Genetic features of myelodysplastic syndrome and aplastic anemia in pediatric and young adult patients

Siobán B. Keel,[1]* Angela Scott,[2,3,4]* Marilyn Sanchez-Bonilla,[5] Phoenix A. Ho,[2,3,4] Suleyman Gulsuner,[6] Colin C. Pritchard,[7] Janis L. Abkowitz,[1] Mary-Claire King,[6] Tom Walsh,[6] and Akiko Shimamura[5]****

[1]Department of Medicine, Division of Hematology, University of Washington, Seattle, WA; [2]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA; [3]Department of Pediatric Hematology/Oncology, Seattle Children's Hospital, WA; [4]Department of Pediatrics, University of Washington, Seattle, WA; [5]Boston Children's Hospital, Dana Farber Cancer Institute, and Harvard Medical School, MA; [6]Department of Medicine and Department of Genome Sciences, University of Washington, Seattle, WA; and [7]Department of Laboratory Medicine, University of Washington, Seattle, WA, USA

**SBK and ASc contributed equally to this work*

***TW and ASh are co-senior authors*

 doi:10.3324/haematol.2016.149476

Received: May 16, 2016.

Accepted: July 13, 2016.

Pre-published: July 14, 2016.

Correspondence: akiko.shimamura@childrens.harvard.edu or sioban@u.washington.edu

Supplementary materials

Supplementary methods

Retrospective chart review

Patient data were collected from medical records by two investigators blinded to the results of genetic testing. The following information was collected: date of birth, transplant, death, and last follow-up, cause of death, sex, physical anomalies, cancer prior to or post-transplant (excluding relapse), family history of a related phenotype or cancer in any 1st or 2nd degree relative (including any malignancy, cytopenia, or anomaly suggestive of an inherited marrow failure syndrome such as café au lait spots, pulmonary fibrosis, liver fibrosis or cirrhosis, history of premature graying, thumb abnormalities), donor source, transplant conditioning regimen, post-transplant complications within 6-months of transplant defined as graft failure, intubation, bronchiolitis obliterans organizing pneumonia, liver sinusoidal obstruction syndrome, or other complications captured by description in Tables 1 and 3 and which were deemed atypical for the post-transplant period on joint review by SK, AScott, and AS. Physical anomalies were defined as those anomalies deemed suggestive of an inherited marrow failure syndrome on joint review by SK, AScott, and AS among all abnormal physical exam or radiographic findings extracted from medical records. Additionally, for the aplastic anemia cohort, information on any prior history of immunosuppressive or androgen therapies, and the presence of a paroxysmal nocturnal hemoglobinuria clone by flow cytometric analysis or Ham test were extracted from the medical record.

Genomics

Pathogenic variants were classified as pathogenic or likely pathogenic based on the American College of Medical Genetics and Genomics' Standards and Guidelines (Supplementary Table 4)[1]. All pathogenic and likely pathogenic variants were validated by Sanger sequencing.

Supplementary results

Aplastic anemia genetics

Two patients carried hemizygous mutations in *DKC1* which encodes dyskerin, a component of the telomerase complex. Mutations in *DKC1* result in X-linked DC[2]. Patient AA3 carried a *DKC1* mutation (c.196A>G [T66A]) located in exon 4. This mutation has been described previously in a family with DC[3] and results in decreased telomerase expression and activity[4]. Patient AA45 carried a previously described *DKC1* promoter mutation (c.-142 C>G). This mutation falls within a putative Sp1 transcription binding site and has been reported in DC[5].

Two patients carried mutations in the *MPL* gene which is associated with Congenital amegakaryocytic thrombocytopenia (CAMT)[2]. The first patient, AA25, carried damaging compound heterozygous mutations in *MPL* (c.G1545A [W515X] and (c.G305C [R102P]). The first mutation is a nonsense mutation causing a premature stop codon. This allele has not been described previously but is predicted to create a damaging truncation in the thrombopoietin receptor. The R102P allele has been previously described in CAMT patients. It is located in the thrombopoietin binding domain and has been demonstrated to impair signal transduction[6]. The second patient, AA37, carried a homozygous missense mutation in *MPL* (c.C1180T [P394S]). This mutation is located in exon 8 and has been described previously in a Pakistanian family with CAMT[7]. Functional studies of this variant have not been reported. AA79 carried a pathogenic mutation in *TP53*

(c.G587A [p.R196Q]). This mutation has previously been reported in solid tumors. The mutation is located in the DNA binding domain of TP53 and impairs transcriptional transactivation[8].

Myelodysplastic syndrome genetics

Compound heterozygous mutations in *FANCA* were identified in HIP12286 (c.C1645T [p.Q549X] and c.3349-1G>A). FANCA p.Q549X has been previously reported and confirmed to be pathologic by complementation assay[9]. *FANCA* c.3349-1G>A has also been previously reported and occurs at a highly conserved nucleotide resulting in disruption of a canonical splice acceptor site in exon 34[10]. Increased mitomycin C and diepoxybutane chromosomal breakage of patient-derived lymphoblasts confirmed the diagnosis of Fanconi anemia.

HIP08919 carried a heterozygous frameshift mutation in *GATA2* (c.302delG [p.G101Afs*]). HIP17707 carried a heterozygous *GATA2* mutation predicted to affect the exon 4 splice donor site (c.1017+2T>C). This mutation is novel; however a de novo *GATA2* c.1017+2T>G mutation at this nucleotide position has been reported in a man with MDS characterized by monocytopenia and warts[11]. HIP18921 carried a nonsense mutation that truncates the protein in the first zinc finger domain (*GATA2* c.988C>T [p.R330X]) which has previously been reported in patients with GATA2 deficiency[12, 13]. HIP18952 carried a missense mutation (*GATA2* c.1061C>T [p.T354M]), which was previously reported in several families with familial AML/MDS and shown to encode a mutant GATA2 with reduced DNA binding affinity which also impaired wild-type GATA2 function in a dominant-negative fashion[14]. HIP20476 carried a missense mutation in *GATA2* which altered a highly-conserved residue in the second zinc finger domain previously reported in a 26 year-old woman with MDS and a history of atypical mycobacterial infections (*GATA2* c.1081C>T [p.R361C])[15].

HIP05737 carried compound heterozygous mutations in *MPL*. Biallelic constitutional mutations in *MPL* cause congenital amegakaryocytic thrombocytopenia (CAMT)[16]. This patient and her family have been previously reported and illustrate the phenotypic heterogeneity of inherited marrow failure that complicates the accurate diagnosis of specific syndromes based on clinical findings alone[17]. The proband's clinical history is notable for a nonclassical presentation of CAMT at a late age (11 years-old) with pancytopenia and marrow hypocellularity concerning for AA. Her seemingly healthy two brothers were evaluated as potential stem cell donors and found to have thrombocytopenia and also carried both mutations in trans. One allele (c.235_236del [p.L79Efs*]) causes a frameshift in exon 3 resulting in premature polypeptide termination and a lack of cell surface expression of the receptor. The second mutation (c.393+5G>C) disrupts a conserved exon 3-intron 3 splice donor site causing diminished *MPL* expression.

Four patients carried mutations in genes affecting telomere maintenance. Two patients carried compound heterozygous mutations in *RTEL1*, which encodes a helicase essential for telomere maintenance and regulation of homologous recombination. Several recent reports have described heterozygous, compound heterozygous, and homozygous *RTEL1* mutations in a severe form of Dyskeratosis congenita called Hoyeraal-Hreidarsson syndrome (HHS)[18-21]. Additionally, heterozygous germline mutations in *RTEL1* are reported in familial interstitial pneumonia and these cases exhibit short telomere lengths in peripheral blood mononuclear cells[22]. HIP17561 carried a novel missense mutation affecting a highly conserved residue in a helicase domain

(*RTEL1* c.347A>G [p.H116R]) and a novel missense mutation (*RTEL1* c.167C>T [p.T56M]) that did not affect any known functional domain; however, a methionine at that position might provide an alternative start codon for translation. The PolyPhen2 and Gerp scores for these two variants are 1.0/4.84 and 1.0/4.94, respectively suggesting the mutations are damaging. HIP02696 harbored a missense mutation in a helicase domain of RTEL1 (*RTEL1* c.1476G>T [p.M492I]) that has been previously reported, in the compound heterozygous state with *RTEL1* c.2992C>T (p.R998X)[21, 23]. HIP02696 carried a second missense mutation (*RTEL1* c.3791G>A [R1264H]) in the C-terminus of the protein, distal to the helicase domain and previously reported as a germline homozygous mutation in two families with HHS[19]. We were unable to confirm definitively that this variant was constitutional as we had discordant results from Sanger sequencing of DNA isolated from skin and GI tissues. The available clinical histories of these two patients with compound heterozygous *RTEL1* mutations were strikingly different. HIP02696 was an only child with a family history notable for a paternal grandmother and great-aunt with chronic lymphocytic leukemia. His clinical history was classic for HHS and included a history of intrauterine growth retardation, oligohydramnios, reversal of placental flows and decreased fetal activity which prompted cesarean-section delivery at 30 weeks gestation and brief oxygen support at delivery. He had microcephaly, cerebellar hypoplasia, hypogonadism and was characterized as having axial hypotonia and postural difficulties that caused difficulty feeding early in life. Around age 18 months, he was found to have mild peripheral blood cytopenias and marrow hypocellularity and went on to develop progressive marrow failure and ultimately underwent a nonmyeloablative unrelated donor peripheral blood HSCT at 2 years of age complicated by graft failure. He underwent a second nonmyeloablative unrelated donor peripheral blood HSCT from the same donor two months after his first transplant that also culminated in graft failure and ultimately engrafted after transplantation without conditioning of bone marrow hematopoietic stem cells from the same donor. He passed away approximately 98 months after his initial transplant at age 10 years of multi-organ system failure presumed secondary to infectious complications in a chronically immunosuppressed patient. HIP17561 initially presented at age 33 years with easy bruising, thrombocytopenia (platelets 11,000/uL) and idiopathic macrocytosis. He was treated with steroids and intravenous immunoglobulin for presumed immune thrombocytopenic purpura without response and was subsequently found to have a hypocellular marrow with dysplastic changes. He underwent an HLA-matched sibling HSCT with peripheral blood stem cells at age 34 years with a myeloablative cyclophosphamide and ATG conditioning regimen. Approximately 12 years post-transplant, he died of liver failure, which was presumed alcohol-related. This outcome is intriguing given the association of liver disease with telomeropathies. His family history was notable for a mother with thrombocytopenia attributed to immune thrombocytopenic purpura that reportedly responded to splenectomy. Telomere length testing was not available for either of these patients.

HIP02099 carried compound heterozygous mutations in *SBDS* (c.A184T [p.K62X] and c.258+2T>C). Mutations in *SBDS* cause Shwachman–Diamond syndrome (SDS), an autosomal recessive disorder with clinical features that include pancreatic exocrine dysfunction, hematological dysfunction, skeletal abnormalities, and leukemia predisposition. The missense mutation is located in exon 2 of the *SBDS* gene and introduces a stop codon at amino acid 62. The intron 2 splice site mutation disrupts a donor splice site leading to a frameshift that prematurely terminates translation of the SBDS protein[24]. The *SBDS* mutations in HIP02099 remained experimentally unconfirmed as constitutional. The presence of an adjacent pseudogene sharing 97%

homology to the SBDS gene precluded confirmatory sequencing of paraffin embedded tissues due to the small quantities of short fragmented DNA available from paraffin embedded tissue samples; additionally, the small quantity of DNA isolated precluded distinguishing pseudogene from gene using our targeted gene capture and NGS panel[25]. However, HIP02099's clinical history of cytopenias and steatorrhea requiring pancreatic enzyme replacement supported the diagnosis of Shwachman-Diamond Syndrome.

HIP02687 carried a heterozygous missense mutation in *TERT* (c.C2110T [P704S]), which has been previously reported in 2 unrelated patients. This variant was heterozygous in one patient with a history of recurrent AA after ATG and cyclosporine therapy[26] and homozygous in another patient who presented with mucocutaneous features classic for DC. Both patients had reduced telomere length in peripheral blood mononuclear cells and reduced telomerase activity[27].

HIP05477 carried a heterozygous missense mutation in *TINF2* (c.G845A [R282H]). The protein product of *TINF2,* TIN2, is a central component of shelterin, the protein complex that stabilizes telomeres[28]. Savage S. et al. reported heterozygous mutations in *TINF2* that co-segregated with the phenotypic finding of very short (<1st percentile) telomere lengths in leukocyte subsets in a family with multiple members with clinical DC and in a subset of unrelated DC probands [29].

HIP21264 carried a heterozygous deletion-frameshift mutation in *TP53* (c.626delGA [R209Ffs*]). HIP21264 initially presented at age 15 years-old with a rib osteosarcoma and received alkylator-based chemotherapy. Six years- later she was diagnosed with therapy-related MDS and ultimately underwent an URD peripheral blood HSCT around age 23 years with a conditioning regimen that included busulfan and cyclophosphamide. Her disease relapsed ~ 49 months post-transplant. The family was known to have Li-Fraumeni syndrome. Their family history was notable for the father having leiomyosarcoma, papillary carcinoma of the thyroid, and Burkitt lymphoma. Three of the proband's four siblings also carried the *TP53* mutation - one brother had rhabdomyosarcoma and subsequent therapy-related AML, another brother developed osteosarcoma and subsequent therapy-related MDS, and a third brother carrying the *TP53* mutation remained cancer-free at last known follow-up. The paternal grandfather died of some form of lymphoma in his 50s. HIP01569 carried a heterozygous missense mutation in *TP53* (c.626delGA [G245S]) which is a well-described mutation in the DNA-binding domain[30]. Li-Fraumeni syndrome is an autosomal dominant familial cancer predisposition syndrome associated with germline *TP53* mutations. Leukemia, most commonly acute lymphoblastic leukemia (ALL) and less commonly AML, as well as reports of MDS or AML as secondary malignancies have been reported in Li-Fraumeni families[31-34]. HIP01569 initially presented at 3 years-old with pro-B cell ALL and subsequently developed therapy-related MDS at 7 years of age. His family history was notable for a mother with breast cancer and a brother who passed away at the age of 12-years from rhabdomyosarcoma.

Supplementary Table 1. Inherited bone marrow failure and myelodysplastic syndrome genes included on the targeted gene capture panel.

Diamond-Blackfan anemia	Dyskeratosis congenita	Congenital neutropenia	Familial MDS & leukemia	Fanconi anemia	Other inherited marrow failure
GATA1	*CTC1*	*ELANE*	*CBL*	*FANCA*	*AK2*
RPL11	*DKC1*	*G6PC3*	*CEBPA*	*FANCB*	*ANKRD26*
RPL35a	*NHP2*	*GFI1*	*ETV6*	*FANCC*	*ATM*
RPL5	*NOP10*	*HAX1*	*GATA2*	*FANCD1 (BRCA2)*	*ATR*
RPS10	*RTEL1*	*JAGN1*	*PAX5*	*FANCD2*	*ATRX*
RPS17	*TERC*	*TCIRG1*	*RUNX1*	*FANCE*	*C15ORF41*
RPS19	*TERT*	*VPS45*	*TP53*	*FANCF*	*CDAN1*
RPS24	*TINF2*	*WAS*		*FANCG*	*LIG4*
RPS26	*WRAP53*			*FANCI*	*MPL*
RPS7				*FANCJ (BRIP1)*	*NBN*
				FANCL	*RMRP*
				FANCM	*SBDS*
				FANCN (PALB2)	*SRP72*
				FANCO (RAD51C)	
				FANCP (SLX4)	
				FANCQ (ERCC4)	

MDS, myelodysplastic syndrome.

Supplementary Table 2. Characteristics of patients diagnosed with AA.

Characteristic	≤18 Years Old n=53	> 18 Years Old n=45	Total n=98
Female	47% (25)	40% (18)	44% (43)
Male	53% (28)	60% (27)	56% (55)
Median age*, years (range)	10 (1-18)	29 (19-40)	18 (1-40)
Family history**	38% (20)	42% (19)	40% (39)
Physical anomalies	11% (6)	11% (5)	11% (11)
HLA-matched sibling donor tx	43% (23)	33% (15)	39% (38)
Alternative donor tx	57% (30)	67% (30)	61% (60)

*Age at transplant; **family history indicates family history of related phenotype or cancer in 1st or 2nd degree relative.
AA, idiopathic acquired aplastic anemia; tx, transplant

Supplementary Table 3. Characteristics of patients diagnosed with MDS.

Characteristic	≤18 Years Old n=46	> 18 Years Old n=64	Total n=110
Female, percent (n)	41% (19)	45% (29)	44% (48)
Male, percent (n)	59% (27)	55% (35)	56% (62)
Median age*, years (range)	9 (1-18)	37 (18-46)	29 (1-46)
Family history**, percent (n)	48% (22)	55% (35)	52% (57)
Physical anomalies, percent (n)	24% (11)	23% (15)	24% (26)

*Age at transplant; **family history indicates family history of related phenotype or cancer in 1st or 2nd degree relative.
MDS, myelodysplastic syndrome

Supplementary Table 4. Summary of genetic variants.

HIP#	Gene	Mutation	Class	Effect	RefSeq	Chr	Coord	Ref	Var	Type	PP	Gerp	EVS	1000G	ExAC	Reads	Inh/Dz	PMID
HIP12286	*FANCA*	c.C1645T	P	p.Q549X	NM_000135	16	89846347	G	A	nonsense	_	4.36	_	_	0.000008238	125/250	AR FA	19367192
	FANCA	c.3349-1G>A	P	splice	NM_000135	16	89813299	C	T	splice	_	5.09	_	_	0.00004435	135/248		21826217
HIP08919	*GATA2*	c.302delG	P	p.G101Afs*	NM_032638	3	128205139	C	_	deletion/ frameshift	_	-	_	_	_	242 | 120	AD MDS/AML	_
HIP17707	*GATA2*	c.1017+2T>C	LP	splice	NM_001145662	3	128202701	A	G	splice	_	5.15	_	_	_	248 | 118	AD MDS/AML	22147895
HIP18921	*GATA2*	c.C988T	P	p.R330X	NM_032638	3	128202732	G	A	nonsense	_	-	_	_	_	247/110	AD MDS/AML	23223431 and 24782121
HIP18952	*GATA2*	c.C1061T	P	p.T354M	NM_032638	3	128200744	G	A	missense	1	-	_	_	_	250/141	AD MDS/AML	21892162
HIP20476	*GATA2*	c.C1081T	LP	p.R361C	NM_032638	3	128200724	G	A	missense	1	4.95	_	_	_	114/246	AD MDS/AML	24227816
HIP05737	*MPL*	c.235_236del	P	p.L79Efs*	NM_005373	1	43804235	CT	_	deletion/ frameshift	_	_	_	_	4.94E-05	234 | 112	AR CAMT	16219544
	MPL	c.393+5G>C	P	splice	NM_005373	1	43804396	G	C	missense	_	5.56	_	_	0.0001484	249 | 117		16219544
HIP17561	*RTEL1*	c.C167T	US	T56M	NM_032957	20	62292715	C	T	missense	1	4.94	_	_	0.000008299	134/246	AR DC/HH	_
	RTEL1	c.A347G	LP	H116R	NM_032957	20	62293248	A	G	missense	1	4.84	_	_	_	119/243		_
HIP02696	*RTEL1*	c.G1476T	P	M492I	NM_001283009	20	62319118	G	T	missense	0.079	5.48	0.000077	_	0.0000558	244/119	AR DC/HH	23453664 and 19461895
	RTEL1	c.G3791A	P	R1264H	NM_001283009	20	62326972	G	A	missense	0.994	3.73	_	_	0.0000766	139/61		24009516
HIP14128	*RUNX1*	c.C958T	P	R293X	NM_001754.4	21	36171607	G	A	nonsense	_	_	_	_	_	246 | 150	AD MDS/AML	19808697 (somatic)
HIP02099	*SBDS*	c.A184T	P	K62X	NM_016038	7	66459273	T	A	nonsense	_	5	_	_	0.0004118	249 | 79	AR SDS	12496757
	SBDS	c.258+2T>C	P	splice	NM_016038	7	66459197	A	G	splice	_	4.27	_	_	0.003946	249 | 140		12496757
HIP02687	*TERT*	c.C2110T	P	P704S	NM_198253	5	1279426	G	A	missense	0.999	2.47	_	_	_	122/248	AD DC	18931339 and 18042801
HIP05477	*TINF2*	c.G845A	LP	R282H	NM_012461	14	24709841	C	T	missense	0.76	5.2	_	_	_	250 | 113	AD DC	18252230
HIP21264	*TP53*	c.626_627del GA	P	R209Ffs*	NM_000546	17	7,578,222	TC	_	deletion/ frameshift	_	_	_	_	_	234 | 125	AD Li-Fraumeni	-

HIP01569	*TP53*	c.G733A	P	G245S	NM_000546	17	7577548	C	T	missense	1	4.6	-	-	8.24E-06	249 | 127	AD Li-Fraumeni	24122735
AA3	*DKC1*	c.A196AG	P	T66A	NM_001363	X	153,994,206	A	G	missense	0.295	-	-	-	-	441 | 431	AD DC	10364516, 10591218
AA25	*MPL*	c.G305C	P	R102P	NM_005373	1	43,804,305	G	C	missense	1	-	-	-	-	657 | 321	AR CAMT	18422784
AA25	*MPL*	c.G1545A	P	W515stop	NM_005373	1	43,815,010	G	A	nonsense	-	-	-	-	-	385 | 186	AR CAMT	-
AA37	*MPL*	c.C1180T	P	P394S	NM_005373	1	43,812,477	C	T	missense	1	-	-	-	-	541| 530	AR CAMT	-
AA45	*DKC1*	c.-142C>G	P	c.-142C>G	NM_001363	X	153,991,099	C	G	promoter	NA	-	-	-	-	73| 73	AD DC	11054058
AA79	*TP53*	c.G587A	P	R196Q	NM_00112613	17	7,578,262	C	T	missense	1	4.4	-	-	-	250|124	AD Li-Fraumeni	12826609

RefSeq, RefSeq transcript number; Chr, chromosome; Coord, coordinate; Ref, reference base; Var, variant base; PP, PolyPhen-2 score; EVS, exome variant sequence allele frequency; 1000G, 1000 Genomes allele frequency; ExAC, Exome Aggregation Consortium allele frequency; Inh/Dz, inheritance/disease; FANCA, Fanconi anemia type A; AR, autosomal recessive; FA, Fanconi anemia; AD, autosomal dominant; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CAMT, Congenital amegakaryocytic thrombocytopenia; DC, Dyskeratosis congenita; HH, Hoyeraal-Hreidarsson syndrome; SDS, Shwachman-Diamond syndrome; P, pathogenic; LP, likely pathogenic; US, uncertain significance

Supplementary Table 5. Transplant outcomes in MDS and AA patients with mutations in Dykeratosis congenita genes.

ID	Sex	Age *	Transplant year	Gene	Mutation	Donor source	Conditioning regimen	Time to death (months post-transplant)	Cause of death
HIP02696	M	2	2002	*RTEL1*	p.M516I; R1264H	HLA-matched URD PBSC	TBI 200cGy + fludarabine	95	Multi-organ system failure
HIP17561	M	34	2002	*RTEL1*	T56M; H116R	HLA-matched sibling PBSC	Busulfan + 120 mg/kg cyclophosphamide	150	Liver failure (no chronic GVHD)
HIP02687	M	41	2002	*TERT*	P704S	HLA-matched URD PBSC	Busulfan + 120 mg/kg cyclophosphamide	90	Acute myeloid leukemia
HIP05477	F	6	1993	*TINF2*	R282H	HLA-matched URD PBSCT	Busulfan + 120 mg/kg cyclophosphamide	36	Pulmonary failure (BOOP + infection)
AA3	M	33	1994	*DKC1*	T66A	HLA-matched sibling	200mg/kg cyclophosphamide + 90mg/kg ATG	21	Colon Cancer
AA45	M	9	2005	*DKC1*	c.-142C>G	HLA-matched sibling	200mg/kg cyclophosphamide + 90mg/kg ATG	-	-

*Age at transplant, MDS, myelodysplastic syndrome; AA, idiopathic acquired aplastic anemia; URD, unrelated donor; PBSCT, peripheral blood stem cell transplant; TBI total body irradiation; GVHD, graft versus host disease; BOOP, Bronchiolitis obliterans organizing pneumonia

Supplementary references

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine : official journal of the American College of Medical Genetics. 2015;17(5):405-424.
2. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood reviews. 2010;24(3):101-122.
3. Knight SW, Heiss NS, Vulliamy TJ, et al. X-linked dyskeratosis congenita is predominantly caused by missense mutations in the DKC1 gene. Am J Hum Genet. 1999;65(1):50-58.
4. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature. 1999;402(6761):551-555.
5. Dokal I. Dyskeratosis congenita in all its forms. Br J Haematol. 2000;110(4):768-779.
6. Tijssen MR, di Summa F, van den Oudenrijn S, et al. Functional analysis of single amino-acid mutations in the thrombopoietin-receptor Mpl underlying congenital amegakaryocytic thrombocytopenia. Br J Haematol. 2008;141(6):808-813.
7. Walne AJ, Dokal A, Plagnol V, et al. Exome sequencing identifies MPL as a causative gene in familial aplastic anemia. Haematologica. 2012;97(4):524-528.
8. Kato S, Han SY, Liu W, et al. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci USA. 2003;100(14):8424-8429.
9. Moghrabi NN, Johnson MA, Yoshitomi MJ, et al. Validation of Fanconi anemia complementation Group A assignment using molecular analysis. Genetics in medicine : official journal of the American College of Medical Genetics. 2009;11(3):183-192.
10. Meier D, Schindler D. Fanconi anemia core complex gene promoters harbor conserved transcription regulatory elements. PLoS One. 2011;6(8):e22911.
11. Kazenwadel J, Secker GA, Liu YJ, et al. Loss-of-function germline GATA2 mutations in patients with MDS/AML or MonoMAC syndrome and primary lymphedema reveal a key role for GATA2 in the lymphatic vasculature. Blood. 2012;119(5):1283-1291.
12. Pasquet M, Bellanne-Chantelot C, Tavitian S, et al. High frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMac syndrome, myelodysplasia, and acute myeloid leukemia. Blood. 2013;121(5):822-829.
13. Fujiwara T, Fukuhara N, Funayama R, et al. Identification of acquired mutations by whole-genome sequencing in GATA-2 deficiency evolving into myelodysplasia and acute leukemia. Ann Hematol. 2014;93(9):1515-1522.
14. Hahn CN, Chong CE, Carmichael CL, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet. 2011;43(10):1012-1017.
15. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014;123(6):809-821.
16. Ihara K, Ishii E, Eguchi M, et al. Identification of mutations in the c-mpl gene in congenital amegakaryocytic thrombocytopenia. Proc Natl Acad Sci USA. 1999;96(6):3132-3136.
17. Gandhi MJ, Pendergrass TW, Cummings CC, Ihara K, Blau CA, Drachman JG. Congenital amegakaryocytic thrombocytopenia in three siblings: molecular analysis of atypical clinical presentation. Exp Hematol. 2005;33(10):1215-1221.
18. Ballew BJ, Yeager M, Jacobs K, et al. Germline mutations of regulator of telomere elongation helicase 1, RTEL1, in Dyskeratosis congenita. Hum Genet. 2013;132(4):473-480.

19. Ballew BJ, Joseph V, De S, et al. A recessive founder mutation in regulator of telomere elongation helicase 1, RTEL1, underlies severe immunodeficiency and features of Hoyeraal Hreidarsson syndrome. PLoS genetics. 2013;9(8):e1003695.
20. Deng Z, Glousker G, Molczan A, et al. Inherited mutations in the helicase RTEL1 cause telomere dysfunction and Hoyeraal-Hreidarsson syndrome. Proc Natl Acad Sci USA. 2013;110(36):E3408-3416.
21. Walne AJ, Vulliamy T, Kirwan M, Plagnol V, Dokal I. Constitutional mutations in RTEL1 cause severe dyskeratosis congenita. Am J Hum Genet. 2013;92(3):448-453.
22. Cogan JD, Kropski JA, Zhao M, et al. Rare variants in RTEL1 are associated with familial interstitial pneumonia. American journal of respiratory and critical care medicine. 2015;191(6):646-655.
23. Lamm N, Ordan E, Shponkin R, Richler C, Aker M, Tzfati Y. Diminished telomeric 3' overhangs are associated with telomere dysfunction in Hoyeraal-Hreidarsson syndrome. PLoS One. 2009;4(5):e5666.
24. Boocock GR, Morrison JA, Popovic M, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet. 2003;33(1):97-101.
25. Zhang MY, Keel SB, Walsh T, et al. Genomic analysis of bone marrow failure and myelodysplastic syndromes reveals phenotypic and diagnostic complexity. Haematologica. 2015;100(1):42-48.
26. Du HY, Pumbo E, Ivanovich J, et al. TERC and TERT gene mutations in patients with bone marrow failure and the significance of telomere length measurements. Blood. 2009;113(2):309-316.
27. Du HY, Pumbo E, Manley P, et al. Complex inheritance pattern of dyskeratosis congenita in two families with 2 different mutations in the telomerase reverse transcriptase gene. Blood. 2008;111(3):1128-1130.
28. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev. 2005;19(18):2100-2110.
29. Savage SA, Giri N, Baerlocher GM, Orr N, Lansdorp PM, Alter BP. TINF2, a component of the shelterin telomere protection complex, is mutated in dyskeratosis congenita. Am J Hum Genet. 2008;82(2):501-509.
30. Giacomazzi J, Selistre SG, Rossi C, et al. Li-Fraumeni and Li-Fraumeni-like syndrome among children diagnosed with pediatric cancer in Southern Brazil. Cancer. 2013;119(24):4341-4349.
31. Gadalla SM, Sales-Bonfim C, Carreras J, et al. Outcomes of allogeneic hematopoietic cell transplantation in patients with dyskeratosis congenita. Biol Blood Marrow Transplant. 2013;19(8):1238-1243.
32. Felix CA, Hosler MR, Provisor D, et al. The p53 gene in pediatric therapy-related leukemia and myelodysplasia. Blood. 1996;87(10):4376-4381.
33. Anensen N, Skavland J, Stapnes C, et al. Acute myelogenous leukemia in a patient with Li-Fraumeni syndrome treated with valproic acid, theophyllamine and all-trans retinoic acid: a case report. Leukemia. 2006;20(4):734-736.
34. Kuribayashi K, Matsunaga T, Sakai T, et al. A patient with TP53 germline mutation developed Bowen's disease and myelodysplastic syndrome with myelofibrosis after chemotherapy against ovarian cancer. Intern Med. 2005;44(5):490-495.