/10.1002/ajmg.a.37637

Alter, B. P., Giri, N., Savage, S. A., & Rosenberg, P. S. (2018). Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up. *Haematologica*, *103*(1), 30–39. https://doi.org/10.3324/haematol.2017.178111

Alter, B. P., & Rosenberg, P. S. (2013). VACTERL-H association and fanconi anemia. *Molecular Syndromology*, *4*(1–2), 87–93. https://doi.org/10.1159/000346035

Ameziane, N., Sie, D., Dentro, S., Ariyurek, Y., Kerkhoven, L., Joenje, H., Dorsman, J. C., Ylstra, B., Gille, J. J. P., Sistermans, E. A., & de Winter, J. P. (2012). Diagnosis of fanconi anemia: mutation analysis by next-generation sequencing. *Anemia*, *2012*, 132856. https://doi.org/10.1155/2012/132856

Aslan, D., Ameziane, N., & De Winter, J. P. (2015). Molecular diagnosis of Fanconi anemia with next-generation sequencing in a case with subtle signs and a negative chromosomal breakage test. *The Turkish Journal of Pediatrics*, *57*(3), 282–285.

Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K. V., Altshuler, D., Gabriel, S., & DePristo, M. A. (2013). From FastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1), 43. https://doi.org/10.1002/0471250953.bi1110s43

Aymun, U., Iram, S., Aftab, I., Khaliq, S., Nadir, A., Nisar, A., & Mohsin, S. (2017). Screening for mutations in two exons of FANCG gene in Pakistani population. *Biomedical Papers*, *161*(2), 158–163. https://doi.org/10.5507/bp.2017.030

Ballew, B. J., Yeager, M., Jacobs, K., Giri, N., Boland, J., Burdett, L., Alter, B. P., & Savage, S. A. (2013). Germline mutations of regulator of telomere elongation helicase 1, RTEL1, in Dyskeratosis Congenita. *Human Genetics*, *132*(4), 473–480. https://doi.org/10.1007/s00439-013-1265-8

Balta, G., de Winter, J. P., Kayserili, H., Pronk, J. C., & Joenje, H. (2000). Fanconi anemia A due to a novel frameshift mutation in hotspot motifs: lack of FANCA protein. *Human Mutation*, *15*(6), 578. https://doi.org/10.1002/1098-1004(200006)15:6<578:AID-HUMU12>3.0.CO;2-Q

Castella, M., Pujol, R., Callén, E., Trujillo, J. P., Casado, J. A., Gille, H., Lach, F. P., Auerbach, A. D., Schindler, D., Benítez, J., Porto, B., Ferro, T., Muñoz, A., Sevilla, J., Madero, L., Cela, E., Beléndez, C., de Heredia, C. D., Olivé, T., … Surrallés, J. (2011). Origin, functional role, and clinical impact of Fanconi anemia FANCA mutations. *Blood*, *117*(14), 3759–3769. https://doi.org/10.1182/blood-2010-08-299917

Cervenka, J., Arthur, D., & Yasis, C. (1981). Mitomycin C test for diagnostic differentiation of idiopathic aplastic anemia and Fanconi anemia. *Pediatrics*, *67*(1), 119–127.

Chandrasekharappa, S. C., Lach, F. P., Kimble, D. C., Kamat, A., Teer, J. K., Donovan, F. X., Flynn, E., Sen, S. K., Thongthip, S., Sanborn, E., Smogorzewska, A., Auerbach, A. D., & Ostrander, E. A. (2013). Massively parallel sequencing, aCGH, and RNA-Seq technologies provide a comprehensive molecular diagnosis of Fanconi anemia. *Blood*, *121*(22), e138–148. https://doi.org/10.1182/blood-2012-12-474585

de Winter, J. P., Léveillé, F., van Berkel, C. G. M., Rooimans, M. A., van der Weel, L., Steltenpool, J., Demuth, I., Morgan, N. V., Alon, N., Bosnoyan-Collins, L., Lightfoot, J., Leegwater, P. A., Waisfisz, Q., Komatsu, K., Arwert, F., Pronk, J. C., Mathew, C. G., Digweed, M., Buchwald, M., & Joenje, H. (2000). Isolation of a cDNA representing the Fanconi anemia complementation group E gene. *American Journal of Human Genetics*, *67*(5), 1306–1308. https://doi.org/10.1016/S0002-9297(07)62959-0

DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., del Angel, G., Rivas, M. A., Hanna, M., McKenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel, S. B., Altshuler, D., & Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, *43*(5), 491–498. https://doi.org/10.1038/ng.806

Desmet, F. O., Hamroun, D., Lalande, M., Collod-Beroud, G., Claustres, M., & Beroud, C. (2009). Human Splicing Finder: An online bioinformatics tool to predict splicing signals. *Nucleic Acids Research*, *37*(9), e67. https://doi.org/10.1093/nar/gkp215

Donovan, F. X., Solanki, A., Mori, M., Chavan, N., George, M., Okuno, Y., Muramastsu, H., Yoshida, K., Shimamoto, A., Takaori-Kondo, A., & Yabe, H. (2020). A founder variant in the South Asian population leads to a high prevalence of FANCL Fanconi anemia cases in India. *Human Mutation*, *41*, 122–128. https://doi.org/10.1002/humu.23914

Dorsman, J. C., Levitus, M., Rockx, D., Rooimans, M. A., Oostra, A. B., Haitjema, A., & Joenje, H. (2007). Identification of the Fanconi anemia complementation group I gene, FANCI. *Cell Oncology*, *29*(3), 211–218. https://doi.org/10.1155/2007/151968

Esmail Nia, G., Fadaee, M., Royer, R., Najmabadi, H., & Akbari, M. R. (2016). Profiling fanconi anemia gene mutations among Iranian patients. *Archives of Iranian Medicine*, *19*(4), 236–240.

Fargo, J. H., Rochowski, A., Giri, N., Savage, S. A., Olson, S. B., & Alter, B. P. (2014). Comparison of chromosome breakage in non-mosaic and mosaic patients with Fanconi anemia, relatives, and patients with other inherited bone marrow failure syndromes. *Cytogenetic and Genome Research*, *144*(1), 15–27. https://doi.org/10.1159/000366251

Fiesco-Roa, M. O., Giri, N., McReynolds, L. J., Best, A. F., & Alter, B. P. (2019). Genotype-phenotype associations in Fanconi anemia: A literature review. *Blood Reviews*, *37*, 100589. https://doi.org/10.1016/j.blre.2019.100589

Fortier, N., Rudy, G., & Scherer, A. (2018). Detection of CNVs in NGS data using VS-CNV. *Methods in Molecular Biology*, *1833*, 115–127. https://doi.org/10.1007/978-1-4939-8666-8_9

Ghazwani, Y., AlBalwi, M., Al-Abdulkareem, I., Al-Dress, M., Alharbi, T., Alsudairy, R., Alomari, A., Aljamaan, K., Essa, M., Al-Zahrani, M., & Alsultan, A. (2016). Clinical characteristics and genetic subtypes of Fanconi anemia in Saudi patients. *Cancer Genet*, *209*(4), 171–176. https://doi.org/10.1016/j.cancergen.2016.02.003

Gille, J. J., Floor, K., Kerkhoven, L., Ameziane, N., Joenje, H., & de Winter, J. P. (2012). Diagnosis of fanconi anemia: mutation analysis by multiplex ligation-dependent probe amplification and PCR-based sanger sequencing. *Anemia*, *2012*, 603253. https://doi.org/10.1155/2012/603253

Golden Helix, I. (2018a). GenomeBrowse® v2.1. Golden Helix, Inc. http://www.goldenhelix.com

Golden Helix, I. (2018b). VarSeq™ v2.1. Golden Helix, Inc. http://www.goldenhelix.com

Gregory, J. J., Wagner, J. E., Verlander, P. C., Levran, O., Batish, S. D., Eide, C. R., Steffenhagen, A., Hirsch, B., & Auerbach, A. D. (2001). Somatic mosaicism in Fanconi anemia: evidence of genotypic reversion in lymphohematopoietic stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(5), 2532–2537. https://doi.org/10.1073/pnas.051609898.

Gross, M., Hanenberg, H., Lobitz, S., Friedl, R., Herterich, S., Dietrich, R., Gruhn, B., Schindler, D., & Hoehn, H. (2002). Reverse mosaicism in Fanconi anemia: Natural gene therapy via molecular self-correction. *Cytogenetic and Genome Research*, *98*(2–3), 126–135. https://doi.org/10.1159/000069805

Hussain, R., & Bittles, A. H. (1998). The prevalence and demographic characteristics of consanguineous marriages in Pakistan. *Journal of Biosocial Science*, *30*(2), 261–275. https://doi.org/10.1017/s0021932098002612

National Institute of Population Studies, I. I. (2013). Pakistan Demographic and Health Survey.

Inano, S., Sato, K., Katsuki, Y., Kobayashi, W., Tanaka, H., Nakajima, K., Nakada, S., Miyoshi, H., Knies, K., Takaori-Kondo, A., Schindler, D., Ishiai, M., Kurumizaka, H., & Takata, M. (2017). RFWD3-Mediated ubiquitination promotes timely removal of both RPA and RAD51 from DNA damage sites to facilitate homologous recombination. *Molecular Cell*, *66*(5), 622–634e628. https://doi.org/10.1016/j.molcel.2017.04.022

Ioannidis, N. M., Rothstein, J. H., Pejaver, V., Middha, S., McDonnell, S. K., Baheti, S., Musolf, A., Li, Q., Holzinger, E., Karyadi, D., Cannon-Albright, L. A., Teerlink, C. C., Stanford, J. L., Isaacs, W. B., Xu, J., Cooney, K. A., Lange, E. M., Schleutker, J., Carpten, J. D., … Sieh, W. (2016). REVEL: An ensemble method for predicting the pathogenicity of rare missense variants. *American Journal of Human Genetics*, *99*(4), 877–885. https://doi.org/10.1016/j.ajhg.2016.08.016

Kalb, R., Neveling, K., Hoehn, H., Schneider, H., Linka, Y., Batish, S. D., Hunt, C., Berwick, M., Callén, E., Surrallés, J., Casado, J. A., Bueren, J., Dasí, Á., Soulier, J., Gluckman, E., Zwaan, C. M., van Spaendonk, R., Pals, G., de Winter, J. P., … Schindler, D. (2007). Hypomorphic mutations in the gene encoding a key Fanconi anemia protein, FANCD2, sustain a significant group of FA-D2 patients with severe phenotype. *American Journal of Human Genetics*, *80*(5), 895–910. https://doi.org/10.1086/517616

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., … MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, *581*(7809), 434–443. https://doi.org/10.1038/s41586-020-2308-7. Epub 2020 May 27. Erratum in: Nature. 2021 Feb;590(7846):E53. PMID: 32461654; PMCID: PMC7334197.

Kim, S., Jhong, J. H., Lee, J., & Koo, J. Y. (2017). Meta-analytic support vector machine for integrating multiple omics data. *BioData Min*, *10*, 2. https://doi.org/10.1186/s13040-017-0126-8

Kimble, D. C., Lach, F. P., Gregg, S. Q., Donovan, F. X., Flynn, E. K., Kamat, A., Young, A., Vemulapalli, M., Thomas, J. W., Mullikin, J. C., Auerbach, A. D., Smogorzewska, A., & Chandrasekharappa, S. C. (2018). A comprehensive approach to identification of pathogenic FANCA variants in Fanconi anemia patients and their families. *Human Mutation*, *39*(2), 237–254. https://doi.org/10.1002/humu.23366

Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J., Cooper, G. M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics*, *46*(3), 310–315. https://doi.org/10.1038/ng.2892

Kitao, H., & Takata, M. (2011). Fanconi anemia: A disorder defective in the DNA damage response. *International Journal of Hematology*, *93*(4), 417–424. https://doi.org/10.1007/s12185-011-0777-z

Knies, K., Inano, S., Ramirez, M. J., Ishiai, M., Surralles, J., Takata, M., & Schindler, D. (2017). Biallelic mutations in the ubiquitin ligase RFWD3 cause Fanconi anemia. *Journal of Clinical Investigation*, *127*(8), 3013–3027. https://doi.org/10.1172/JCI92069

Koc, A., Pronk, J. C., Alikasifoglu, M., Joenje, H., & Altay, C. (1999). Variable pathogenicity of exon 43del (FAA) in four Fanconi anaemia patients within a consanguineous family. *British Journal of Haematology*, *104*(1), 127–130. https://doi.org/10.1046/j.1365-2141.1999.01156.x

Kottemann, M. C., & Smogorzewska, A. (2013). Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature*, *493*(7432), 356–363. https://doi.org/10.1038/nature11863

Krausz, C., Riera-Escamilla, A., Chianese, C., Moreno-Mendoza, D., Ars, E., Rajmil, O., Pujol, R., Bogliolo, M., Blanco, I., Rodríguez, I., Badell, I., Ruiz-Castañé, E., & Surrallés, J. (2019). From exome analysis in idiopathic azoospermia to the identification of a high-risk subgroup for occult Fanconi anemia. *Genetics in Medicine*, *21*(1), 189–194. https://doi.org/10.1038/s41436-018-0037-1

Landrum, M. J., Lee, J. M., Benson, M., Brown, G. R., Chao, C., Chitipiralla, S., Gu, B., Hart, J., Hoffman, D., Jang, W., Karapetyan, K., Katz, K., Liu, C., Maddipatla, Z., Malheiro, A., McDaniel, K., Ovetsky, M., Riley, G., Zhou, G., … Maglott, D. R. (2018). ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Research*, *46*(D1), D1062–D1067. https://doi.org/10.1093/nar/gkx1153

Levran, O., Erlich, T., Magdalena, N., Gregory, J. J., Batish, S. D., Verlander, P. C., & Auerbach, A. D. (1997). Sequence variation in the Fanconi anemia gene FAA. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(24), 13051–13056. https://doi.org/10.1073/pnas.94.24.13051

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, Q., & Wang, K. (2017). InterVar: Clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *American Journal of Human Genetics*, *100*(2), 267–280. https://doi.org/10.1016/j.ajhg.2017.01.004

Manichaikul, A., Mychaleckyj, J. C., Rich, S. S., Daly, K., Sale, M., & Chen, W. M. (2010). Robust relationship inference in genome-wide association studies. *Bioinformatics*, *26*(22), 2867–2873. https://doi.org/10.1093/bioinformatics/btq559

Mobarak, A. M., Chaudhry, T., Brown, J., Zelenska, T., Khan, M. N., Chaudry, S., Wajid, R. A., Bittles, A. H., & Li, S. (2019).

Estimating the health and socioeconomic effects of cousin marriage in South Asia. *Journal of Biosocial Science*, *51*(3), 418–435. https://doi.org/10.1017/S0021932018000275

Moghadam, A. A., Mahjoubi, F., Reisi, N., & Vosough, P. (2016). Investigation of FANCA gene in Fanconi anaemia patients in Iran. *Indian Journal of Medical Research*, *143*(2), 184–196. https://doi.org/10.4103/0971-5916.180206

Rodriguez, A., & D'Andrea, A. (2017). Fanconi anemia pathway. *Current Biology*, *27*(18), R986–R988. https://doi.org/10.1016/j.cub.2017.07.043

Salem, A. M., El-Bassyouni, H. T., El-Kamah, G. Y., Zarouk, W. A., Eid, M. M., Mosaad, R. M., Sayed, A. A., & Temtamy, S. A. (2014). Screening for common mutations in four FANCA gene exons in Egyptian Fanconi anemia patients. *Middle East Journal of Medical Genetics*, *3*(1), 24–30. https://doi.org/10.1097/01.MXE.0000438179.47299.3c

Shahid, M., Firasat, S., Satti, H. S., Satti, T. M., Ghafoor, T., Sharif, I., & Afshan, K. (2019). Screening of the FANCA gene mutational hotspots in the Pakistani fanconi anemia patients revealed 19 sequence variations. *Congenit Anom (Kyoto)*, 2020 Jan;60(1):32–39. doi: 10.1111/cga.12331. Epub 2019 Mar 24. PMID: 30809872.

Shamseldin, H. E., Elfaki, M., & Alkuraya, F. S. (2012). Exome sequencing reveals a novel Fanconi group defined by XRCC2 mutation. *Journal of Medical Genetics*, *49*(3), 184–186. https://doi.org/10.1136/jmedgenet-2011-100585

Shukla, P., Rao, A., Ghosh, K., & Vundinti, B. R. (2013). Identification of a novel large intragenic deletion in a family with Fanconi anemia: first molecular report from India and review of literature. *Gene*, *518*(2), 470–475. https://doi.org/10.1016/j.gene.2013.01.016

Solanki, A., Kumar Selvaa, C., Sheth, F., Radhakrishnan, N., Kalra, M., & Vundinti, B. R. (2017). Characterization of two novel FANCG mutations in Indian Fanconi anemia patients. *Leukemia Research*, *53*, 50–56. https://doi.org/10.1016/j.leukres.2016.11.013

Solanki, A., Mohanty, P., Shukla, P., Rao, A., Ghosh, K., & Vundinti, B. R. (2016). FANCA gene mutations with 8 novel molecular changes in Indian Fanconi anemia patients. *PLoS One*, *11*(1), e0147016. https://doi.org/10.1371/journal.pone.0147016

Tamary, H., Bar-Yam, R., Shalmon, L., Rachavi, G., Krostichevsky, M., Elhasid, R., Barak, Y., Kapelushnik, J., Yaniv, I., Auerbach, A. D., & Zaizov, R. (2000). Fanconi anaemia group A (FANCA) mutations in Israeli non-Ashkenazi Jewish patients. *British Journal of Haematology*, *111*(1), 338–343. https://doi.org/10.1046/j.1365-2141.2000.02323.x

Tamary, H., Dgany, O., Toledano, H., Shalev, Z., Krasnov, T., Shalmon, L., Schechter, T., Bercovich, D., Attias, D., Laor, R., Koren, A., & Yaniv, I. (2004). Molecular characterization of three novel Fanconi anemia mutations in Israeli Arabs. *European Journal of Haematology*, *72*(5), 330–335. https://doi.org/10.1111/j.1600-0609.2004.00240.x

Vaz, F., Hanenberg, H., Schuster, B., Barker, K., Wiek, C., Erven, V., Neveling, K., Endt, D., Kesterton, I., Autore, F., Fraternali, F., Freund, M., Hartmann, L., Grimwade, D., Roberts, R. G., Schaal, H., Mohammed, S., Rahman, N., Schindler, D., & Mathew, C. G. (2010). Mutation of the RAD51C gene in a Fanconi anemia-like disorder. *Nature Genetics*, *42*(5), 406–409. https://doi.org/10.1038/ng.570

Vundinti, B. R. (2014). Chromosomal instability and molecular mutations in multi spectrum disease of Fanconi anemia. *Molecular Cytogenetics*, *7*(Suppl 1), I47. https://doi.org/10.1186/1755-8166-7-S1-I47

Waisfisz, Q., Altay, C., Leegwater, P. A., de Winter, J. P., Komatsu, K., Evans, G. R., Wegner, R.-D., Reis, A., Joenje, H., Arwert, F., Mathew, C. G., Pronk, J. C., Saar, K., Morgan, N. V., & Digweed, M. (1999). The Fanconi anemia group E gene, FANCE, maps to chromosome 6p. *American Journal of Human Genetics*, *64*(5), 1400–1405. https://doi.org/10.1086/302385

Wang, A. T., & Smogorzewska, A. (2015). SnapShot: Fanconi anemia and associated proteins. *Cell*, *160*(1–2), 354–354 e351. https://doi.org/10.1016/j.cell.2014.12.031

Wang, K., Li, M., & Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, *38*(16), e164. https://doi.org/10.1093/nar/gkq603

Wang, P. P., Parker, W. T., Branford, S., & Schreiber, A. W. (2016). BAM-matcher: a tool for rapid NGS sample matching. *Bioinformatics*, *32*(17), 2699–2701. https://doi.org/10.1093/bioinformatics/btw239

Wegner, R. D., Henrichs, I., Joenje, H., & Schroeder-Kurth, T. (1996). Fanconi anemia complementation group E: Clinical and cytogenetic data of the first patient. *Clinical Genetics*, *50*(6), 479–482. https://doi.org/10.1111/j.1399-0004.1996.tb02716.x

Wijker, M., Morgan, N. V., Herterich, S., van Berkel, C., Tipping, A. J., Gross, H. J., Gille, J., Pals, G., Savino, M., Altay, C., Mohan, S., Dokal, I., Cavenagh, J., Marsh, J., Van Weel, M., Ortega, J. J., Schuler, D., Samochatova, E., Karwacki, M., Bekassy, A. N., Abecasis, M., Ebell, W., Kwee, M. L., de Ravel, T., Gibson, R. A., Gluckman, E., Arwert, F., Joenje, H., Savoia, A., Hoehn, H., Pronk, J. C., & Mathew, C. G. (1999). Heterogeneous spectrum of mutations in the Fanconi anaemia group A gene. *European Journal of Human Genetics*, *7*(1), 52–59. https://doi.org/10.1038/sj.ejhg.5200248

Yang, J., Benyamin, B., McEvoy, B. P., Gordon, S., Henders, A. K., Nyholt, D. R., Madden, P. A., Heath, A. C., Martin, N. G., Montgomery, G. W., Goddard, M. E., & Visscher, P. M. (2010). Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, *42*(7), 565–569. https://doi.org/10.1038/ng.608

Zareifar, S., Dastsooz, H., Shahriari, M., Faghihi, M. A., Shekarkhar, G., Bordbar, M., Zekavat, O. R., & Shakibazad, N. (2019). A novel frame-shift deletion in FANCF gene causing autosomal recessive Fanconi anemia: a case report. *BMC Medical Genetics*, *20*(1), 122. https://doi.org/10.1186/s12881-019-0855-2

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.