Germline Predisposition to Hematolymphoid Neoplasia

2017 Society for Hematopathology/European Association for Haematopathology Workshop Report

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ABSTRACT

Objectives: *The 2017 Workshop of the Society for Hematopathology/European Association for Haematopathology aimed to review clinical cases with germline predisposition to hematolymphoid neoplasms.*

Methods: *The Workshop Panel reviewed 51 cases with germline mutations and rendered consensus diagnoses. Of these, six cases were presented at the meeting by the submitting pathologists.*

Results: *The cases submitted to the session covering germline predisposition included 16 cases with germline* GATA2 *mutations, 10 cases with germline* RUNX1 *mutations, two cases with germline* CEBPA *mutations, two germline* TP53 *mutations, and one case of germline* DDX41 *mutation. The most common diagnoses were acute myeloid leukemia (15 cases) and myelodysplastic syndrome (MDS, 14 cases).*

Conclusions: *The majority of the submitted neoplasms occurring in patients with germline predisposition were myeloid neoplasms with germline mutations in* GATA2 *and* RUNX1. *The presence of a germline predisposition mutation is not sufficient for a diagnosis of a neoplasm until the appearance of standard diagnostic features of a hematolymphoid malignancy manifest: in general, the diagnostic criteria for neoplasms associated with germline predisposition disorders are the same as those for sporadic cases.*

Myeloid neoplasms generally present sporadically in older adult populations. With increasing use of molecular profiling, an evolving number of inherited cancer predisposition syndromes have now been described. The diagnosis of a myeloid neoplasm with genetic predisposition dictates a different approach for clinical management of the affected patient, impacting donor selection for stem cell transplantation, genetic counseling, and disease surveillance. The revised fourth edition (2016) World Health Organization (WHO) *Classification of Tumours of Haematopoietic and Lymphoid Tissues*[1] has included myeloid neoplasms with germline predisposition syndromes as a provisional category. The 2017 Workshop of the Society for Hematopathology/European Association for Haematopathology (SH/EAHP) included a session dedicated to these germline predisposition syndromes (session 1) and a total of 51 cases were submitted to this session. The diagnoses and genetic features of the cases from session 1 are summarized in Table 1, Table 2, Table 3, and Table 4 and presenting symptoms, clinical history, and family history in Table 5. The majority of these cases (42 cases) comprised myeloid neoplasms, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), with fewer cases of mixed myelodysplastic/myeloproliferative neoplasms (MDS/MPN) such as chronic myelomonocytic leukemia (CMML), and myeloproliferative neoplasm (MPN) such as essential thrombocythemia (ET). Lymphoid neoplasms with germline mutations submitted to this session included three cases of B-lymphoblastic leukemia (B-ALL) as well

Table 1

Features of Cases Submitted With Germline *GATA2* Mutation

Case No.	Age, y	Sex	*GATA2* Mutation: Genomic	*GATA2* Mutation: Protein	*GATA2* Mutation: Type	*GATA2* Mutation: Familial or Sporadic	Infections	Other	Prior Disease	PB Cytopenias	Bone Marrow Diagnosis	Karyotype	NGS Additional Mutations
20	18	M	c.821delC	p.274fs	FS PS	F	NR	Family history of solid tumors	No	P, blasts	AML-MRC	–7, +8, add	NR
40	17	M	c.1081C>T	p.R361C	M ZF2	NR	NR	Presented post MVA	No	Monocytosis	CMML	–7	ASXL1 R693X, KRAS G12V, STAT3 S614R
48	6	M	c.610C>T	p.Arg204*	PS	F	NR	Megakaryoblasts	No	P	AML-MRC	–7	WT1
52	22	F	c.1114G>A	p.A372T	M ZF2	S	PNA, cellulitis, cervical HPV	Lymphedema	Yes	P	MDS-EB2	–7	NR
87	5	M	c.1143 + 200_1198del		Lg Del	NR	cellulitis, warts	Lymphedema	Yes	N, B-NK	RCC	N	ASXL1
105	57	M	c.1085G>A	p.Arg362Gln	M ZF2	F	NR	Patient was donor for brother with AML 20 y prior	Yes	P	MDS-MLD	N	NR
138	13	F	c.16delG	p.6fs	FS	S	NR	2-y prior history of anemia	Yes	A, N	MDS-MLD	N	NR
157	45	F	c.1045T>C	p.C349R	M ZF2	NR	HPV-carcinomas	Onset age 16, prior diagnosis of AA	Yes	P	MDS-MLD	+21	NR
176	10	F	c.154_179del,	p.H51fs*	FS PS	NR	NR	Lymphedema, onset age 6	Yes	N	MDS-EB1	–7	ASXL1, NRAS
236	30	M	c.706A>G	p.Met236Val	M VUS	F	NR	BT, pure erythroid leukemia	Yes, BT	P	AML-MRC	Complex	NR
258	62	F	c.1193G>A	p.R398Q	M ZF2	NR	MAI	0nset 52 y cytopenias	Yes	A, Mo, L	HLHBMID	+8	NR
266	16	F	c.521del	p.174fs	FS	NR	NR	h/o MDS 1 y prior	Yes	P	AML-MRC	N	FANC
275	44	F	c.1192C>T	p.R398W	M ZF2	F	Pulmonary aspergillosis, MAI, cervical HPV	Mother died colon cancer 31 y; sister died 36 y infection; AFB + marrow	Yes	T, Mo, L	BMID	N	STAG2 p.R614X
307	12	M	3.1-3.3 Mb deletion; nt 128198265-128212030		Lg Del	S	PNA		No	A,N,Mo, rare Blasts	RCC	–7	CRLF2 p.N63S
337	31	F	c.1017 + 572C>T		R/I	F	Panniculitis, PNA, HPV, MAI	Granulomata in marrow	Yes	P, Mo, B, NK	MDS-MLD	+8	NR
381	17	F	c.1084C>T;	p.R362*	PS ZF2	NR	NR		No	A, N	RCC	Der(1;7), +8	NR

A, anemia; AA, aplastic anemia; AFB, acid-fast bacilli; AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; B, B-lymphopenia; BMID, bone marrow and immunodeficiency disorder; BT, β-thalassemia; cellu, cellulitis; cerv, cervical; CMML, chronic myelomonocytic leukemia; F, familial; FS, frameshift; HLH, hemophagocytic lymphohistiocytosis; HPV, human papillomavirus; L, lymphopenia; Lg Del, large deletion; M, missense; Mo, monocytopenia; MAI, *Mycobacterium avium-intracellulare*; MDS-EB1, myelodysplastic syndrome with excess blasts I; MDS-EB2, myelodysplastic syndrome with excess blasts II; MDS-MLD, myelodysplastic syndrome with multilineage dysplasia; MVA, motor vehicle accident; N, neutropenia; NGS, next-generation sequencing; NK, NK-lymphopenia; NR, not reported; P, pancytopenia; PNA, pneumonia; PNA, pneumonia; PS, premature stop; RCC, refractory cytopenia of childhood; R/I, regulatory/intronic; S, sporadic; T, thrombocytopenia; VUS, variant of unknown significance; ZF2, zinc finger 2.

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Table 2
Myeloid Neoplasms With Germline Predisposition Syndrome

Case No.	Panel Diagnosis	Pathogenic Genetic Alterations
Acute Myeloid Leukemia (AML)		
230	AML with biallelic mutations of *CEBPA* (one germline, one somatic)	*CEBPA* p.Q41fs (G) *CEBPA* p.K313dup *WT1* p.V379fs*72
283	AML with biallelic mutations of *CEBPA* (one germline, one somatic)	*CEBPA* p.E57 (G)*
234	AML with inv(3)(p21:3q26.2); *GATA2, MECOM* [with germline constitutional t(8;21)]	*RUNX1* p.L219* *FLT3-ITD* *ASXL1* p.G646fs inv(3)(p21:3q26.2)
38	AML with mutated *RUNX1* (germline)	*RUNX1* p.Y260*(G)
284	AML with mutated *RUNX1* (possibly germline)	*RUNX1* c.351 + 1G>C (G) *NRAS* p.G12D *BCOR* p.K839fs t(2;21)(q23;q22)
264	AML MRC (in a patient with Maffucci syndrome with mosaic *IDH1* mutation)	*IDH1* p.R132C *NRAS* p.G13A *WT1* p.R445Q
253	AML MRC with germline *BLM* mutation	*BLM* p.Y736Lfs (G) *NF1* p.I679Dfs *WT1* c.616 + 1dup
20	AML MRC with germline *GATA2* mutation	*GATA2* c.821del(G)
48	AML MRC with germline *GATA2* mutation	*GATA2* p.R204* (G) *WT1* p.P376fs* *WT1* p.A314fs*4 *JAK2* p.V617F *CSF3R* p.Y814* *KRAS* p.G12D
236	AML MRC with germline *GATA2* mutation	*GATA2* p.M236V (G)
266	AML MRC with germline *GATA2* mutation	*GATA2* p.P174fs (G)
318	AML, NOS with germline *DDX41* mutation	*DDX41* p.M1? (G)
55	AML, NOS with germline *NF1* mutation	*NF1* p.L380R (G)
225	Therapy-related AML [myeloid sarcoma, with t(8;16)(p11:2;p13.3);*KAT6A-CREBBP*] with germline 9q22.32-9q31.1 microdeletion of unknown significance	KAT6A-CREBBP PTCH TGRB1 microdeletion (G)
167	Therapy-related AML with germline *TP53* mutation (Li-Fraumeni syndrome)	*TP53* p.E204Sfs*43
Myelodysplastic Syndrome (MDS)		
176	MDS-EB1 with germline *GATA2* mutation	*GATA2* p.H51fs* (G) *PTPN11* p.A72T *ASXL1* p.D879fs* *NRAS* p.G12D *NRAS* p.G13D
52	MDS EB-2 with germline *GATA2* mutation	*GATA2* p.A372T (G)
170	MDS EB-2 with germline *RBM8A* mutation	*RBM8A* mutation (G)
196	MDS MLD with germline *G6P3* mutation	*G6PC3* c.218 + 1G>A (G)
105	MDS MLD with germline *GATA2* mutation	*GATA2* p.R362Q (G)
138	MDS MLD with germline *GATA2* mutation	*GATA2* p.E6fs (G)
337	MDS MLD with germline *GATA2* mutation	*GATA2* c.1017 + 572C>T (G)
157	MDS MLD with germline *GATA2* mutation	*GATA2* p.C349R (G)
39	MDS MLD with germline *RUNX1* mutation	*RUNX1* p.Y189* (G)
339	MDS MLD with germline *RUNX1* mutation	*RUNX1* p.K194N (G)
87	RCC with germline *GATA2* mutation	*GATA2* c.365del (G) *ASXL1* p.T880Qfs*2
307	RCC with germline *GATA2* mutation	*GATA2* 3.1-3.3 Mb deletion (G)
381	RCC with germline GATA2 mutation	*GATA2* p.R362*(G)
80	RCC with germline *SAMD9* mutation	*SAMD9* p.E1136Q (G)
Other Myeloid Neoplasms		
40	CMML-1 with germline *GATA2* mutation	*GATA2* p.R361C (G) *KRAS* p.G12V *ASXL1* p.R693* *KRAS* p.K117N *NF1* p.I679fs*21 *SETBP1* p.I871T *STAT3* p.S614R *WT1* p.A382fs*4 *WT1* p.S381*
273	MDS/MPN, unclassifiable with germline *SAMD9* mutation	*SAMD9* p.R685Q (G) *SAMD9* p.C835R (G)
42	ET with germline *SH2B3* mutation	*SH2B3* p.D231fs (G) *FANCE* p.K475R *EPHB1* p.S690N *AXIN2* p.R834Q
292	JMML in a patient with Noonan syndrome	PTPN11 p.T73I (G)

CMML, chronic myelomonocytic leukemia; EB, excess blasts; ET, essential thrombocythemia; G, germline; JMML, juvenile myelomonocytic leukemia; MLD, multilineage dysplasia; MPN, myeloproliferative neoplasm; MRC, myelodysplasia related changes; NOS, not otherwise specified; RCC, refractory cytopenia of childhood.

as one case each of Burkitt lymphoma, follicular lymphoma, and T-cell large granular lymphocytic leukemia. The remaining cases involved patients presenting with thrombocytopenia (six cases), bone marrow and immunodeficiency disorder in patients with GATA2 deficiency (two cases), and thrombocytosis with germline *MPL*

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Table 3
Lymphoid Neoplasms With Germline Predisposition Syndrome

Case No.	Panel Diagnosis	Pathogenic Genetic Alterations
	B-ALL	
194	B-ALL with germline *ELA2* mutation (congenital neutropenia)	*ELANE* (G)
101	B-ALL with germline *PAX5* mutation	*PAX5* p.G183S (G)
99	B-ALL with hypodiploidy in a patient with Noonan syndrome (germline *SHOC2* mutation)	*SHOC2* p.S2G (G)
	Lymphoid neoplasms	
209	Follicular lymphoma grade 1-2 of 3 in a patient with germline *TP53* mutation (Li-Fraumeni syndrome)	*TP53* p.R158H (G)
106	Burkitt lymphoma in a patient with X-linked lymphoproliferative syndrome (germline *SH2D1A* mutation)	*SH2D1A* c.163C>T (G)
342	T-cell large granular lymphocytic leukemia with pure red cell aplasia and with germline *CTLA4* mutation	*CTLA4* p.R51* (G)

B-ALL, B-lymphoblastic leukemia; G, germline.

Table 4
Other Disorders With Germline Predisposition Syndromes

Case No.	Panel Diagnosis	Pathogenic Alterations
268	Thrombocytopenia with germline *ANKRD26* mutation	*ANKRD26* c.-119C>G
271	Thrombocytopenia with germline *RUNX1* mutation	*RUNX1* exon 6 deletion *ATM* K2253T
309	Thrombocytopenia with germline *RUNX1* mutation	*RUNX1* p.I366_G367dup *TET2* p.M533T
364	Thrombocytopenia with germline *RUNX1* mutation	*RUNX1* c.967 + 2_967 + 5delTAAG
219	Thrombocytopenia with germline *RUNX1* variant of unknown significance	*RUNX1* p.R207W
275	Bone marrow and immunodeficiency disorder (monoMAC syndrome) with germline *GATA2* mutation	*GATA2* p.R398W *STAG2* p.R614* *PALB2* p.?splice
258	Hemophagocytic lymphohistiocytosis in a patient with bone marrow and immunodeficiency disorder (MonoMAC syndrome) with germline *GATA2* mutation	*GATA2* p.R398Q (G)
97	Thrombocytosis with germline *MPL* mutation	*MPL* p.P106L (G)
346	1: Classical Hodgkin lymphoma2: Congenital neutropenia (germline *CSF3R* variant of unknown significance)	*CSF3R*p.R583C (G)
320	Transient myeloproliferative disorder in a patient with germline *PTPN11* mutation (Noonan syndrome)	*PTPN11* p.S502L
200	TAFRO (thrombocytopenia, ascites, fibrosis, renal dysfunction, organomegaly), a variant of multicentric Castleman disease	*RUNX1* p.G87C
333	Constitutional t(12;18) of undetermined significance	t(12;18)

G, germline.

mutation. Overall, the most common cases submitted were those involving germline mutations in *GATA2* (16 cases) and *RUNX1* (10 cases). Other germline mutations present in the submitted cases were *CEBPA*, *DDX41*, *TP53*, *AKRD26*, and *PTPN11*. The 2016 WHO classification[1] subdivides myeloid neoplasms with germline predisposition into three main categories: those associated with preexisting platelet disorders (including *RUNX1*, *ANKRD26*, and *ETV6* mutations); those associated with other organ dysfunction (including *GATA2* mutation, bone marrow failure syndromes, Noonan syndrome, neurofibromatosis, and telomere biology disorders such as dyskeratosis congenita); and those without a preexisting platelet disorder or organ dysfunction (including *DDX41* and *CEBPA* mutations). Because germline mutated *GATA2* cases represented the largest number of submitted cases to this workshop, this entity will be discussed first, followed by other mutations associated with germline predisposition to MDS and AML, germline mutations associated with platelet disorders, and finally germline mutations associated with MPN and lymphomas.

Germline Predisposition Associated With Mutated *GATA2*

GATA2, located on chromosome 3q21, encodes a zinc finger transcription factor that is critical for

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Table 5

Myeloid Neoplasia Highlighting Key Clinical, Family History, and Morphologic Findings Associated With the Most Common Genetic Mutations in This Study

Case No.	Diagnosis	Clinical History	Family History	Morphologic Findings
230	AML with biallelic mutations of *CEBPA* (one germline, one somatic)	Two-month history of increasing dyspnea on exertion, associated with a cough, fever, easy bruising, and weight loss	Daughter and younger brother with AML	Medium to large-sized blasts with irregular nuclear contour, fine chromatin, and prominent nucleoli
283	AML with biallelic mutations of *CEBPA* (1 germline, 1 somatic)	One-month history of bruising, chest pain, night sweats	None	Blasts with mostly round nuclei, occasionally indented nuclear contours, fine chromatin, large prominent nucleoli, and moderate amounts of cytoplasm containing occasional granules and Auer rods
38	AML with mutated *RUNX1* (germline)	Easy bruising, with platelets in the low to normal range, but no significant excessive bleeding; presented with high fevers, hypotension, diffuse lymphadenopathy, and splenomegaly	Family history of platelet function defect; affected family members include her 2 older sisters, father, paternal aunt, paternal uncle, cousins, and paternal great-grandfather	Medium to large-sized blasts with high nuclear-to-cytoplasmic ratios, irregular nuclear contours, smooth chromatin, prominent nucleoli, and mild amounts of basophilic cytoplasm with Auer rods
284	AML with mutated *RUNX1* (possibly germline)	History of thrombocytopenia since the age of 4 y, treated with steroids for presumptive ITP, without significant response	None	Predominance of myeloid blasts and small, hypolobated megakaryocytes
39	MDS MLD with germline *RUNX1* mutation	Thrombocytopenia (dating back to the newborn period) and neutropenia in the setting of chronic idiopathic elevation in creatine kinase and myopathy of unclear etiology	Unknown	Hypercellular marrow with trilineage dysplasia and prominent dysmegakaryopoiesis
339	MDS MLD with germline *RUNX1* mutation	Progressive thrombocytopenia and anemia and a life-long history of bruising and mild thrombocytopenia	Father with long-standing history of easy bruising and asymptomatic 4-y-old sister with mild thrombocytopenia	Hypercellular marrow with trilineage dysplasia, decreased megakaryocytes with frequent small hypolobated forms and 5% blasts
20	AML MRC with germline *GATA2* mutation	Fatigue and weight loss	Family history of both solid tumors and hematologic malignancies, including colon cancer in his father and 5 paternal uncles, breast cancer in 3 paternal aunts, and leukemia in his sister and a paternal aunt	Intermediate to large in size blasts with open chromatin, round to slightly irregular nuclear contours, occasional prominent nucleoli, and scant cytoplasm; no cytoplasmic granules or Auer rods were seen
48	AML MRC with germline *GATA2* mutation	Pancytopenia	None	Numerous blasts with abundant dysplastic megakaryocytes
236	AML MRC with germline *GATA2* mutation	Pancytopenia, lymphadenopathy, and splenomegaly	Unknown	Hypercellular bone marrow with increased and slightly dysplastic erythroids, moderate reticulin fibrosis, and rare hemophagocytic histiocytes
266	AML MRC with germline *GATA2* mutation	Pancytopenia with macrocytosis	Unknown	Large blasts with round to irregular nuclear contours, variably prominent nucleoli, and large amounts of cytoplasm; a subset of the blasts had cytoplasmic granules
176	MDS-EB1 with germline *GATA2* mutation	Hemihypertrophy developed right lower extremity lymphedema over several years now presented with high WBC count, anemia, and thrombocytosis.	None	Hypercellular marrow with increased megakaryocytes showing prominently separated nuclear lobes; erythroid elements exhibited megaloblastic features with irregularly shaped nuclei; myeloid cells exhibited left-shifted maturation with abnormal nuclear lobation

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Table 5 (cont)

Case No.	Diagnosis	Clinical History	Family History	Morphologic Findings
52	MDS EB-2 with germline *GATA2* mutation	Pancytopenia and recent multiple infections including pneumonia, bladder and sinus infections, and cellulitis; medical history notable for occasional left lower extremity edema and human papillomavirus of the cervix	None	Hypocellular bone marrow with 16% blasts, rare dysplastic erythroid forms, and left-shifted myeloid elements with hypolobation and hypogranulation and absent megakaryocytes
105	MDS MLD with germline *GATA2* mutation	Cytopenias	Brother with AML	Hypercellular bone marrow with trilineage dysplasia along with <15% ring sideroblasts and 2.6% marrow blasts without identifiable Auer rods
138	MDS MLD with germline *GATA2* mutation	2-year history of anemia of unknown origin	None	Bone marrow aspirate showed trilineage dysplasia and <5% blasts
337	MDS MLD with germline *GATA2* mutation	Sudden onset of fatigue at 21 and was found to be pancytopenic	Maternal family history is significant for MDS and warts; patient's sister has mild cytopenias	Hypocellular bone marrow with trilineage dysplasia and <5% blasts; granulomas seen at later time point
157	MDS MLD with germline *GATA2* mutation	History of worsening cytopenias diagnosed at age 16, ultimately progressing to transfusion dependence and aplastic anemia	None	Hypercellular marrow with increased ratio of myeloid cells to erythroid cells, abnormal erythroid maturation including atypical forms; left-shifted and dysplastic myelopoiesis, rare hypolobated megakaryocytes; markedly increased iron stores with rare ringed sideroblasts
87	RCC with germline *GATA2* mutation	Recurrent group A streptococcal lymphangitis/cellulitis, streptococcal bacteremias, lymphangiectasis in the right thigh region; HPV-driven warts, mainly involving the fingers and the toes; recurrent scrotal and penile lymphedema	None	Hypocellular bone marrow with relative erythroid predominance, left-shifted granulocytes with toxic appearance, no increase in blasts or hemophagocytosis, occasional abnormal megakaryocytes (small, monolobated and widely separated nuclei)
307	RCC with germline *GATA2* mutation	3 days of fever, cough, and a diagnosis of pneumonia; CBC showed macrocytic anemia, leukopenia with neutropenia, monocytopenia, and rare circulating blasts	None	Hypocellular marrow with dysplasia
381	RCC with germline *GATA2* mutation	May-Thurner syndrome and pancytopenia; initially felt to have megaloblastic anemia due to folate deficiency	None	Hypocellular marrow with megaloblastic changes and dyserythropoiesis
40	CMML-1 with germline *GATA2* mutation	Motor vehicle accident resulting in postoperative course complications	None	Hypercellular marrow with dysplasia and monocytosis

AML, acute myeloid leukemia; HPV, human papillomavirus; ITP, immune thrombocytopenia; MDS, myelodysplastic syndrome; MLD, multilineage dysplasia; MRC, myelodysplasia related changes; RCC, refractory cytopenia of childhood.

hematopoiesis,[2] and maintenance of hematopoietic stem cells.[3] Depending on the isoform, it consists of six or seven exons and its transcription is initiated from the first two exons. GATA2 protein contains two highly conserved zinc finger domains (C-ZnF and N-ZnF) responsible for its DNA-binding ability and interaction with other proteins.[3] Additionally, other nonfinger domains are distinguished: two transactivation domains, a nuclear localization signal, and a negative regulatory domain.[4] Germline heterozygous autosomal dominant and sporadic mutations in *GATA2* result in haploinsufficiency[4] and are the underlying genetic cause of a number of previously described syndromes with overlapping features, including monocytopenia and *Mycobacterium avium*

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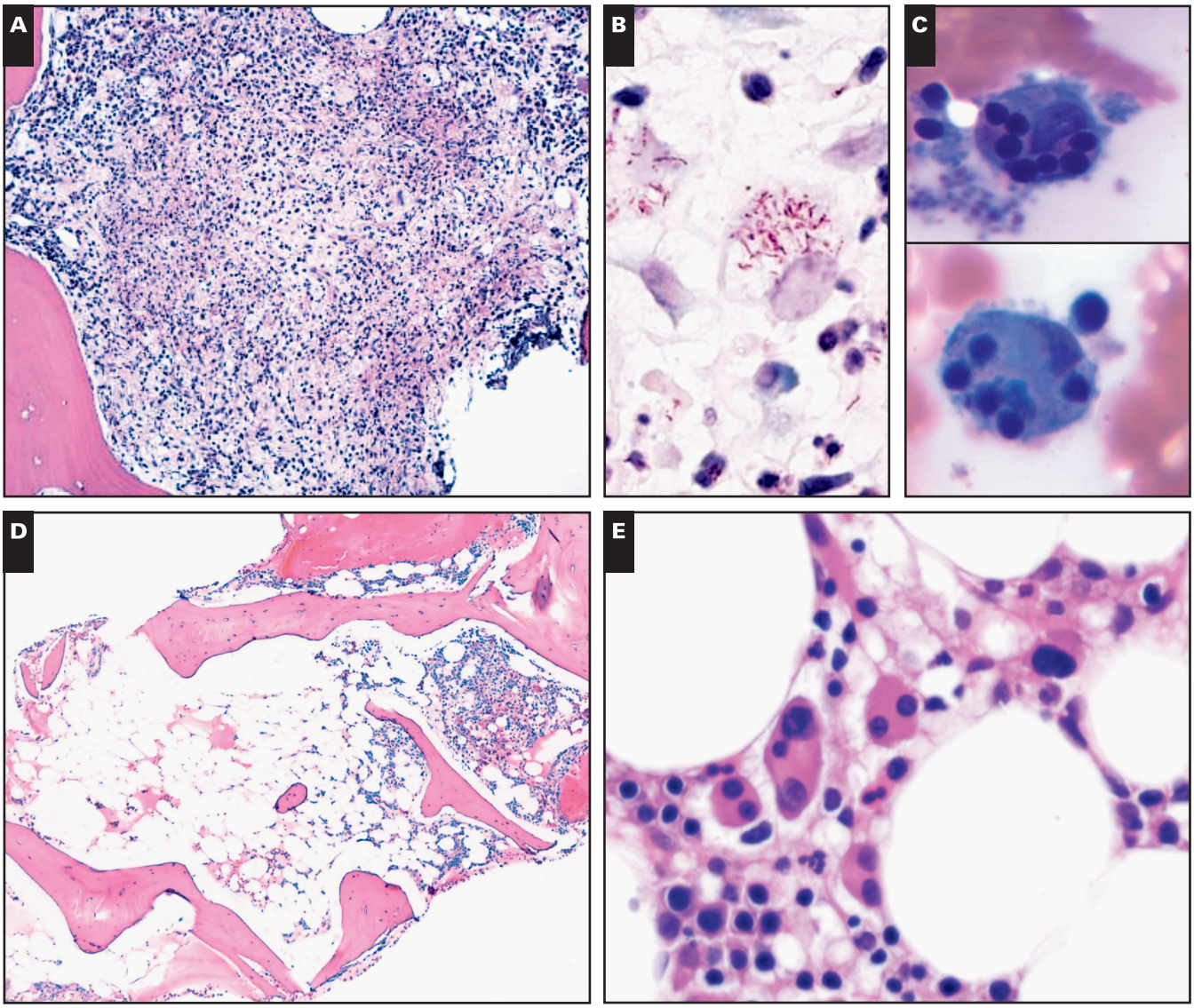

Image 1 Spectrum of bone marrow features in GATA2 deficiency, part 1. A subset of patients have bone marrows with granulomatous inflammation (**A**) and acid-fast bacilli-positive organisms (**B**), as in this patient with *Mycobacterium avium-intracellulare* infection (case 275). Secondary hemophagocytic lymphohistiocytosis with hemophagocytic macrophages (**C**) is a severe complication of GATA2 deficiency and was reported in case 258. Myelodysplasia in the setting of germline GATA2 mutation can present with hypocellular/hypoplastic bone marrow, as reported in half of submitted GATA2 cases (**D**, case 52). Megakaryocytic atypia and dysplasia is a common feature seen on core biopsies and aspirates of GATA2 marrows, as demonstrated in case 307 (**E**).

complex (monoMAC)[5,6] MDS EB2; dendritic cell, monocyte, B and NK lymphoid deficiency (DCML)[7]; Emberger syndrome[8]; and familial MDS/AML.[9] More recently, it has been shown that germline mutations in *GATA2* are present in up to 7% of primary pediatric MDS and 37% of pediatric MDS with monosomy 7.[10] Patients with germline *GATA2* mutations may also present with chronic neutropenia[11] or with features overlapping between aplastic anemia and hypoplastic MDS.[12] Overall disease penetrance is high, and it has been estimated that individuals harboring germline *GATA2* mutations have a 90% risk of clinical complications by age 60,[13] with an estimated 50% to 75% developing myeloid neoplasia.[14,15] The phenotype and age at presentation are variable. In one large study, the median age at presentation was 20 years with a broad range of 4 to 78 years.[14] However, the most common feature of all reported cohorts is the high propensity to develop myeloid neoplasms, particularly MDS, AML, and CMML. For this reason, the syndromes and entities involving germline mutations in *GATA2* are now

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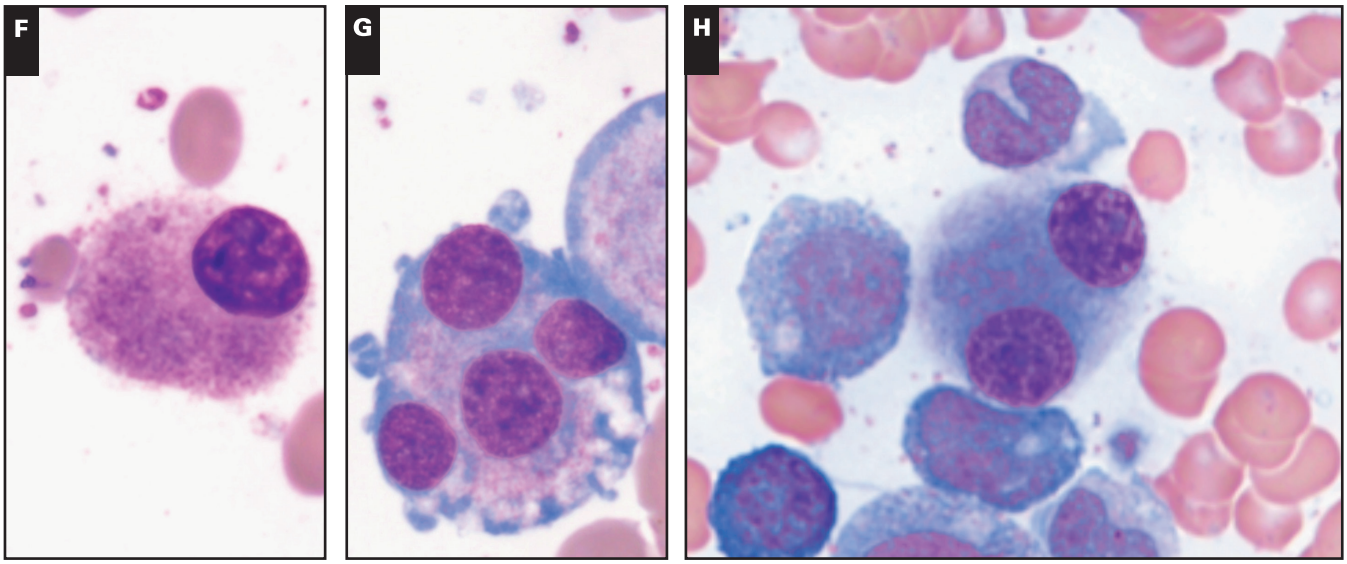

Image 1 (cont) Cases 307 (**F** and **G**) and 20 (**H**).

referred to as a single protean disorder termed GATA2 deficiency.[13-16]

A total of 16 cases with germline *GATA2* mutations were submitted to the 2017 SH workshop. The age at the time of initial presentation, family history, clinical history of immunodeficiency, infections, cytopenias, and type of myeloid neoplasm varied among the cases (Table 1). The age range was 5 to 62 years (median 17.5 years), with seven pediatric cases (under the age of 18 years). The male:female ratio was 7:9. There were four cases of AML, two cases of MDS with excess blasts (MDS-EB), four cases of MDS with multilineage dysplasias (MDS-MLD), three cases of refractory cytopenia of childhood (RCC), and one case of CMML. Two cases (cases 258 and 275) displayed abnormal bone marrow pathology but did not meet formal WHO criteria for a diagnosis of MDS or any other myeloid neoplasm and were diagnosed as bone marrow and immunodeficiency disorder with germline *GATA2* mutation. In nine cases, patients presented with a history of infections and/or cytopenias preceding the development of myeloid neoplasm (MDS/AML, [case 52], RCC [cases 87 and 381], AML [case 266], RCC [case 381], and other MDS subtypes [cases 105, 138, 157, 176, and 337]). In five cases, patients presented with a myeloid neoplasm without any recognized preceding period of cytopenias or infections (AML [cases 20 and 48], CMML [case 40], RCC [case 307], and MDS with hemophagocytic syndrome [case 236]).

Cellular depletion and immunodeficiency with loss of monocytes, B cells, and NK cells is a feature often seen in GATA2 deficiency prior to the development of overt myeloid neoplasia.[14,16] The presenting phenotypic of immunodeficiency and severe infections was reported in six submitted cases. There were three cases with infections involving *Mycobacterium avium complex* (cases 258, 275, and 337), with granulomata in the bone marrow and acid-fast bacilli-positive organisms in case 275 **Image 1A** and **Image 1B**. Warts and severe human papillomavirus (HPV) infection, as previously noted in monoMAC, DCML, and Emberger syndromes,[14,17] were reported in four submitted cases, including HPV-related vulvar and/or anal carcinoma (cases 157 and 175), cervical HPV resulting in hysterectomy (case 275), epidermal dysplasia verruciformis (case 157), and warts (case 337). Cellulitis and panniculitis are disease manifestations of GATA2 deficiency[18] and were reported in at least three submitted cases (cases 52, 87, and 337). Pulmonary infections were present in four cases (case 275 [pulmonary aspergillosis], 157 [*Klebsiella*], 307, and 337). Lymphedema was noted in cases 52 and 87. Scattered hemophagocytic cells are often seen in the bone marrow of patients with GATA2 deficiency[19] without clinical evidence of hemophagocytic lymphohistiocytosis (HLH). However, severe secondary HLH can be a serious complication in the setting of GATA2 deficiency,[20] as demonstrated in cases 258, 236, and 275 **Image 1C**. Other manifestations of GATA2 deficiency reported in the literature that were not represented in the cases submitted include pulmonary alveolar proteinosis[21] and complications of Epstein-Barr virus (EBV) infection.[22] While monocytopenia is a common feature of the monoMAC and DCML phenotypes, GATA2 deficiency patients can develop monocytosis with disease progression to CMML or MDS/MPN,[23] or may present initially with monocytosis due to CMML, as reported in case 40.

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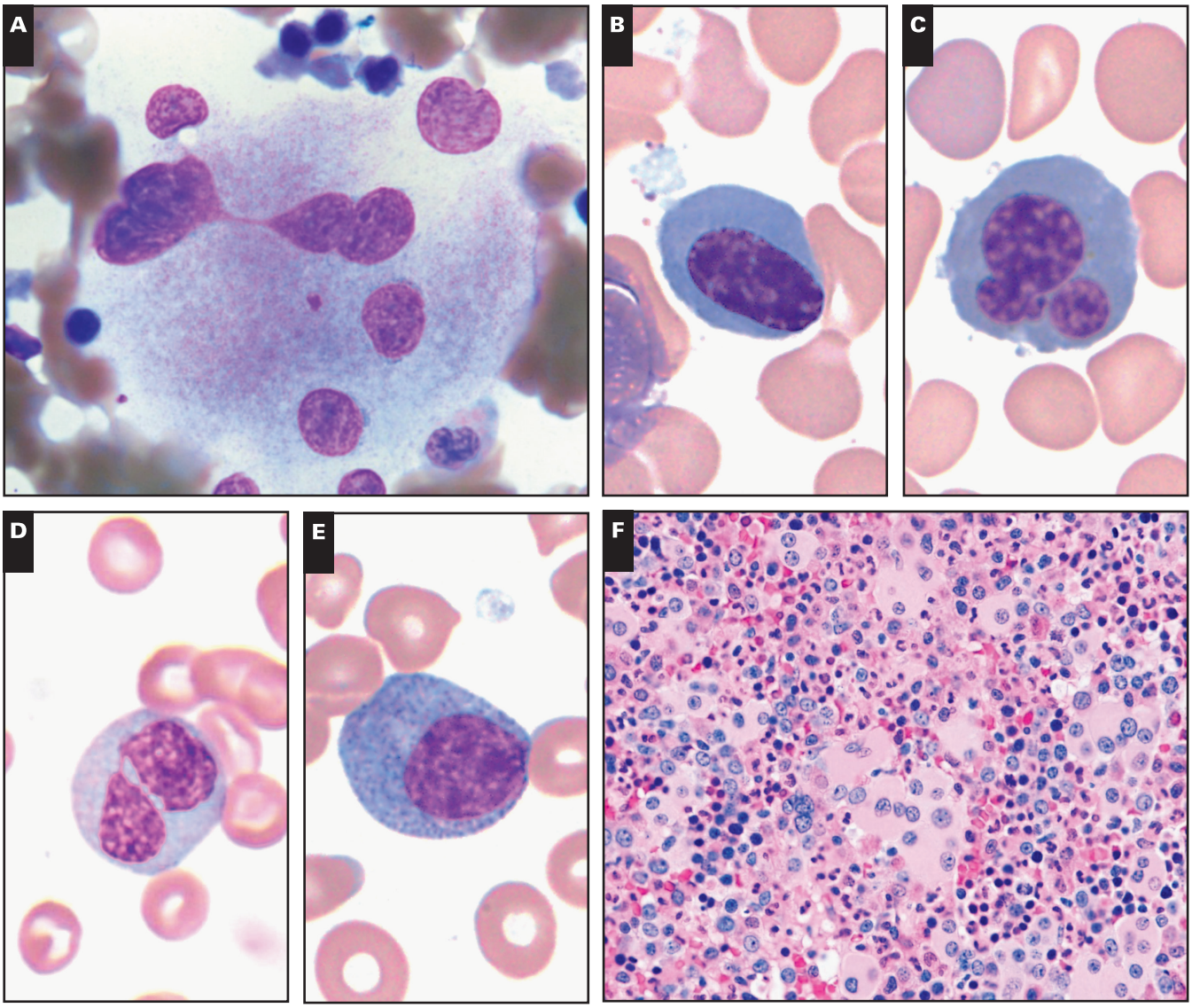

Image 2 Spectrum of bone marrow features in GATA2 deficiency, part 2. Very large giant abnormal megakaryocytes with detached nuclei can often be seen on aspirate smears as shown in case 138 (**A**). Multilineage dysplasia, with dyserythropoiesis (**B**, **C**, case 105) and dysmyelopoiesis (**D**, case 52; and **E**, case 105) is common in GATA2 deficiency-associated myelodysplastic syndrome (MDS). High-grade MDS and acute myeloid leukemia (AML) are often associated with hypercellularity as demonstrated in case 176 diagnosed as MDS-EB2 with a hypercellular marrow with marked megakaryocytic dysplasia (**F**).

Myelodysplasia in GATA2 deficiency is often hypoplastic, as seen in half of the submitted cases **Image 1D**. Atypical/dysplastic megakaryocytes with separated nuclear lobes and micromegakaryocytes are a common feature in the bone marrow from patients with GATA2 deficiency and may precede the development of overt MDS.[19] Atypical/dysplastic megakaryocytes were present in 12 of 16 (75%) of the submitted cases **Image 1E**, **Image 1F**, **Image 1G**, and **Image 1H**, including some cases with very large giant megakaryocytes with abnormal detached nuclei **Image 2A**. Dysplastic features were also common in erythroid **Image 2B** and **Image 2C** and myeloid lineages **Image 2D** and **Image 2E**. A subset of cases showed normocellular or hypercellular marrows, particularly those with advanced myeloid neoplasms such as MDS-EB **Image 2F** and **Image 2G**, AML **Image 2H**, CMML, or those with granulomatous inflammation (Image 1A). In the setting of pancytopenia, some GATA2 marrows are markedly hypocellular (less than 10% cellularity) and these cases may meet criteria for a diagnosis of aplastic anemia (AA), as indicated in the clinical history for case 157. The distinction between

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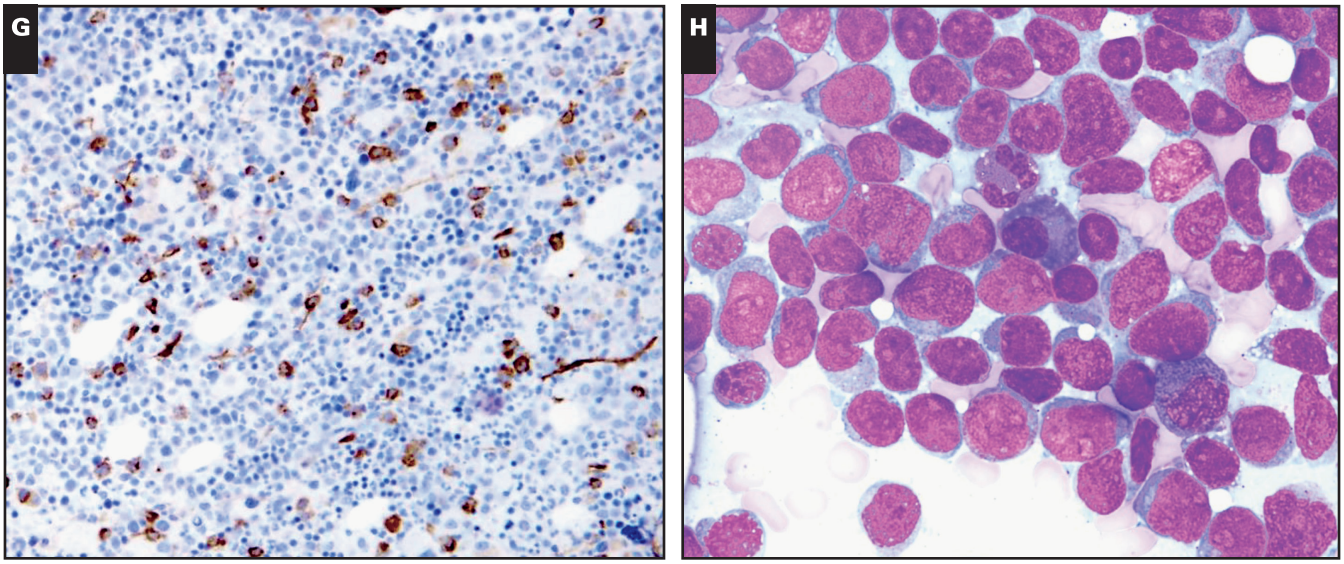

Image 2 (cont) Increased CD34-positive blasts by immunohistochemistry (**G**), and case 20 diagnosed as AML with 80% cellularity on the core biopsy (not shown) and sheets of myeloblasts on the aspirate smear (**H**).

idiopathic AA and GATA2 deficiency is important for proper patient management, as GATA2 deficiency patients may have a poor outcome when treated with immunosuppression. Bone marrow flow cytometry analysis[12] can be helpful in identifying patients with pancytopenia and hypocellular marrows that may harbor *GATA2* mutations, while awaiting genetic sequencing results: patients with GATA2 deficiency often show a disproportionate loss of, or even absent, bone marrow B cells and B-cell precursors,[24] NK-cells, plasmacytoid dendritic cells, and monocytes in comparison to AA and control patients.[12] Those presenting with hypocellular marrows without overt dysplasia may progress to overt MDS as demonstrated in case 157, which showed progression to MDS in a GATA2-deficient patient previously diagnosed with AA. Patients with GATA2 deficiency who develop MDS may subsequently progress to overt AML, as demonstrated in cases 236 and 266. The majority of cases with increased blasts reported blasts with a myeloid phenotype expressing CD34, CD117, and MPO, with or without aberrant expression of CD7. Two notable exceptions included case 48, AML with myelodysplasia-related changes (MRC) with megakaryoblasts that were positive for CD61 and negative for CD34; and case 236, pure erythroid leukemia with blasts that expressed E-cadherin and glycophorin A, and were negative for CD34 and CD117.

Germline mutations in *GATA2* comprise pathogenic variants in exonic and in intronic/regulatory regions of the gene.[10,14] Exonic missense mutations frequently involve the second zinc finger domain and were present in six cases (Table 1). Frameshift mutations and/or mutations resulting in premature stop codons were reported in six cases. There was one mutation involving the regulatory region in intron 5, and two cases with large deletions. One case reported a *GATA2* variant of unknown significance involving a missense change upstream of the zinc finger domains. Germline mutations in *GATA2* result in haploinsufficiency and all appear to confer predisposition to myeloid malignancy; however, a greater incidence of viral infections and lymphedema is reported in individuals with large deletions, premature stop codons, or frameshift mutations.[13] However, the age of disease onset, severity, and phenotype can vary widely in patients with germline *GATA2* mutations, even among family members harboring the same mutation, and the phenotype is not a reliable predictor of mutation status. Ideally, it is important to confirm the germline nature of the *GATA2* mutation, particularly in cases without family history of disease. Sequencing of cultured skin fibroblasts (as performed in case 157) is preferable to buccal swabs that may be contaminated with circulating peripheral blood cells harboring somatic mutations (which can be seen in the *GATA2* gene in sporadic myeloid neoplasms).

Many patients with germline *GATA2* mutations have family history of disease and mutations that are inherited. However, a significant number of GATA2 deficiency patients (up to 71%) harbor de novo rather than inherited germline mutations, which are not detected in family members.[6,10] Cases 52, 138, and 307 documented de novo/sporadic *GATA2* mutations, detected in the patient and not in the parents (including cases 52, 138, and 307). An increased incidence of solid tumors is also associated with GATA2 deficiency, with most related to underlying HPV

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or EBV infections; however, breast cancer, other solid tumors, and melanoma are also reported.[14,25] Cases 20 and 275 reported a family history of solid tumors, some of which were diagnosed in the third decade.

GATA2 is highly expressed in immature hematopoietic cells and declines with blood cell maturation. It is essential for the maintenance and proliferation of hematopoietic stem cells by the complex interactions with a network of other hematopoietic transcription factors, including RUNX1, SCL/TAL1, MYB, GFI1, FLI1, LYL1, or PU.1.[25] Balanced expression of GATA2 is essential for proper hematopoiesis and the disruption of its structure and/or activity can contribute to leukemogenesis. GATA2 overexpression is associated with development of AML and correlates with poor prognosis in this setting.[26]

Targeted next-generation sequencing myeloid sequencing panels were performed for many of the submitted cases (Table 1). Somatic acquired *ASXL1* mutations have been previously reported in myeloid neoplasms with germline *GATA2* mutations[23,26] and were detected in several cases (cases 40, 87, and 176). Other genes with detected somatic variants/mutations included *STAG2* (case 44), *CRLF2* (case 307), *WT1* (case 48), *NRAS* (case 176), and *KRAS* (case 40).

Hematopoietic stem cell transplantation is the only definitive cure for GATA2 deficiency-associated immunodeficiency and myeloid neoplasms (MDS/AML/CMML).[27-30]

However, care must be taken in selecting the donor, as illustrated in case 105 that describes a patient diagnosed with MDS-MLD with germline *GATA2* mutation at the age of 57 years. This patient had been a healthy marrow donor for his brother who was diagnosed with AML 20 years prior. The brother also harbored the germline *GATA2* mutation (assessed by sequencing archived bone marrow) and ultimately died posttransplant after receiving the *GATA2* mutation-positive donor marrow. Further details are not provided, but this case shows two siblings with the same mutation that develop MDS/AML over 20 years apart, and underscores the need for screening all potential related donors for the patient's *GATA2* mutation prior to stem cell transplantation regardless of the state of health of the related donor.[31]

Noonan Syndrome

Noonan syndrome is a relatively common developmental disorder that is found in one of 2000 births. Patients with Noonan syndrome have characteristic dysmorphic features such as low-set, posteriorly rotated ears, down-slanting palpebral fissures, widely spaced nipples, and increased nuchal tissue.[32] These patients have an increased risk of hematologic malignancy, including juvenile myelomonocytic leukemia (JMML) and acute lymphoblastic leukemia (ALL) among others.[32] JMML is a myeloproliferative disorder of early childhood that originates from the multipotent hematopoietic stem cell and is characterized by overproduction of myeloid cells. Hyperactive RAS signaling is assumed to be the main driving event in JMML and is thought to be caused by somatic mutations in *KRAS*, *NRAS*, or *PTPN11* in about 50% of patients.[32] Case 292 reported a 49-day-old infant with Noonan syndrome who presented with mild hepatosplenomegaly and persistent leukocytosis (50-97 × 10^9/L) after treatment for an abscess. Peripheral blood showed neutrophilia and a monocytosis with 6% circulating blasts. Next generation sequencing identified a pathogenic heterozygous *PTPN11* mutation, which in this setting is diagnostic of JMML associated with Noonan syndrome. Case 99 presented a 19-month-old child with Noonan syndrome with a more rare *SHOC2* mutation who developed B-ALL.

Up to 10% of Noonan syndrome patients develop a JMML-like transient myeloproliferative disorder during the neonatal or early infantile period.[32] This is morphologically indistinguishable from JMML but usually regresses spontaneously over months or years, as was seen in case 320. The treatment recommendation for transient myeloproliferative disorder in this setting is to monitor closely with consideration for only mild cytoreductive therapy in severe cases. The patient in case 320 was closely monitored without treatment and had clinically stable disease with resolving leukocytosis and monocytosis at 1 year.

Other Myeloid Neoplasms With Germline Predisposition Associated With Other Organ Dysfunction

Five AML cases that developed in individuals with other congenital disorders were submitted to the workshop, and these patients ranged in age from 4 months to 53 years (Table 2). Most of these cases were AML-MRC or therapy-related AML. Case 55 described a 4-month-old child who presented with splenomegaly, hepatomegaly, leukocytosis, and anemia and was found to have 29% myeloid blasts in bone marrow **Image 3A**. Next-generation sequencing targeting JMML-associated genes revealed *NF1* mutation c.1139T>G p.Leu380Arg with an allele burden of 90% in bone marrow and 49% in buccal sampling, suggesting unrecognized neurofibromatosis type I (NF1) due to germline mutation in

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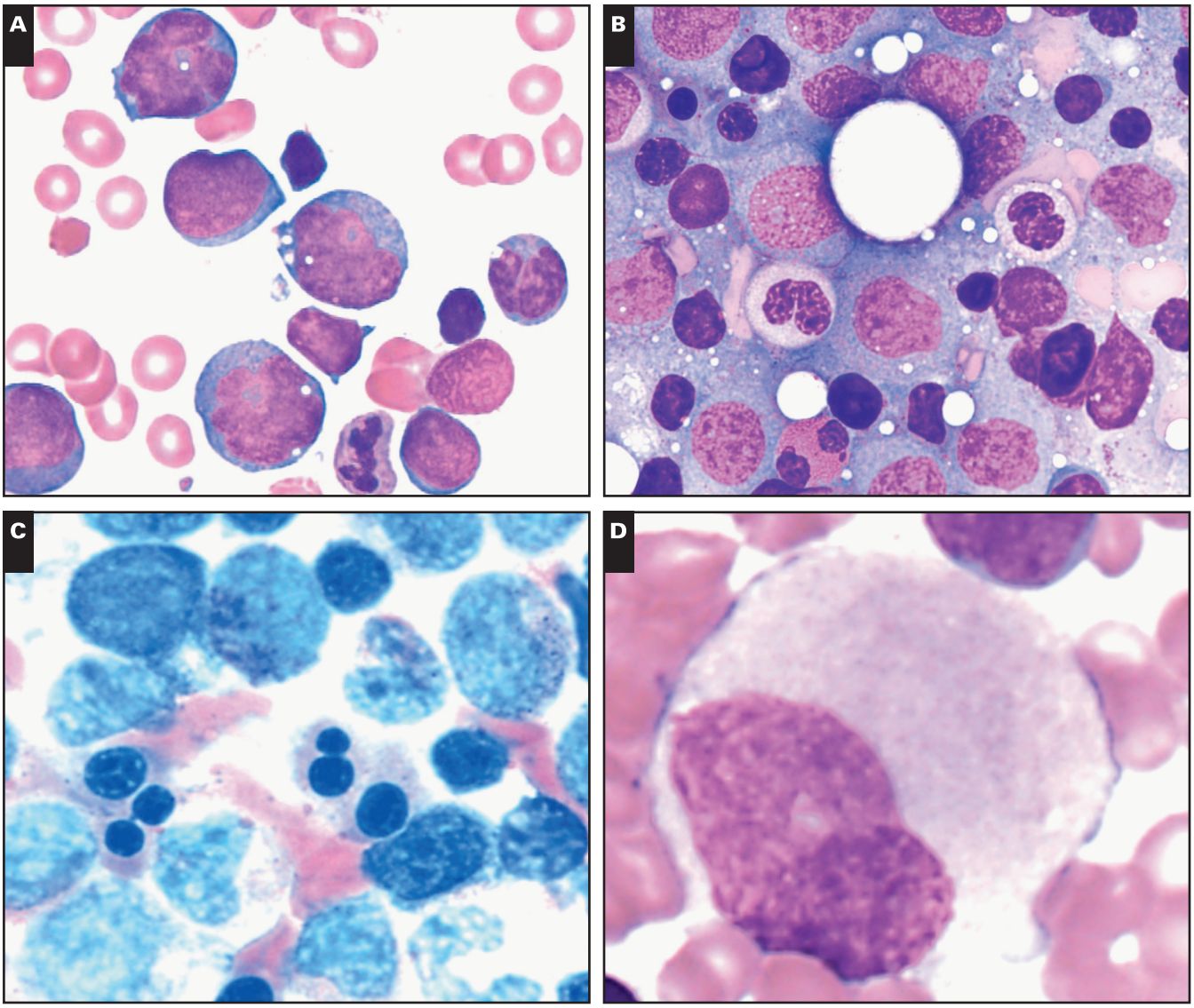

Image 3 Bone marrow morphology findings in germline predisposition syndromes. **A**, Acute myeloid leukemia not otherwise specified with germline *NF1* mutation shows prominent monocytic differentiation (case 55). **B**, Prominent background dysplasia is seen in a case of therapy-related acute myeloid leukemia with germline *TP53* mutation (Li-Fraumeni syndrome, case 167) **C**, Myelodysplastic/myeloproliferative neoplasm, unclassifiable with germline *SAMD9* mutation (MIRAGE syndrome [myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, enteropathy]). **D**, Small hypolobated megakaryocytes seen in case of thrombocytopenia with germline *RUNX1* mutation (case 271).

the *NF1* that encodes neurofibromin.[33] AML cases were submitted describing patients with other cancer predisposition syndromes including Bloom syndrome (case 253) and Maffucci syndrome (case 264). Cancer risk is generally high in patients with Bloom syndrome and is a leading cause of death; AML accounts for about 12.5% of all tumors.[34] Maffucci syndrome is a rare nonhereditary disorder caused by somatic mosaic isocitrate dehydrogenase 1 or 2 (*IDH1* or *IDH2*) mutations characterized by enchondromatosis with an increased risk for skeletal deformity and sarcomatous transformation, but also with rare AML cases reported.[35]

Case 167 described a 44-year-old woman with multiple malignancies who presented with AML with complex karyotype and germline *TP53* mutation, representing a new diagnosis of Li-Fraumeni syndrome. Morphology showed numerous blasts in a background of prominent dysplastic erythroid cells and megakaryocytes Image 3B. Li-Fraumeni syndrome is a rare autosomal dominant syndrome that is characterized by a high incidence of a variety of malignant neoplasms (usually solid tumors, less frequently acute leukemias) within affected families.[36,37] Case 170 described an 11 year old with thrombocytopenia absent radius (TAR) syndrome, an extremely rare

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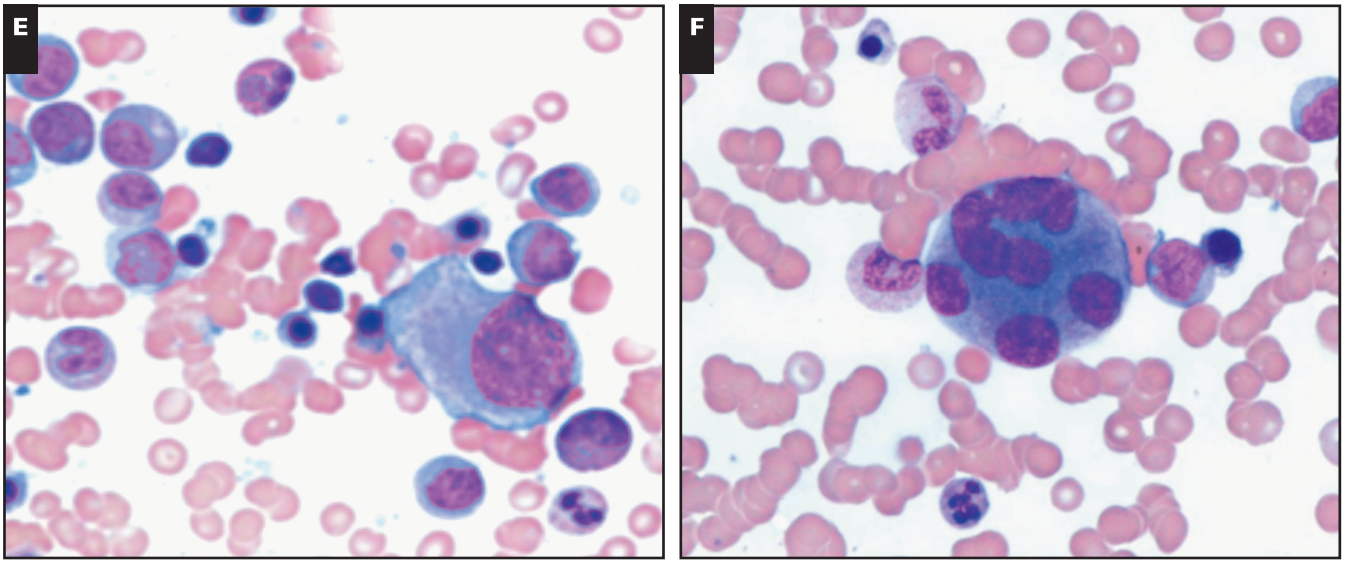

Image 3 (cont) **E**, **F**, Abnormal megakaryocytes seen in a case of myelodysplastic syndrome with multilineage dysplasia with germline *RUNX1* mutation including small size, hypolobation and separated nuclear lobes (case 39).

congenital disorder involving biallelic loss of function of *RBM8A* (RNA binding motif protein 8A) gene, who developed MDS-EB while being treated for Langerhans cell histiocytosis. TAR syndrome is not generally associated with bone marrow failure or malignancy and development of myeloid neoplasms are only rarely reported in this syndrome.[38]

Two cases (case 80 and 273) were submitted that involved *SAMD9* mutations, which have been recently associated with a clinical spectrum of disorders including the MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, enteropathy) syndrome, ataxia-pancytopenia syndrome, and myelodysplasia and leukemia with monosomy 7 syndrome (Table 2). MIRAGE syndrome is a multisystem disorder associated with MDS and monosomy 7.[39] Germline mutations in the related gene *SAMD9L* have also been reported in ataxia pancytopenia syndrome and in families with immune deficiencies, neurologic abnormalities, and MDS.[40] Case 80 was RCC and case 273 was MDS/MPN, unclassifiable, both showing monosomy 7 on karyotype **Image 3C**.

Myeloid Neoplasms With Germline Predisposition and Preexisting Platelet Disorders

Runt-related transcription factor 1 (*RUNX1*) is a protein-coding gene located in 21q22.12, which encodes the DNA-binding subunit of the core-binding factor transcription complex that is essential for normal hematopoiesis.[41] *RUNX1* point mutations were first identified in leukemia in 1999 in six families.[41] Many subsequent studies documented frequent somatic mutations in *RUNX1* in MDS, AML, ALL, and other myeloid neoplasms. Germline *RUNX1* mutations are associated with familial platelet disorders with an increased risk of myeloid malignancy, and these patients have variable thrombocytopenia from birth, with or without bleeding tendencies.[42] Decreased platelet aggregation to collagen and epinephrine may also be seen. A total of four cases submitted to the workshop showed germline *RUNX1* mutation and thrombocytopenia (Table 2). These patients ranged in age from 3 to 37 years and one of these patients had an extensive family history of AML. Most cases showed some degree of morphologic dysplasia, which was overall insufficient for a diagnosis of MDS. In case 309, the *RUNX1* mutation was an incidental finding discovered by targeted sequencing that eventually led to germline confirmation. Prior to the recognition of germline *RUNX1* mutation familial platelet disorders, most of these patients were considered to have immune thrombocytopenia. Thus, it is important to consider the possibility of germline mutations in patients presenting with isolated thrombocytopenia, particularly if there is evidence of megakaryocytic atypia, such as seen in case 271 **Image 3D**. Patients with germline *RUNX1* mutation have a lifetime risk of 35% to 44% of developing AML or MDS, although the risk depends on the specific *RUNX1* mutation[43,44] and likely requires acquisition of additional mutations. Three cases submitted to the workshop were AML and MDS arising

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in the setting of an underlying germline *RUNX1* mutation and one of these cases (case 284) showed additional somatic *NRAS* and *BCOR* mutations (Table 2). Clinical findings and relevant family history are listed in Table 5. Prominent megakaryocytic dysplasia, including hypolobated megakaryocytes with eccentric nuclei and megakaryocytes with separated nuclear lobes, are often seen in cases of MDS with germline *RUNX1* mutation Image 3E and Image 3F

A variety of *RUNX1* mutations have been described throughout the gene, including frameshift or nonsense mutations. Germline *RUNX1* mutations generally cluster in the N-terminal region, resulting in disruption of DNA binding, and are less frequent in the C-terminal region, which maintains DNA binding but lacks a functional transactivation domain.[44] Often novel mutations are seen, such as in case 219, which showed a 37-year-old man with persistent isolated thrombocytopenia and a *RUNX1* mutation that resulted in p.Arg207Trp amino acid change that had not been previously reported (Table 4).

Making a definitive diagnosis in patients with inherited thrombocytopenia is important because different forms of this disorder differ with respect to prognosis and treatment. Thrombocytopenia 2 is characterized by germline mutations in *ANKRD26*, located on chromosome band 10p12.1, and is an autosomal dominant disorder characterized by moderate thrombocytopenia and increased risk of developing MDS or AML.[45] Case 268 is of a 43-year-old woman who presented with history of thrombocytopenia. Genetic testing on a skin culture specimen showed a sequence change in the 5′ untranslated region ANKRD26 c.-119C>G (Table 4). Of note, this is an overall exceedingly rare form of inherited thrombocytopenia.[45]

Myeloid Neoplasms With Germline Predisposition Without a Preexisting Disorder or Organ Dysfunction: *CEPBA* and *DDX41* Mutations

Two cases (cases 283 and 230) were AML with germline *CEBPA* mutations, both with classic germline N-terminal *CEBPA* frameshift mutation and somatic C-terminal insertion mutation (Table 2 and Table 5).[46] Both cases had CD7 expression on myeloblasts, often observed in these patients. Blasts in AML with germline *CEBPA* mutations often show Auer rods Image 4A. Both patients were in remission at the latest follow-up, consistent with reported favorable clinical outcomes seen in this entity. One submitted case of AML with germline *DDX41* mutation (case 138) was in a 67-year-old man who had slowly decreasing peripheral blood counts and an extensive family history of leukemias. Unlike many other germline mutations, patients with germline mutations in *DDX41* tend to present with myeloid neoplasms in adulthood, but with similar clinical course to other myeloid neoplasm presenting with similar genetics.[47]

Other possible germline predisposition mutations without a prior phenotype included a therapy-related AML in a patient with germline *PTCH TGRB1* microdeletion of unknown significance (case 225) and AML in a patient with a germline t(8;21) translocation encompassing the *RUNX1* gene and somatic *RUNX1* mutation (case 234). Case 234 is of a 53-year-old man who was found to have leukocytosis without other symptoms and no family history of hematologic disorders. The t(8;21) translocation encompassed the *RUNX1* gene and was seen in all metaphases regardless of disease involvement, suggestive of a constitutional abnormality. Concurrent detection of a somatic *RUNX1* loss-of-function mutation suggests a two-hit mechanism of *RUNX1* loss.

Germline Predisposition to Myeloproliferative Neoplasms

The vast majority of myeloid neoplasms associated with the above germline predisposition conditions are MDS, MDS/MPN, or AML. However, rare examples of MPN can also be associated with germline mutations. Case 42 describes a 6-year-old girl with history of B-ALL, autoimmune hepatitis, and progressive splenomegaly since birth that was out of proportion to her liver disease. She underwent splenectomy; splenic infarcts were noted but otherwise histology was unremarkable. After splenectomy, the patient's platelet count rose to 2,131 × 10^9/L within 9 days and remained elevated. Bone marrow biopsy showed atypical large megakaryocytes with hyperlobated nuclei, frequently occurring in clusters, features consistent with ET Image 4B. Testing confirmed the presence of a homozygous frameshift mutation in the *SH2B3* gene. *SH2B3* encodes an adaptor (LNK) that inhibits the JAK/STAT pathway and negatively modulates signaling of several cytokine receptors, including MPL. This mutation had been shown in vivo to increase JAK2/STAT3 phosphorylation and increase growth and proliferation, and *SH2B3* knockout mice have been shown to have accelerated leukemogenesis.[48] The germline loss-of-function variant in *SH2B3* likely played a role in the development of ALL and MPN in this patient.

Case 97 was of a 6-year-old boy who presented for evaluation as a bone marrow donor candidate for his older sibling with relapsed classical Hodgkin lymphoma. He was noted to have thrombocytosis and bone marrow examination showed an increased number of megakaryocytes with

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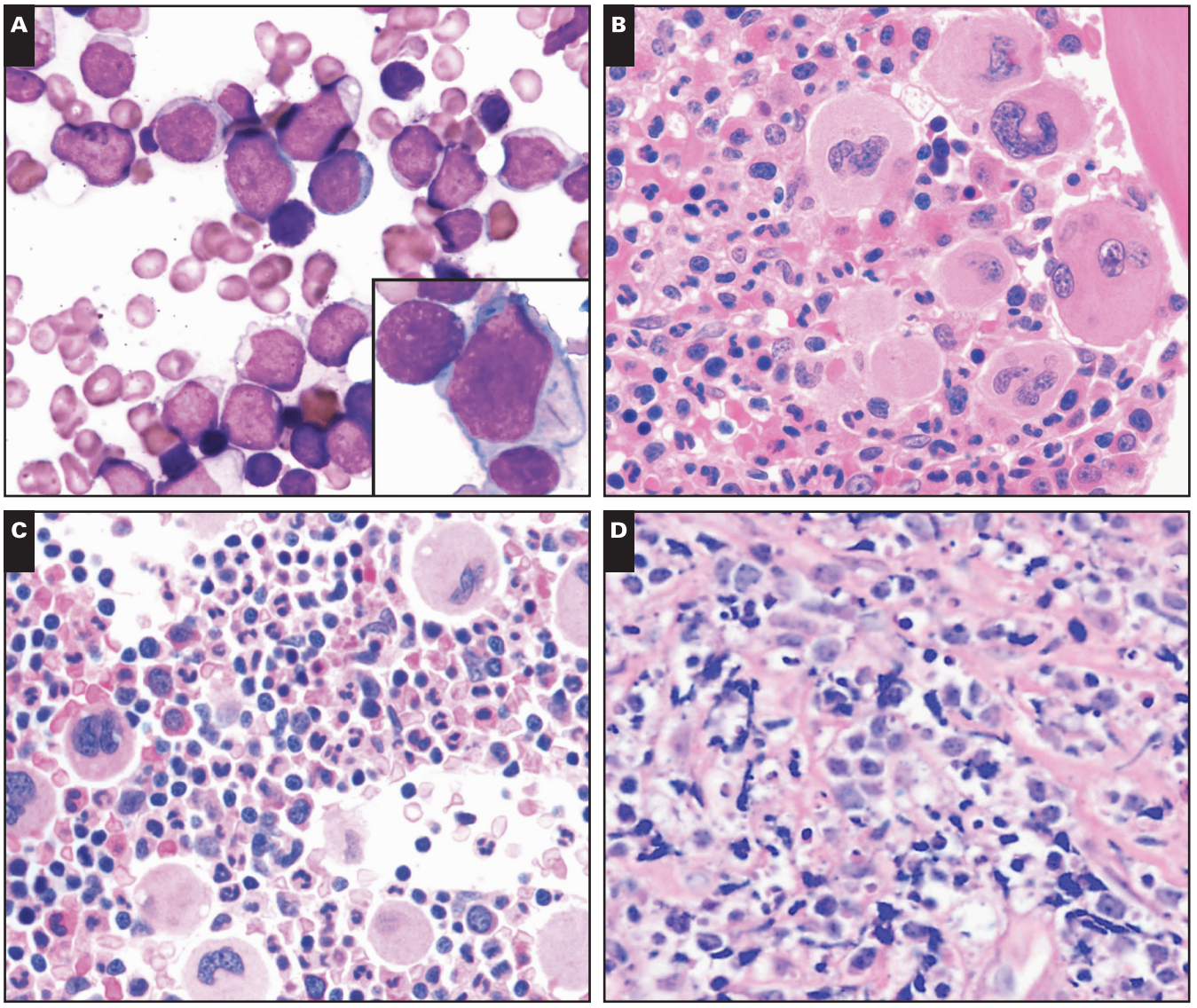

Image 4 Other neoplasms with germline predisposition syndromes. **A**, Blasts in acute myeloid leukemia with germline *CEBPA* mutations often show Auer rods (case 230). Inset, prominent Auer rods. **B**, Proliferation of large atypical megakaryocytes in essential thrombocythemia with germline *SH2B3* mutation (case 42). **C**, Increased number of mostly normal-appearing megakaryocytes in thrombocytosis with *MPL* mutation (case 97). **D**, Sheets of intermediate-sized lymphoid cells in Burkitt lymphoma (case 106) in a patient with X-linked lymphoproliferative disorder.

normal morphology **Image 4C**. Whole-exome sequencing showed a germline homozygous *MPL* P106L activating mutation. This *MPL* variant, present in 6% of individuals of Arab ancestry, has been associated with thrombocytosis in the homozygous state and normal or mildly elevated platelet counts in the heterozygous state. Although definite myeloproliferative disorders have not yet developed in the families reported with this mutation, it may have an oncogenic potential, as the mutation appears to affect thrombopoietin binding to the MPL receptor.[49,50] A 19 year old with longstanding neutropenia and recurrent multiple systemic infections since childhood, diagnosed with classical Hodgkin lymphoma and a germline *CSF3R* mutation, was identified (case 346). Although somatic *CSF3R* mutations are associated with the myeloid neoplasm chronic neutrophilic leukemia and leukocytosis, the germline mutations are associated with neutropenia, and the discovery of this mutation shed light on the etiology of the patient's longstanding neutropenia and infections. Acquired somatic truncating mutations in *CSF3R*, analogous to those found in chronic neutrophilic leukemia, have been described in up to 30% to 40% of patients with severe congenital neutropenia concurrent with inherited, pathogenically relevant mutations such as *ELANE* or *HAX1*.[51]

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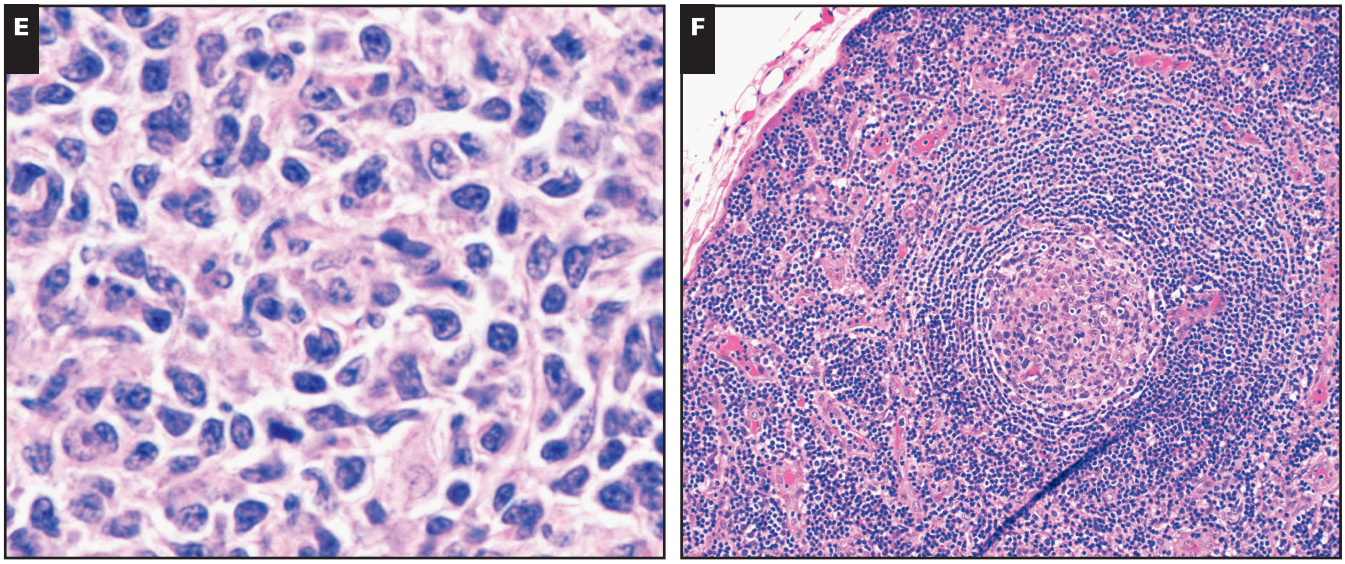

Image 4 (cont) **E**, Follicular lymphoma, grade 1 to 2 of 3, in a patient with germline *TP53* mutation (Li-Fraumeni syndrome; case 209). **F**, TAFRO (thrombocytopenia, ascites, fibrosis, renal dysfunction, organomegaly), a variant of multicentric Castleman disease (case 200).

Germline Predisposition to Lymphoid Malignancies

Lymphoid neoplasms can also develop in the setting of germline predisposition syndromes (Table 3). Case 106 reported a 6-year-old boy who presented with typical Burkitt lymphoma Image 4D and family history of lymphoma in siblings, which ultimately led to a diagnosis of X-linked lymphoproliferative disorder. X-linked lymphoproliferative disease type 1 (XLP1) is a rare primary immunodeficiency caused by the mutation in *SH2D1A* located on the long arm of the X chromosome. About one-third of XLP1 patients present with lymphoma, and of these up to 50% are Burkitt lymphoma.[52] Case 101 and 194 were B-ALLs in young children arising in setting of germline *PAX5* and *ELANE* mutations, respectively (Table 3). Case 342 described a 40 year old with recurrent respiratory and gastrointestinal infections who presented with T-cell large granular lymphocytic leukemia in association with a germline *CTLA4* mutation. Morphologic evaluation of the bone marrow showed numerous lymphoid aggregates and increased large granular lymphocytes. Germline mutations in the *CTLA4* gene have been recently discovered in patients with autoimmunity and immunodeficiency.[53] Case 209 detailed a 38-year-old female patient with a documented history of Li-Fraumeni syndrome who had undergone a prophylactic bilateral mastectomy. Routine surveillance with annual magnetic resonance imaging disclosed an enlarging left inguinal lymph node that on excision showed follicular lymphoma, grade 1 to 2 Image 4E. Li-Fraumeni syndrome patients tend to develop tumors for which the sporadic counterparts also carry a high incidence of *TP53* mutations: while 2% of these patients develop lymphomas, there are no previously reported cases of follicular lymphoma in the setting of Li-Fraumeni syndrome.

The syndrome of thrombocytopenia, ascites, fibrosis, renal dysfunction, organomegaly (TAFRO) is a variant of multicentric Castleman disease. Its etiology is still unknown but it likely represents a systemic inflammatory disorder mediated by redundant cytokines including interleukin-6 (IL-6), vascular endothelial growth factor, and IL-10.[54] Case 200 described a 3-year-old boy with no known family history who presented with fever, generalized lymphadenopathy, hepatosplenomegaly, bilateral pleural effusions, ascites, thrombocytopenia, and anemia. An excised lymph node showed preserved nodal architecture with scattered reactive follicles, some with expanded mantle zones and regressed germinal centers Image 4F. The overall findings were diagnostic of TAFRO syndrome; an underlying germline *RUNX*1 mutation was identified, which had not been previously reported in TAFRO syndrome.

Conclusions and Future Directions: Testing and Recognition of Germline Predisposition in Clinical Practice

Although any single cancer predisposition syndrome is rare, recent studies have demonstrated that germline cancer predisposition mutations as a whole may not be as rare as previously presumed. A group from St Jude

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Children's Research Hospital, Memphis, TN,[55] analyzed a panel of 565 genes in 1,120 patients with pediatric cancer and identified germline mutations in 8.5% of patients, including in 4.4% of leukemia patients. Strikingly, only 23% of patients with an identified mutation had a family history that was suggestive of cancer predisposition.[55] Similarly, a study that used a targeted panel of 28 genes identified germline mutations in 18% of adult patients.[56] Inherited and acquired alterations of genes encoding hematopoietic transcription factors are central events in the pathogenesis of ALL. Recent studies have identified germline mutations in *TP53, PAX5, ETV6,* and *IKZF1* in families with familial ALL.[57] Forty-nine genes with a (presumed) causative role in lymphomagenesis have been described, and germline variants in diffuse large B-cell lymphoma have been identified.[58] The cases submitted to this workshop appear to reflect the variety of myeloid neoplasms with germline predisposition that have been described in the literature. Although lymphoid neoplasms with germline predisposition are less frequent, they reflect the overall distribution of cases in this workshop.

Germline mutations in an increasing number of genes are known to be associated with increased risk for development of myeloid or lymphoid malignancies. With more frequent and widespread use of molecular testing in clinical diagnostics, an increasing number of patients and families will likely be recognized as having an inherited cancer predisposition syndrome. Clinical history that would prompt consideration for the presence of a germline predisposition syndrome includes a personal history of multiple cancers and early onset of malignancies, longstanding thrombocytopenia and/or platelet dysfunction, and multiple cases of myeloid malignancies or solid tumors occurring within two generations in the family.[59,60] Detailed family histories should be obtained by physicians or genetic counselors knowledgeable in these syndromes.

When a clear clinical phenotype strongly suggests germline predisposition syndrome, targeted sequencing for mutation in the suspected single gene or a sequential series of genes based on the phenotype is a reasonable strategy.[60] However, phenotypic features often overlap between multiple different syndromes, genetic disorders exhibit broad phenotypic variability and penetrance, and clinical or familial findings to inform diagnosis are often lacking. Hematologic malignancy gene panels used in the clinical setting are designed to target specific gene regions known to be somatically mutated and often omit regions of the gene that are mutated in germline hematologic malignancy disorders. Therefore, targeted gene panels that are designed for somatic mutation analysis of myeloid neoplasm should not be relied upon for diagnostic evaluation of germline mutations. Increasingly, gene panels specifically designed to evaluate genes associated with germline predisposition are becoming available. Whole-exome sequencing is also a powerful tool for evaluation, and the breadth of this coverage can be helpful when traditional targeted panels have not identified a causative gene. However, some genes can harbor mutations in noncoding or intronic regions that are not covered by whole-exome sequencing.

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