Genomic analysis of bone marrow failure and myelodysplastic syndromes reveals phenotypic and diagnostic complexity

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ABSTRACT

Accurate and timely diagnosis of inherited bone marrow failure and inherited myelodysplastic syndromes is essential to guide clinical management. Distinguishing inherited from acquired bone marrow failure/myelodysplastic syndrome poses a significant clinical challenge. At present, diagnostic genetic testing for inherited bone marrow failure/myelodysplastic syndrome is performed gene-by-gene, guided by clinical and laboratory evaluation. We hypothesized that standard clinically-directed genetic testing misses patients with cryptic or atypical presentations of inherited bone marrow failure/myelodysplastic syndrome. In order to screen simultaneously for mutations of all classes in bone marrow failure/myelodysplastic syndrome genes, we developed and validated a panel of 85 genes for targeted capture and multiplexed massively parallel sequencing. In patients with clinical diagnoses of Fanconi anemia, genomic analysis resolved subtype assignment, including those of patients with inconclusive complementation test results. Eight out of 71 patients with idiopathic bone marrow failure or myelodysplastic syndrome were found to harbor damaging germline mutations in *GATA2, RUNX1, DKC1*, or *LIG4*. All 8 of these patients lacked classical clinical stigmata or laboratory findings of these syndromes and only 4 had a family history suggestive of inherited disease. These results reflect the extensive genetic heterogeneity and phenotypic complexity of bone marrow failure/myelodysplastic syndrome phenotypes. This study supports the integration of broad unbiased genetic screening into the diagnostic workup of children and young adults with bone marrow failure and myelodysplastic syndromes.

Introduction

Timely and accurate diagnosis of inherited bone marrow failure (BMF) and inherited myelodysplastic syndromes (MDS) is essential to ensure appropriate medical management and treatment.[1,2] Early diagnosis of an underlying inherited BMF/MDS allows clinical monitoring for signs of clonal evolution in order to initiate hematopoietic stem cell transplantation prior to leukemia development. Secondary leukemias arising in these clinical contexts carry particularly poor prognoses. Many of these syndromes require reduced intensity transplantation regimens to avoid excessive toxicities. Furthermore, the recognition of an inherited disorder informs hematopoietic stem cell donor selection as it allows unambiguous identification of affected siblings and guides appropriate genetic counseling of family members.

Evidence-based guidelines for genetic screening for inherited BMF/MDS are scarce. A family history of MDS or leukemia is a clue to an underlying inherited cause. Physicians also rely on clinical stigmata of inherited BMF/MDS to guide diagnosis. Substantial phenotypic overlap between different syndromes, as well as absent, subtle, or previously unreported clinical findings of inherited BMF/MDS, render diagnosis challenging.[3] The clinical benefit of applying next generation sequencing approaches for comprehensive genetic screening of seemingly idiopathic BMF/MDS remains a critical question.

To investigate the clinical utility of broad genomic analysis for cryptic or atypical presentations of inherited BMF/MDS, we developed a targeted capture gene panel coupled with high-throughput, multiplexed, massively parallel sequencing. This panel, referred to here as MarrowSeq, includes 85 genes responsible for inherited and acquired marrow failure syndromes and MDS. Using this assay, we queried patients deemed to have idiopathic disease for an underlying genetic cause of their BMF and MDS.

Methods

Subjects

The study was conducted in accordance with a protocol approved by the Institutional Review Board of Seattle Children's Hospital and the Declaration of Helsinki. Informed consent was obtained for all study subjects. Samples were obtained from pediatric and adult patients with idiopathic BMF or MDS treated between 2000 and 2013

 doi:10.3324/haematol.2014.113456
The online version of this article has a Supplementary Appendix.
Manuscript received on July 22, 2014. Manuscript accepted on September 15, 2014.
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Table 1. Inherited bone marrow failure and myelodysplastic syndrome genes.

Dyskeratosis congenita	Fanconi	Diamond-Blackfan anemia	Congenital neutropenia	Other inherited BMF/MDS	Familial MDS/leukemia	AML and MDS	
CTC1	*FANCA*	*GATA1*	*ELANE*	*ABCB7*	*CBL*	*ABL1*	*NPM1*
DKC1	*FANCB*	*RPS7*	*G6PC3*	*AK2*	*CEBPA*	*ASXL1*	*NRAS*
NHP2	*FANCC*	*RPS10*	*GFI1*	*ANKRD26*	*GATA2*	*BCL2L11*	*PML*
NOP10	*BRCA2 (FANCD1)*	*RPS17*	*HAX1*	*ATR*	*PAX5*	*BCOR*	*PRPF40B*
RTEL1[a]	*FANCD2*	*RPS19*	*WAS*	*LIG4*	*RUNX1*	*BCR*	*RARA*
TERC	*FANCE*	*RPS24*		*MPL*		*BRAF*	*RPS14*
TERT	*FANCF*	*RPS26*		*NBN*		*DNMT3A*	*SF1*
TINF2	*FANCG*	*RPL5*		*RMRP*		*ETV6*	*SF3A1*
WRAP53	*FANCI*	*RPL11*		*SBDS*		*FLT3*	*SF3B1*
	BRIP1 (FANCJ)	*RPL35A*		*SRP72*		*IDH1*	*SRSF2*
	FANCL					*IDH2*	*TET2*
	FANCM					*JAK2*	*TP53*
	PALB2 (FANCN)					*KIT*	*U2AF1*
	RAD51C (FANCO)					*KRAS*	*U2AF2*
	SLX4 (FANCP)					*MET*	*WT1*
	ERCC4 (FANCQ)[a]					*MLL*	*ZRSR2*

BMF: bone marrow failure; MDS: myelodysplastic syndromes; AML: acute myeloid leukemia. [a]*Genes added after initial screens.*

at Seattle Children's Hospital, Seattle Cancer Care Alliance, University of Washington Medical Center, and Boston Children's Hospital. Patients had been previously tested for mutations in various individual inherited marrow failure syndrome genes based on clinical history and physical findings but remained unclassified after genetic workup. Pediatric inclusion criteria were presentation to a pediatric hematology clinic with idiopathic marrow failure (hypoproductive cytopenias including any of the following: absolute neutrophil count less than $1.5x10^9$/L, hemoglobin low for age, platelet count <150 $x10^9$/L, hypocellular marrow for age) or MDS defined by WHO criteria.[4] Adult inclusion criteria were presentation to an adult hematology clinic with marrow failure or MDS (defined as above) and either young age (<40 years) at presentation, or family history suggestive of inherited BMF/MDS regardless of age. Genomic DNA was isolated from peripheral blood, marrow mononuclear cells, and/or fibroblasts using the Allprep DNA/RNA kit (Qiagen) or as previously described.[5]

Genomics

Oligonucleotide probes were designed using the eArray website (Agilent Technologies). Probes of 120 basepairs (bp) tiled each region with 3x depth and covered coding regions, 20 bp of intronic sequence flanking each exon, as well as the promoter of *DKC1*[6] and the 5'UTR of *ANKRD26*.[7] Pathogenic variants in an intronic region of *GATA2* were described subsequently[8] so all samples were screened for these mutations by Sanger sequencing. Mutations in *RTEL1* and *ERCC4* leading to dyskeratosis congenita[9] and Fanconi anemia,[10] respectively, were reported after completion of the initial screens and have been incorporated into subsequent gene capture pools.

Targeted gene capture and sequencing were performed as previously described.[11,12] Reads were aligned to the human reference genome (hg19) using the Burrows-Wheeler aligner.[13] Single nucleotide and small insertion-deletion (indel) variants were called by three independent bioinformatics pipelines, as previously described[12,14,15] and results jointly analyzed. Alignment to the whole genome facilitated exclusion of variants that fell in pseudogenes. Copy number variants (CNVs) were identified as previously described.[16]

Variants were classified by predicted effect on protein function, as previously described.[11,12] Variants previously reported as either germline or somatic pathogenic alleles for BMF/MDS were specifically noted. Loss-of-function or likely damaging mutations in genes not previously reported in inherited BMF/MDS were considered variants of unknown clinical significance. Variants of unknown clinical significance were evaluated as follows: 1) the likely impact of haploinsufficiency for truncating mutations was assessed based on published data regarding the function for the host gene and on modes of inheritance of phenotypes associated with the gene; and 2) *in silico* structural modeling for missense variants at highly conserved sites in critical domains.

Potentially damaging variants were validated by Sanger sequencing. Variants were confirmed as germline by sequencing fibroblast DNA. This analysis focused on identification of constitutional mutations in BMF/MDS genes.

Additional details on the design and methods of this study are provided in the *Online Supplementary Methods*.

Results

Validation of gene capture assay

Genes known to contribute to inherited bone marrow failure and myelodysplastic syndromes were selected for capture and sequencing (Table 1). Since acquired mutations that cause MDS may also be pathogenic when present as an inherited mutation, and vice versa, a broad gene panel was designed. Identified mutations were evaluated based on variant allele read fraction and by analysis of DNA sequences from a non-hematologic tissue to determine whether a mutation was constitutional. For all samples evaluated, median coverage across the 383kb targeted region was 549X, with 97.8% of bases having over 50X coverage and 98.2% of bases having over 10X coverage. This depth of coverage enabled identification of all classes of mutations, including point mutations, small indels, copy number variants, and genomic rearrangements. The assay was validated by blinded analysis of genomic DNA

Table 2. Characteristics of patients with idiopathic BMF/MDS.

Characteristic	Pediatric patients (n = 58)	Adult patients (n = 13)
Sex		
Male	32 (57%)	5 (38%)
Female	26 (43%)	8 (62%)
Median age in years (range)	8.5 (1-18)	24 (20-67)
BMF and MDS		
BMF only	51 (88%)	12 (92%)
MDS only	4 (7%)	1 (8%)
BMF evolving to MDS	3 (5%)	0 (0)
Family history of related phenotypes	26/56 (46%)	6/11 (55%)
Congenital physical or structural anomalies	28 (48%)	2 (15%)
Short telomeres*	8/38 (21%)	3/10 (30%)

*BMF: bone marrow failure; MDS: myelodysplastic syndrome. *Telomeres less than 1st percentile for age in at least 3 lymphocyte subsets.*[17]

from 14 patients with known mutations in nine genes representing a variety of mutation classes (*Online Supplementary Table S1*). All mutations, including copy number variants (Figure 1A), were correctly identified.

Genetic determination of Fanconi anemia subtype

Simultaneous targeted capture and sequencing of all Fanconi anemia (FA) genes was applied to 6 patients who had been clinically diagnosed with Fanconi anemia. By prior complementation studies, 4 patients had been reported as subtype A (FA-A), and 2 patients remained unclassified after clinical complementation testing for A, C, and G subtypes. The critical mutations were unknown for all 6 patients. We identified biallelic deleterious mutations in *FANCA* in FH-9, FH-73, FH-124, and FH-241 (Figure 2A), confirming the results of their complementation tests. Three of the eight *FANCA* mutations were copy number variants (Figure 1B-D).

Patient FH-42, without a previous subtype assignment, carried two damaging mutations in *FANCD2*: c.2715+1G>A (p.Glu906Ilefs*4) and c.2048T>C (p.Leu683Pro) (*Online Supplementary Table S2*). Sequencing of subcloned cDNA from this patient indicated that the two mutations were in trans and indicated the insertion of 27 base pairs of intron 28 in the FANCD2 transcript, consistent with aberrant splicing. Immunoblotting of protein from patient-derived fibroblast lysates showed nearly absent levels of FANCD2 protein (Figure 2B), confirming subtype D2 for this patient.

Clinical complementation testing of Patient FH-3 was negative for FA subtypes A, C, and G. However, genetic analysis revealed two mutations in *FANCA* for FH-3 (Figure 2A). No other pathogenic mutations were found in any other FA genes. By immunoblot analysis, fibroblasts of FH-3 were deficient in FANCD2 monoubiquitination, which was restored by the introduction of wild-type *FANCA* (Figure 2C), confirming the FA-A subtype for this patient.

Broad genetic screening identifies cryptic presentations of inherited BMF/MDS

We next tested for BMF/MDS gene mutations in 71 patients deemed to have idiopathic disease after clinical, laboratory, and clinically-directed genetic evaluation

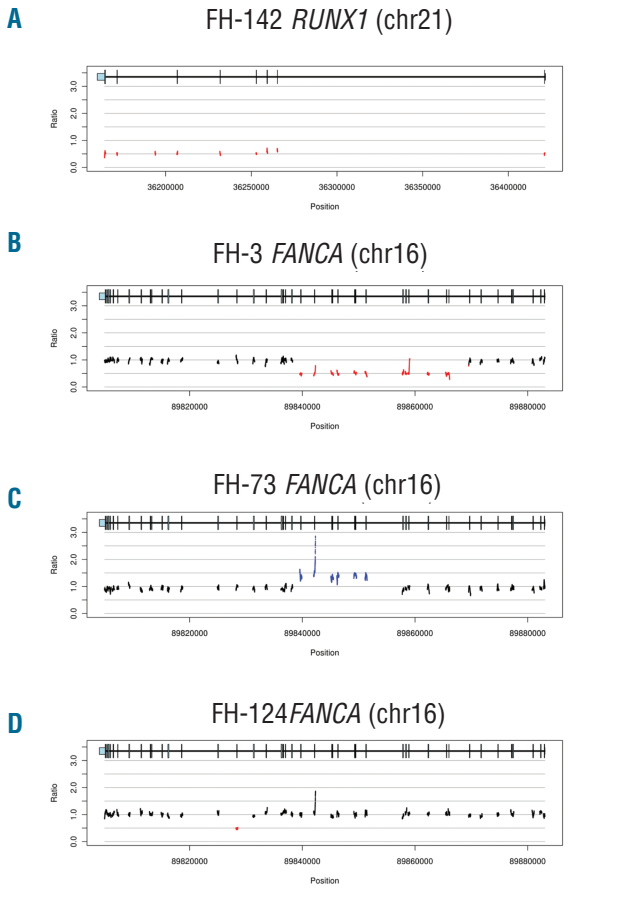

Figure 1. Detection of genomic copy number variants. Ratios of sample to median corrected depth of coverage within a flow cell lane are plotted across targeted genomic regions of the indicated gene. Diploid bases are shown in black. Deletions and duplications are shown in red and blue, respectively. Genomic positions of exons (vertical bars) and untranslated regions (light blue rectangles) are shown above ratio plots. (A) Whole gene deletion of *RUNX1*. No diploid bases were present in this region. (B) Deletion of *FANCA* exons 9-22. (C) Amplification of *FANCA* exons 15-22. (D) Deletion of *FANCA* exon 29.

(Table 2 and *Online Supplementary Table S3*). Marrow failure in these patients remained unclassified after directed testing of candidate genes, chromosomal breakage testing for possible Fanconi anemia,[18] telomere length analysis for possible dyskeratosis congenita,[17] and tests of pancreatic enzyme levels for possible Shwachman-Diamond syndrome.[19] Of the 71 subjects, 58 patients were age 18 years or younger and drawn from the pediatric clinic, and 13 patients were older than 18 years and drawn from the adult clinic. Thirty-two patients, including 6 of the 13 adults, had a positive family history. Eight patients carried damaging germline mutations in one of the BMF/MDS genes (Table 3). Five patients were heterozygous for damaging mutations in the hematopoietic transcription factor *GATA2*, despite the absence of clinical features of MonoMac[20-23] or Emberger syndrome.[24] *GATA2* contains two Cys4 zinc finger domains, the first of which is responsible for interaction with co-regulator FOG1 (Friend of GATA1) and the second for mediating DNA binding. Two patients with idiopathic MDS carried nonsense mutations that truncate the protein in the first zinc finger

Table 3. Clinical features and genetic diagnoses of patients with previously unclassified BMF/MDS.

ID	Sex	Age (years)	Marrow pathology	Hematologic	Immunological	Transplant	Family history	Congenital physical anomalies	Other clinical features	Initial diagnosis	Gene	Mutation
FH-181	F	22	Hypocellular, transient trisomy 8, no morphological dysplasia	Neutrophils 900-1200/μL, HCT 34% (normocytic), monocytes 0-20/μL (reference range 0-800)	Normal quantitative immunoglobulins	–	MDS/AML (maternal uncle), hematologic malignancies (maternal niece and maternal grandmother)	None	2 episodes of pneumonia, 1 episode of severe skin infection of thumb requiring debridement	Marrow failure *vs.* hypocellular MDS	*GATA2*	c.988C>T (p.R330*)
CH-119	M	12	Hypocellular, transient trisomy 8, mild erythroid and myeloid morphological dysplasia	Pancytopenia, monocytes 0-32/μL (reference range 0-900)	–	Mismatched unrelated donor transplant complicated by engraftment failure, died during second transplant with matched unrelated donor	Leukopenia (mother, maternal aunt), neutropenia (maternal cousins)	None	ICU admission for febrile illness and pulmonary complications (pulmonary hemorrhage and bronchiolitis obliterans)	Marrow failure	*GATA2*	c.1078T>A (p.W360R)
FH-202	F	12	Hypocellular, mild tri-lineage, morphological dysplasia	Transfusion-dependent platelets 70k/μL, neutrophils 500/μL, monocytes 10-40/μL (reference range 0-900)	Decreased B-cell precursors with normal mature B-cell numbers	–	Pre-B ALL (maternal cousin, age 3 years), breast cancer (maternal aunt, age 45 years)	None	Hypothyroidism, psoriasis, erythema multiforme, abdominal migraine, dysplastic nevus	Marrow failure	*GATA2*	c.1082G>A (p.R361H)
FH-154	F	17	Hypocellular, mild multi-lineage morphological dysplasia	Pancytopenia, red cell macrocytosis, monocytes 90/μL (reference range 0-900)	–	–	None	Strabismus, hearing loss	Hematoma after tooth extraction	Marrow failure	*GATA2*	c.1084C>T (p.R362*)
FH-82	F	16	Hypocellular, mild morphological tri-lineage dysplasia	Neutrophils 740/μL, monocytes 0-171/μL (reference range 0-900)	Lymphocytes 860/μL, $CD2^+$ 834/μL, $CD19^+$ 17/μL, abnormal lymphocyte stimulation with tetanus and candida	–	None	Sensorineural hearing loss, pseudotumor cerebri, onycholysis	Developmental delay, anxiety, recurrent abdominal pain and vomiting, hepatic transaminitis, abdominal nevus	Marrow failure	*GATA2*	c.1084C>T (p.R362*)
FH-178	M	12	Hypocellular, del(5)(q15q33) clone	Platelets 60-70k/μL, Neutrophils 900-1200/μL	–	–	Unknown (adopted)	Chiari I malformation, scoliosis, umbilical hernia	Myopathy, chronic obstructive pulmonary disease	MDS	*RUNX1*	c.567C>G (p.Y189*)
FH-70	M	15	Hypocellular, no morphological dysplasia	Neutrophils 1000-1500/μL	–	–	Cytopenias, marrow hypocellularity, diarrhea (2 siblings)	Short stature, laryngomalacia, Narrow rib cage	eczema, steatorrhea, clubbed digits, chronic cough, encephalitis, angioedema, chronic joint pain, dry eyes	Shwachman-Diamond syndrome	*DKC1*	c.-141C>G (hemizygous)
CH-103	M	10	Hypocellular, tri-lineage morphological dysplasia. Progressed to MDS and AML with del(7) q21q31-32)[9], idem +21[10]_46,XY[1]	Transfusion-dependent anemia and thrombocytopenia, Neutrophils <500μL, macrocytosis (MCV 10^{6}fL)	Low IgG/IgM, low B- and T-cell counts, normal lymphocyte stimulation with mitogens	Matched unrelated donor, death from transplant complications	None	Primordial dwarfism, bird-like facies, undescended testes	Intrauterine growth retardation, failure to thrive, otitis media, developmental delay, renal dysplasia	Atypical Seckel syndrome	*LIG4*	c.[2440C>T] (p.R814*); [1751_1755del] (p.I584Rfs*2)

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; F: female; HCT: hematocrit; HSCT: hematopoietic stem cell transplant; ICU: intensive care unit; M: male; MCV: mean corpuscular volume; MDS: myelodysplastic syndrome.

domain (*GATA2* c.988C>T [p.Arg330*]) or in the second zinc finger domain (*GATA2* c.1084C>T [p.Arg362*]). GATA2 p.Arg330* was previously reported in an individual with MonoMac characteristics;[23] our patient presented with familial leukemia/myelodysplasia. GATA2 p.Arg362* has not been previously reported. Two other patients carried *GATA2* missense mutations altering highly-conserved residues in the second zinc finger domain: *GATA2* c.1078T>A [p.Trp360Arg] and *GATA2* c.1082G>A [p.Arg361His]. These missense mutations have not been previously reported, but GATA2 p.Arg361Leu and p.Arg361Cys have been reported in Emberger[24] and MonoMac[25] syndromes, respectively, and GATA2 p.Arg361His has been reported as an acquired mutation in acute myelogenous leukemia (AML).[26] Trp360 resides in a LWRR motif that is conserved across N- and C-terminal zinc fingers in all human GATA proteins (*Online Supplementary Figure S1A*). Mutation of the buried hydrophobic Trp360 to a basic arginine residue would be predicted to disrupt the zinc-finger domain folding (*Online Supplementary Figure S1B*). These data support recent reports of cryptic presentations of *GATA2* mutations without features of MonoMac syndrome, Emberger syndrome, or familial MDS in patients with BMF/MDS.[25,27]

One patient (FH-178) presented with MDS. His antecedent medical history was significant for multiple

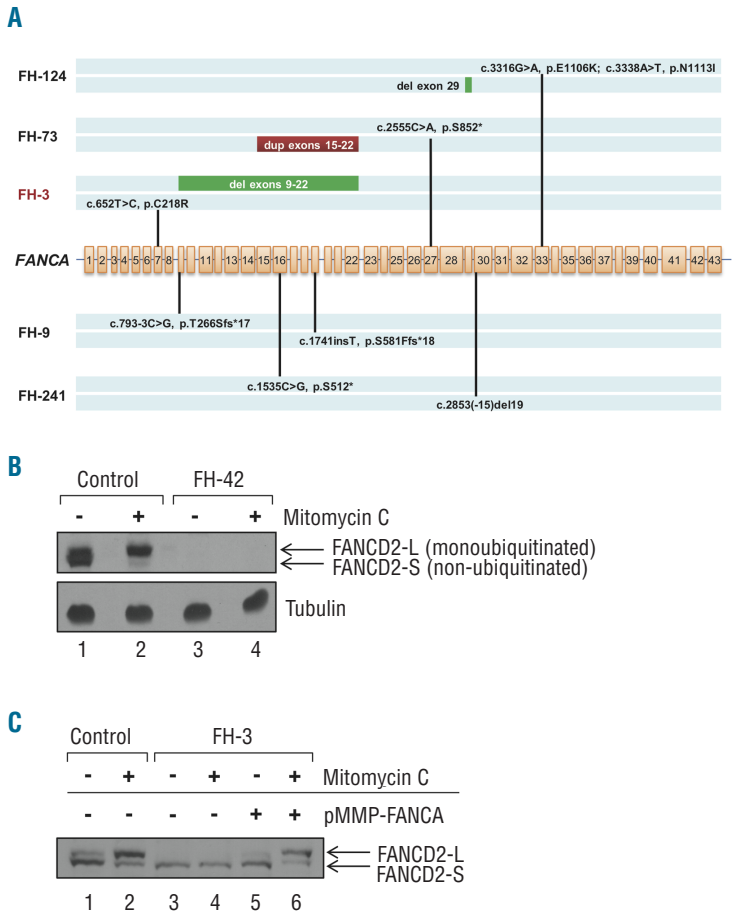

Figure 2. Targeted gene capture correction of Fanconi anemia subtype assignment. (A) Biallelic *FANCA* mutations identified by MarrowSeq in 5 patients. FA patient FH-3 (highlighted in red) was non-ACG subtype by clinical complementation testing. (B) Protein extracts of bone marrow fibroblasts isolated from healthy controls or Fanconi anemia patient FH-42 (*FANCD2*, p.[Leu683Pro];[Glu906Ilefs*4]) were immunoblotted for FANCD2 with or without 24-h mitomycin C treatment. Fibroblasts from FH-42 exhibit low FANCD2 protein expression (lanes 3 and 4) in comparison to cells from controls (lanes 1 and 2). α-tubulin was used to ascertain equivalent protein loading. (C) Functional validation of Fanconi anemia subtype A in FA patient FH-3 (FANCA p.[Cys218Arg];[Val265Leufs*8]). Protein extracts of bone marrow fibroblasts isolated from healthy control or FH-3 were immunoblotted for FANCD2 with or without 24-h mitomycin C treatment. Fibroblasts from healthy control show both non-ubiquitinated (FANCD2-S) and monoubiquitinated (FANCD2-L) FANCD2 forms (lane 1), with an increased ratio of monoubiquitinated FANCD2 relative to non-ubiquitinated FANCD2 upon mitomycin C treatment (lane 2). Fibroblasts from FH-3 show only the non-ubiquitinated FANCD2-S form with and without mitomycin C (lanes 3 and 4). FANCD2 monoubiquitination is restored upon infection with a pMMP retroviral vector encoding the wild-type *FANCA* cDNA (lanes 5 and 6).

congenital anomalies, neutropenia, and thrombocytopenia (Table 3). A heterozygous mutation (c.567C>G [p.Tyr189*]) in *RUNX1* was identified. Heterozygous *RUNX1* mutations cause familial platelet disorder with propensity to myeloid malignancy.[28,29] RUNX1 p.Tyr189* truncates the protein C-terminal to the RUNT domain, with loss of the transactivation domain, and is predicted to function in a dominant negative fashion.[30] One patient (FH-70) was originally diagnosed at an outside hospital with Shwachman-Diamond syndrome based on his clinical presentation of failure to thrive, neutropenia, enzyme-responsive steatorrhea, and low fecal elastase. He lacked mutations in *SBDS* and expressed normal levels of SBDS protein. Telomere lengths fell between the 1st and 10th percentiles for age in all 6 leukocyte subsets tested for FH-70, and hence did not raise prior clinical suspicion for dyskeratosis congenita using published criteria.[18] We identified a hemizygous mutation in *DKC1* (c.-141C>G) located in the promoter of the *DKC1* gene. This promoter mutation was not found in normal control databases dbSNP138, the Exome Variant Server, nor 1000Genomes Project. Whole exome sequencing analysis of this family did not identify alternative candidate genes. This promoter mutation had been previously reported in 2 unrelated kindreds with X-linked dyskeratosis congenita and segregated with the dyskeratosis congenita (DC) phenotype.[6,31] This mutation is located in a GC-rich element required for Sp1 transcription factor binding[32] and leads to reduced transcript levels of *DKC1*.[6] In *DKC1* (c.-141C>G) patient-derived CD34+ cells, expression of $DKC1^{WT}$, but not catalytically-inactive $DKC1^{Asp125Ala}$, rescued hematopoietic colony formation.[6] The absence of telomere lengths shorter than the first percentile was consistent with prior reports of other DC patients carrying this promoter mutation in *DKC1*.[6] Thus, broad genetic screening identified a clinically unsuspected genetic syndrome presenting in an atypical fashion.

Patient CH-103 was originally diagnosed with Seckel syndrome based on his presentation with short stature, microcephaly, bird-like facies, and mental retardation (Table 3). He subsequently developed marrow failure evolving to MDS and AML. Seckel syndrome with marrow failure is associated with mutations in the *ATR* gene, but no deleterious *ATR* mutations were identified for CH-103. Instead, CH-103 harbored biallelic truncating mutations *LIG4* (c.2440C>T [p.Arg814*])[33] and *LIG4* (c.1751_1755delTAAGA [p.Ile584Argfs*2]). *LIG4* encodes DNA ligase IV. *LIG4* mutations are associated with an autosomal recessive syndrome that, like Seckel syndrome, is characterized by microcephaly, facial dysmorphism, growth retardation, developmental delay, and pancytopenia, but MDS and AML have not been previously reported for LIG4 syndrome.[33] DNA ligase IV functions in DNA double-strand break repair via non-homologous end-joining and V(D)J recombination.[34] Patients with LIG4 syndrome exhibit sensitivity to ionizing radiation, so the correct diagnosis informs choice of treatments and imaging modalities. Patients with LIG4 syndrome develop pancytopenia[33,35] and lymphoid malignancies.[36-38] However, MDS and AML were not previously associated with LIG4 syndrome and thus directed testing for *LIG4* mutations

had not been pursued for this patient. Therefore, broad multi-gene sequencing approach overcomes limitations posed by incomplete published knowledge of the range of clinical phenotypes for these syndromes.

Discussion

Currently, the selection of specific genetic tests for a given patient is driven by clinical suspicion based on history, physical examination and laboratory evaluation. Our results demonstrate that multi-gene screening identifies patients harboring mutations in known BMF/MDS genes that were clinically unsuspected and therefore were not diagnostically pursued. Our study subjects were selected after a diagnostic workup including prior screening for cryptic presentations of inherited marrow failure syndromes such as chromosomal breakage testing for Fanconi anemia,[18] telomere length testing for dyskeratosis congenita,[39,40] and pancreatic enzyme testing for Shwachman-Diamond syndrome.[41] Our results, therefore, underestimate the frequency of genetic causes of BMF/MDS in children, young adults, and adults with suggestive family histories.

Of the patients for whom no mutations were found in any of the 85 BMF/MDS genes, 47 patients had family history and/or syndromic features suggestive of inherited, or at least constitutional, disease. Our results, therefore, suggest that additional as yet unidentified genes remain to be found for BMF and MDS. Further investigation with whole exome sequencing or whole genome sequencing may identify new genetic causes of inherited BMF/MDS. Conversely, of the 8 patients for whom constitutional damaging mutations were identified, only 4 had a suggestive family history. Of the genetic diagnoses in the absence of a family history, 2 most likely involve de novo mutation (of *GATA2* in FH-82 and FH-154), one involved recessive inheritance (of *LIG4* in CH-103), and one occurred in an adopted child with no available family history (*RUNX1* in FH-178).

Substantial phenotypic overlap among inherited BMF/MDS syndromes, as well as pleiotropy and variable expressivity within syndromes, further complicate diagnosis based purely on clinical presentation. Identification of causal genetic lesions thus plays a critical role in the diagnostic workup. Our multiplexed genetic approach resolved the diagnosis of 2 patients, FH-70 and CH-103, by simultaneously revealing the absence of mutations in genes associated with their initial clinical diagnoses (SBDS and ATR, respectively) and identifying pathogenic mutations in clinically unsuspected genes (*DKC1* and *LIG4*, respectively).

For the purposes of clinical diagnostic testing, custom targeted gene panels offer important advantages over whole exome sequencing. Clinical genetic testing requires sensitive and accurate mutation detection across a defined set of clinically actionable genes with reporting of results in a timely manner. Whole exome sequencing, while well suited for gene discovery, does not ensure deep coverage across all exons of clinical interest. In contrast, high depth of coverage across all genes of interest is achieved through targeted gene capture.[42] The deep coverage afforded by this targeted sequencing approach detected CNVs with a single assay, in contrast to alternative approaches combining targeted capture, array comparative genomic hybridization (aCGH), and RNA sequencing.[43] This is especially important for inherited BMF/MDS which are frequently caused by deletions in ribosomal protein genes,[44,45] *RUNX1*,[28,46] and *FANCA*.[47,48] Finally, whole exome sequencing queries thousands of genes unrelated to BMF/MDS and thus may reveal incidental mutations not immediately relevant to the diagnosis that prompted the sequencing. Patients for whom a germline cause for his or her disease is not revealed by MarrowSeq may benefit from whole exome sequencing for gene discovery in a research setting. New BMF/MDS genes can be incorporated into probe sets for MarrowSeq as they are reported.

These results demonstrate the clinical utility of broad screening of apparently idiopathic cases of BMF and MDS to identify cryptic presentations of inherited disease. Genomic analysis revealed previously unreported phenotypes due to mutations in known genes for syndromic disease. The integration of genomic analysis with clinical and laboratory evaluation in the diagnosis of patients with BMF/MDS can guide clinical management. Multiplexed, clinically unbiased genetic screening provides a powerful approach to elucidate the genetic heterogeneity and phenotypic complexity of inherited syndromes.

Acknowledgments

The authors would like to thank Marshall Horwitz and Penny Jeggo for helpful discussion, Scott Coats and Sommer Castro for sample processing, Dana Matthews, Blythe Thomson, Barbara Small, Jessica Pollard, Paul Carpenter, Melissa Forouhar, Paul Hendrie, Bart Scott, Pam Becker, and Terry Gernsheimer for patient referrals, Kathleen McGregor for her assistance in the marrow failure/MDS clinic, and James Wacker, Celeste Oglesby, and Lauren DePue for clinical research assistance. Regulatory support to A.S. was obtained through the University of Washington Northwest Institute of Genetic Medicine from Washington State Life Sciences Discovery funds (grant 2065508).

Funding

This work was supported by NIH/NIDDK grants R24DK093425 and R24DK099808 (A.S., M.C.K., J.A.), R24DK094746 (M.D.F., D.A.W.), T32AG000057 (M.Y.Z.), and the Ghiglione Aplastic Anemia Fund and the Julian's Dinosaur Guild at Seattle Children's Hospital (A.S.). M.C.K. is an American Cancer Society professor.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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