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Review Series

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The Journal of Clinical Investigation

Transcription factor mutations as a cause of familial myeloid neoplasms

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The initiation and evolution of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are driven by genomic events that disrupt multiple genes controlling hematopoiesis. Human genetic studies have discovered germline mutations in single genes that instigate familial MDS/AML. The best understood of these genes encode transcription factors, such as GATA-2, RUNX1, ETV6, and C/EBPα, which establish and maintain genetic networks governing the genesis and function of blood stem and progenitor cells. Many questions remain unanswered regarding how genes and circuits within these networks function in physiology and disease and whether network integrity is exquisitely sensitive to or efficiently buffered from perturbations. In familial MDS/AML, mutations change the coding sequence of a gene to generate a mutant protein with altered activity or introduce frameshifts or stop codons or disrupt regulatory elements to alter protein expression. Each mutation has the potential to exert quantitatively and qualitatively distinct influences on networks. Consistent with this mechanistic diversity, disease onset is unpredictable and phenotypic variability can be considerable. Efforts to elucidate mechanisms and forge prognostic and therapeutic strategies must therefore contend with a spectrum of patient-specific leukemogenic scenarios. Here we illustrate mechanistic advances in our understanding of familial MDS/AML syndromes caused by germline mutations of hematopoietic transcription factors.

Introduction

High-throughput sequencing has revolutionized molecular medicine. In hematology, genomic technologies have unveiled the complex genomic landscape of blood pathologies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (1–3). The formidable challenge now is to decipher vast genetic blueprints harboring abundant "variants of undetermined significance" to discover clinically important aberrations. Major efforts focus on elucidating how single mutations or combinations thereof cause MDS and AML and impact treatment responsiveness or resistance.

The study of familial MDS/AML has unique power to identify leukemogenic drivers and elucidate multistep mechanisms in which a risk allele enables acquisition of additional mutations or confers hypersensitivity to secondary insults, either genetic or environmental, thus causing progressive bone marrow (BM) failure and/or MDS/AML. Since the identification of *RUNX1* as the first nonsyndromic monogenic familial MDS/AML gene (4), more than 65 genes with diverse biologic functions have been implicated in contributing to MDS/AML risk (Table 1, Table 2, and refs. 5–7). Efforts to catalog the diverse clinical phenotypes, disease penetrance and latency, germline mutation spectra, and cooperating acquired mutations have provided insight into the organ systems and cell lineages that rely on each gene. These data also inform how each germline mutation uniquely disrupts protein function(s), whether it generates haploinsufficiency, dominant-negative or ectopic protein functions, or amalgamated gain-of-function and loss-of-function defects to disrupt cellular phenotypes and instigate MDS/AML.

Among MDS/AML risk loci, *GATA2*, *RUNX1*, *ETV6*, and *CEBPA* encode transcription factors with vital functions to control hematopoietic stem and progenitor cell (HSPC) development and differentiation. Comparing and contrasting phenotypes caused by germline and somatic mutations in these genes in mouse and human systems has yielded fundamental insights into the complexity and interconnectedness of networks that govern hematopoiesis. This Review describes how germline mutations dysregulating hematopoietic transcription factors in familial MDS/AML inform pathogenesis.

GATA-2 deficiency syndrome

Mechanistic foundations. Germline *GATA2* coding and regulatory element mutations cause immunodeficiency, MDS/AML, lymphatic vascular dysfunction, and other complex phenotypes (Table 3) (8–13). To understand how distinct mutations in one gene instigate diverse pathologies, it is instructive to rigorously establish a foundation for *GATA2* function at the molecular, cellular, and physiological levels.

The discovery of the founding member of the GATA transcription factor family, GATA-1, initiated a richly productive phase of research that unveiled numerous mechanistic insights. As reviewed elsewhere (14, 15), efforts to elucidate mechanisms governing developmental changes in globin gene expression identified a transcription factor termed GATA-1 (16, 17) that bound with specificity to WGATAR-containing DNA (18–20). Targeted deletion of murine *Gata1* abolished definitive erythropoiesis, yielding

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Table 1. Mutations in DNA repair genes implicated in cellular mechanisms of familial MDS/acute leukemia

Base excision repair	Homologous recombination	Mismatch repair	Nonhomologous end joining	Transcription deficiency	Other DNA repair
MBD4	*ATM, FANCA, FANCB, FANCC, BRCA2* (*FANCD1*), *FANCD2, FANCE, FANCF, FANCG, FANCI, BRIP1,* (*FANCJ*), *FANCL, FANCM, PALB2* (*FANCN*), *RAD51C* (*FANCO*), *SLX4* (*FANCP*), *ERCC4* (*FANCQ*), *RAD51* (*FANCR*), *BRCA1* (*FANCS*), *UBE2T* (*FANCT*), *XRCC2* (*FANCU*), *REV7* (*FANCV*)	*EPCAM, MLH1, MSH2, MSH6, PMS2*	*LIG4*	*ERCC6L2*	*BLM, NBN*

anemia and embryonic lethality (21). GATA-1 also controls megakaryocyte (22), eosinophil (23), and basophil (24) differentiation.

In contrast to lineage-restricted GATA-1 functions, targeted deletion of *Gata2* abrogates multilineage hematopoiesis (25). This discovery established GATA-2 as the first protein that endows hematopoietic stem cells (HSCs) with the capacity to generate all blood cells. Studies of mice with conditional deletions of *Gata2* and *Gata2* enhancer mutations (12, 26–31) extended its vital developmental activity to adult hematopoiesis and delineated GATA-2–dependent cellular/molecular steps and genetic networks (Figure 1) controlling hematopoiesis. GATA-1 and GATA-2 function through multimeric complexes assembled on WGATAR motifs or E-box–spacer–WGATAR composite elements (20, 32). Despite having the capacity to function through the same motif at the same locus (in different developmental contexts), the influence of GATA-1 versus GATA-2 on transcription can differ. Furthermore, reducing the levels of GATA factor complex components can differentially impact GATA factor–dependent target gene ensembles (33).

GATA-2 is an unstable protein ($t_{1/2}$ < 1 hour) degraded by the ubiquitin-proteasome system (34, 35). During embryogenesis, BMP4 induces GATA-2 expression (36, 37). The blood- and vascular-regenerative transcription factor ETV2 (38–40), one of the approximately 30-member ETS transcription factor family that also includes ETV6, occupies the *Gata2* locus (41). GATA-2 also occupies conserved noncoding DNA regions in the *Gata2* locus that assemble GATA-2 and GATA-1 complexes in a context-dependent manner (42–44). *Gata2* transcription positively correlates with GATA-2 occupancy at these sites, implying autoregulation (14, 15, 42). In contrast, GATA-1/FOG-1–dependent displacement of GATA-2 from these chromatin sites, deemed a GATA switch, represses *Gata2* expression, which enables erythroid precursors to progressively mature into erythrocytes (14, 43).

Mutant mice lacking individual "GATA switch sites" demonstrate essential activities of enhancers +9.5 and –77 kb relative to the *Gata2* transcription start site (Figure 2) for hematopoiesis and embryogenesis (12, 29). The +9.5 intronic enhancer triggers HSC emergence in hemogenic endothelium of the aorta-gonad-mesonephros (AGM) region of the embryo, and its deletion depletes HSCs (12, 28). The –77 distal enhancer regulates myeloid progenitor differentiation but not HSCs or function in the mouse embryo (29). Individual emergence of deletions of the other GATA switch sites (–1.8, –2.8, and –3.9) (44–46) have little or no impact on hematopoiesis (47–49).

Why do deletions of different enhancers at the same locus yield embryonic lethality, yet differentially affect hematopoiesis? Compound-heterozygous mice lacking one copy each of the +9.5 and –77 enhancers on different chromosomes are embryonic lethal, which resembles homozygous deletions of either enhancer alone (50). These compound heterozygous mice exhibit normal HSC emergence but defective myeloid progenitors. While one +9.5 copy suffices to induce HSC emergence, the +9.5 and –77 enhancers must reside on the same chromosome for normal progenitor biology. This mechanism, in which both enhancers function in concert in progenitors, but not in HSCs, exemplifies the context-dependent nature of GATA-2 regulation.

Downstream of GATA-2 expression, GATA-2–regulated genetic networks are highly context-dependent (12, 28, 29, 50, 51), involving variable composition and activity of coregulators and transcription factors within distinct cell types and even subnuclear domains within a single cell type. For example, in eryth-

Table 2. Mutations in diverse genes implicated in cellular mechanisms of familial MDS/acute leukemia

DNA damage–sensing	Cell proliferation	Cytokine signaling	Hematopoietic transcription factors	Neutrophil biology	Protein ubiquitination	RAS pathway	Ribosome biology	Telomere biology	Unresolved mechanism
TP53	*SAMD9, SAMD9L*	*SH2B3*	*CEBPA, ETV6, GATA1, GATA2, IKZF1, MECOM1, PAX5, RUNX1*	*CSF3R, ELANE, WAS*	*RBBP6*	*CBL, NF1, PTPN11*	*RPL11, RPL5, RPS19, RPS26, SBDS, SRP54*	*ACD, CTC1, DKC1, NAF1, NHP2, NOP10, PARN, POT1, RTEL1, TERC, TERT, TINF2, USB1, WRAP53*	*ANKRD26, ATG2B* (*GSKIP*), *DDX41, SRP72*

This table includes genes in the listed pathways that have been observed in at least one patient who developed MDS or acute leukemia. Additional genes in these pathways that have been observed only in bone marrow failure presentations are not included.

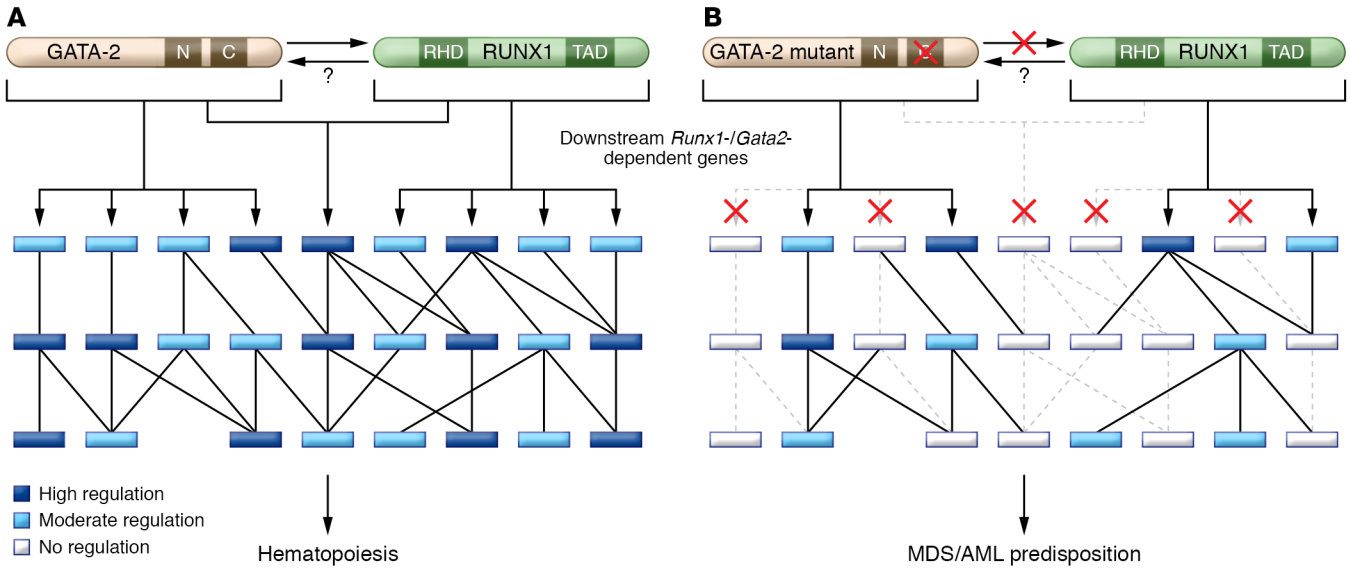

Figure 1. Ensuring normal hematopoiesis by establishing and maintaining genetic network integrity. The diagram on the left depicts the GATA-2–dependent increase in RUNX1 expression (28, 99), and both independent and collective functions of these transcription factors to establish and maintain genetic networks. The question mark denotes uncertainty as to whether RUNX1 might control GATA-2 expression. The diagram on the right depicts how an inhibitory GATA-2 C-finger mutation corrupts network integrity. The model assumes that network integrity ensures normal hematopoiesis, and numerous opportunities exist to corrupt integrity, thereby creating a predisposition or vulnerability to a battery of genetic or environmental insults that trigger pathogenesis. For simplicity, the diagram illustrates GATA-2 and RUNX1 actions, yet numerous components are involved, including other genes and their respective proteins subject to germline mutations that create a predisposition for MDS/AML, e.g., C/EBPα. In addition, there may be instances in which GATA-2–dependent pathogenesis is independent of RUNX1 and vice versa. The rectangles at the bottom represent individual genes, with the colors representing variable levels of gene activity. The white rectangles within the corrupted network reflect little or no transcriptional regulation. Red X, blocked path; hatched line, disrupted regulatory connection. N, N-finger; C, C-finger; RHD, Runt homology domain; TAD, transactivation domain.

roid precursors, GATA-2 induces *Gata1* and *Kit* transcription (50). GATA-1 represses *Kit* transcription (52, 53) to promote prodifferentiation erythropoietin signaling (54) and represses *Gata2* transcription (43, 55–57). In HSPCs, GATA-2 induces 20 G protein–coupled receptors, including GPR65 (58). Downregulating GPR65 increases HSC emergence as a result of a negative-feedback loop that establishes repressive chromatin, restricts Scl/TAL1 occupancy at the +9.5 enhancer, and decreases *Gata2* transcription (58). In the AGM, GATA-2 induces RUNX1 and other transcriptional regulators of hematopoiesis, including Scl/TAL1 and the zinc finger proteins GFI1 and GFI1b (28). Because these transcription factors establish/maintain genetic networks (59–61), and because GATA-2 is expressed in cells containing these factors, the networks are interdigitated, and a subset of the components are coregulated (Figure 1). How these networks parse into circuits involving autoregulatory, feed-forward, and feedback loops and how circuits interdigitate to establish, maintain, buffer, and remodel network integrity to mediate GATA-2 function are of considerable interest. Deciphering the context-dependent mechanisms governing GATA-2 regulation and function will be critical to understand and treat the diverse GATA-2–dependent pathologies in humans.

GATA-2 dysfunction in human pathologies. As expected from the discoveries described above in mouse models, acquired mutations in human *GATA2* are associated with multiple pathologies (Table 3). Although *GATA2* mutations are relatively rare, occurring in less than 5% of MDS/AML cases overall (Table 4), specific subsets are enriched for different *GATA2* mutations. An acquired recurrent mutation (p.L359V) in the DNA-binding carboxy-terminal zinc finger (C-finger) domain was reported in 9% (8 of 85) of accelerated- or blast-phase chronic myeloid leukemia (CML) cases (62, 63). In reporter assays, p.L359V-mutated GATA-2 exhibited greater activation and repression activities, in comparison with wild-type GATA-2, consistent with a gain of function. In mice, p.L359V cooperated with BCR/ABL to induce myelomonocytic leukemia. In contrast, GATA-2 N-terminal zinc finger (N-finger) mutations are acquired in 20%–39% of biallelic C/EBPα-mutated AML (64). However, the function of the N-finger, which differs from the C-finger in not being required for binding to WGATAR sequences (65), and how acquired N-finger mutations alter GATA-2 activity are unclear.

Heterozygous germline *GATA2* mutations cause diverse clinical presentations, including familial MDS/AML alone (9), MonoMAC and DCML syndromes (immunodeficiency syndromes featuring monocytopenia, B cell, NK cell, and dendritic cell deficiencies along with pulmonary alveolar proteinosis and/or unusual or severe infections including disseminated nontuberculosis mycobacterium, extensive HPV-related warts, and opportunistic fungal or viral infections) (10, 11, 66), Emberger syndrome (primary lymphedema with predisposition to MDS/AML) (8), chronic neutropenia (67), pediatric or young adult–onset MDS often with monosomy 7 (68, 69), and aplastic anemia (70). These presentations are now considered to be the spectrum of a single disease entity, termed GATA-2 deficiency syndrome.

Another enigmatic feature of GATA-2 deficiency syndrome is the highly variable time of symptom onset (71). While symptoms can present in young children, adults can be asymptomatic,

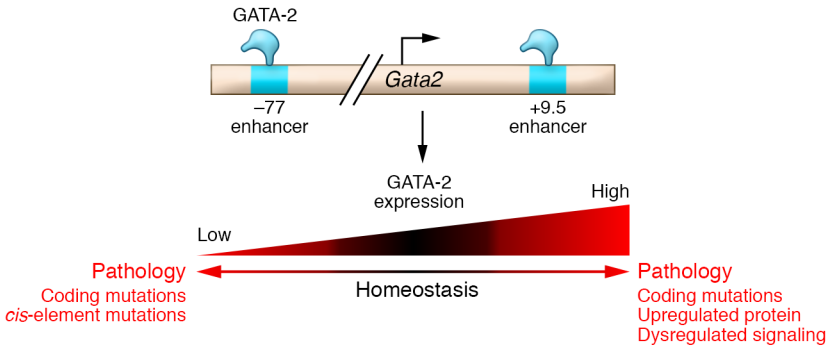

Figure 2. Pathogenic consequences of GATA-2 deficiency and excess. Physiological GATA-2 levels in HSPCs are conferred by the *Gata2* +9.5 and –77 enhancers in the mouse, and these enhancers are conserved in humans (14). While the enhancers are GATA-2–occupied, the ensemble of proteins and mechanisms governing enhancer activities are not yet known. Deviations from a physiological window of GATA-2 expression, either deficiency or excess, are pathogenetic (14). Coding region mutations decrease or increase GATA-2 levels and/or activity, and these alterations can be locus-specific (84). GATA-2 enhancer mutations decrease GATA-2 levels (12, 13). Since GATA-2 hyperphosphorylation increases GATA-2 activity in a locus-specific manner (183, 184), presumably, dysregulated cell signaling mechanisms can also yield excessive GATA-2 activity.

suggesting that a germline heterozygous *GATA2* mutation alone is insufficient for disease. Either mechanisms suppress *GATA2* mutation–instigated pathogenesis, or other genetic or environmental insults constitute the breaking point for overt disease development. In line with this hypothesis, even before peripheral blood (PB) cytopenias develop, clonal hematopoiesis is evident in the PB via skewed X chromosome inactivation (72). This may progress to an aplastic anemia–like presentation (69, 70), reminiscent of HSPC loss in GATA-2–deficient murine models. However, acquisition of a second *GATA2* mutation at the time of MDS or marrow failure in human disease has not been reported.

Also consistent with a mechanism in which additional aberrations are disease triggers, the risk of overt hematologic malignancy development increases with age from about 10% by age 10 to 50%–80% by age 40 (72–74). Most initial presentations involve MDS featuring hypocellularity, atypical megakaryocytes, fibrosis, and acquired cytogenetic abnormalities, of which monosomy 7 and trisomy 8 are the most frequent (68, 73, 74). *ASXL1* mutations are acquired at the time of malignancy in about 30% of cases and correlate with a chronic myelomonocytic leukemia (CMML) phenotype and monosomy 7 (75–79), suggesting cooperation between these genetic lesions and the germline *GATA2* mutation in malignant progression. *SETBP1* and *STAG2* mutations are also recurrent in this setting (77, 80).

A unique case study of multiple members of a family with the recurrent germline p.T354M mutation provided clues regarding mechanisms underlying symptom onset. One family member exhibited monoallelic expression of the mutated allele during a symptomatic period early in life, but regained biallelic expression later when her PB counts and BM normalized (79). She also carried an acquired *ASXL1* mutation that persisted for 6 years without progression to overt malignancy, suggesting that combined *GATA2* and *ASXL1* mutations alone are insufficient for MDS/AML. Her father and uncle, carriers of the familial mutation, had biallelic *GATA2* expression and remained asymptomatic into adulthood. This study implicates epigenetic control of *GATA2* expression and possibly symptom onset in humans with heterozygous mutations, consistent with epigenetic regulation of mouse *Gata2* (43, 46, 58, 81, 82). Environmental factors, e.g., infection or other stresses that can sculpt epigenomes (83), might also trigger pathogenesis, but these remain poorly defined.

More than 100 unique *GATA2* germline mutations have been reported. From the three largest series (67, 68, 73, 74), approximately 45% are missense or small in-frame insertions or C-finger deletions. C-finger residues p.T354, p.R361, p.R396, and p.R398 are recurrent germline missense mutation sites. Forty percent yield truncations prior to or within the C-finger, 5% are whole gene deletions, and 10% reside in the +9.5 enhancer (68, 73, 74). De novo mutations are frequent, seen in 22% (6 of 27) of those presenting with chronic neutropenia or GATA-2 deficiency symptoms and 70% (7 of 10) of children/adolescents with MDS lacking a history of *GATA2*-related symptoms.

Despite growing reports of mutations, the heterogeneous disease presentations, small patient cohorts, rarity of individual mutations, and dearth of biologically instructive in vitro assays have made rigorous genotype/phenotype correlations difficult to establish. A recent analysis of p.T354M and p.R307W function in a genetic complementation assay in primary cells from *Gata2* –77 enhancer mutant mice revealed defective activity at certain loci, with retention of activity or even hyperactivity at other loci (84). The locus-specific dysregulation of GATA-2 activity suggests that disease phenotypes involve an amalgamation of loss-of-function and gain-of-function phenotypes (Figure 2). In vivo correlations observed across two or more human patient series currently include differences in symptom penetrance, immunodeficiency, MDS/AML risk, and lymphedema prevalence. Nearly complete penetrance of GATA-2 deficiency–related symptoms was observed in carriers of truncating or deletion-type mutations, whereas a small subset of carriers of missense and +9.5 enhancer mutations remain asymptomatic into adulthood (68, 79, 85). Interindividual comparisons of disease phenotypes, including in related individuals carrying p.T354M and p.R396Q, have suggested a lower cumulative incidence of immunodeficiency but a higher cumulative incidence of MDS/AML with p.T354M (85). It remains to be determined whether additional shared genetic or environmental risk factors explain these differences or whether the functional consequences of individual mutations can be distinct. Lymphedema is more frequent in carriers of null mutations (72, 74) and with some missense mutations (8, 73). Mutation-specific effects on GATA-2 activity impact its function through the *PROX1* enhancer (86, 87). *PROX1* encodes a lymphatic development regulator (88), and disruption of GATA-2–dependent *PROX1* regulation constitutes a cell type–specific mechanism underlying one facet of GATA-2 deficiency syndrome. Establishing mutation- and context-specific associations involving other components of the pathologies will guide the development of organ-specific therapies.

Table 3. Hematopoietic transcription factors implicated in familial MDS/acute leukemia syndromes: germline presentations

	Hematologic malignancy characteristics						
Gene	Type(s)	Karyotype	Acquired mutations	Other hematologic manifestations	Other organ system manifestations	Inheritance pattern and penetrance	References
CEBPA	AML often with abnormal eosinophils	Normal karyotype	Second *CEBPA* mutation (100%), *GATA2* (56%; in ZF1), *WT1* (33%), *EZH2*, *SMC3*, *TET2*, *NRAS*, others	None	None	AD with near-complete penetrance for AML	171–173
GATA2	MDS, CMML, AML, T cell ALL	Monosomy 7, trisomy 8, normal karyotype, complex or other karyotypes	*ASXL1* (29%), *SETBP1*, *STAG2*, others	Monocytopenia (49%–78%), B lymphopenia (78%–100%), NK cell lymphopenia, $CD4^+$ T lymphopenia, neutropenia, anemia, thrombocytopenia, aplastic anemia (uncommon)	Lymphedema (11%–20%), infections (mycobacterial [20%–50%], viral [10%–20%], fungal [9%–16%], bacterial), HPV-related warts (60%–70%), pulmonary alveolar proteinosis or other lung abnormalities, venous and arterial thromboses, miscarriage, autoimmunity	AD with variable penetrance for all features, but high penetrance for hematologic malignancies	8–13, 66–80
RUNX1	MDS, AML, T cell ALL	Trisomy 21, various others	Second *RUNX1* abnormality is most common; *CDC25*, *TET2*, *CBL*, *TP53*, *FLT3*, *KRAS*, others	Thrombocytopenia, platelet dysfunction, aplastic anemia (uncommon)	Eczema	AD with variable penetrance for low platelets and hematologic malignancies	4, 118–120, 125–129
ETV6	B cell ALL, MDS, CMML, AML, MM	B-ALL: high hyperdiploidy (64%)		Thrombocytopenia (100%), platelet dysfunction, macrocytosis (uncommon)	Mild learning deficits? GI motility deficits or GI cancers?	AD with near-complete penetrance for low platelets; variable penetrance for hematologic malignancies	149–156
IKZF1	Pre–B-ALL, T cell ALL	High hyperdiploidy (38%), *ETV6-RUNX1*, *MLL*, and other rearrangements; 9p loss	*PAX5* (19%), *KRAS* or *NRAS* (26%), *TP53*, *JAK2*, *FLT3*, *MTOR*	Common variable immunodeficiency with low immunoglobulin levels, B and T cell deficits; myeloid deficits also seen with dominant-negative alleles (p.N159T/S in ZF2)	Autoimmunity, infections (bacterial, fungal, viral)	AD with variable penetrance	189–193
PAX5	Pre–B-ALL	9p loss via i(9)(q10) or other		None	None	AD with variable penetrance	194

AD, autosomal dominant; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; ZF, zinc finger.

Disorders caused by *RUNX1* and *ETV6* mutations

The autosomal dominant disorders familial platelet disorder with propensity to myeloid malignancy (FPDMM) and thrombocytopenia 5 (THC5), caused by heterozygous mutations in *RUNX1* and *ETV6*, respectively, share the clinical triad of thrombocytopenia with normal platelet size, mild bleeding tendency, and hematopoietic malignancy predisposition. Given the similarities, it is instructive to consider mechanistic links between these genes and their phenotypes.

Familial platelet disorder with propensity to myeloid malignancy (FPDMM)

Mechanistic foundations. RUNX1, formerly known as acute myeloid leukemia protein 1 (AML1), resembles GATA-2 in functioning as a master regulator of definitive hematopoiesis. RUNX1 encodes the α subunit that dimerizes with a β subunit to yield a functional core binding factor (CBF) heterodimeric transcription factor that binds the DNA consensus YGYGGTY and activates or represses transcription (89, 90). The RUNX1 Runt domain characterizes a small transcription factor family (91) and mediates CBF-β dimerization and DNA binding (92–94).

Targeted deletion of murine *Runx1* is embryonic lethal and is associated with defective fetal liver hematopoiesis, reduced HSPC genesis/function, and hemorrhaging (95–98). During the endothelial-to-hematopoietic transition in the AGM, GATA-2 increases *Runx1* expression (ref. 28 and Figure 1). GATA and ETS motifs within a *Runx1* +23.5-kb intronic enhancer are required for hematopoietic activity in transgenic mice (99), suggesting that GATA-2 directly activates *Runx1*. GATA-2 and RUNX1 can occupy neighboring chromatin sites, along with an ensemble of other factors (100, 101), implying their collective function in certain contexts. As RUNX1 controls HSC emergence from the AGM (102–104) and hemogenic endothelium function in vitro (105), it is attractive to consider a model in which GATA-2 activates RUNX1 expression in hemogenic endothelium and/or its cell progeny, and both factors function collectively (and perhaps independently) at a target gene ensemble to establish genetic networks that orchestrate HSPC

Table 4. Hematopoietic transcription factors implicated in familial MDS/acute leukemia syndromes: germline versus acquired genetic variation

Gene	Hematopoietic phenotypes in knockout mice	Germline mutation types and locations	Germline mutation frequency	Acquired mutation types and locations	Acquired mutation frequency	Impact of acquired mutations on hematologic malignancy prognosis	References
CEBPA	Myeloid maturation block similar to M2 AML	N-terminal frameshift prior to second ATG (majority), C-terminal leucine zipper in-frame insertions or deletions (rare)	AML: 1%	N-terminal frameshift prior to second ATG, C-terminal leucine zipper in-frame insertions or deletions	AML: 4%	AML: favorable prognosis in biallelic *CEBPA*-mutated cases	1, 162, 164, 170–176
GATA2	Embryonic lethal; abrogates multilineage hematopoiesis	Missenses or small in-frame insertions or deletions in C-terminal ZF (45%) (p.T354M, p.R361, p.R396, and p.R398 are recurrent sites); truncating prior to C-terminal ZF (40%); intron 5 ("+9.5") enhancer (10%); large deletions (5%)	Childhood MDS: 7%; chronic neutropenia: 6 of 14 probands with other *GATA2* features; adult MDS/AML/CMML: unknown	Accelerated-/blast-phase CML: 10% and located in C-terminal ZF (p.L349V); biallelic *CEBPA* mutated AML: 20%–39% and located in N-terminal ZF	MDS: <5%; AML: <1%	No clear impact on outcome other than adverse effect of inv(3) translocation that brings *GATA2* enhancer ("–77") near *EVI1* in MDS/AML	1, 25, 62–64, 67, 68, 73, 74, 186
RUNX1	Embryonic lethal; defective fetal liver hematopoiesis	Truncations and large deletions (70%); missenses cluster in RUNT domain; complex 21q abnormalities	MDS/AML: unknown	B cell ALL: translocations, amplifications; MDS: missense, truncating; AML: translocations, missense, truncating	B cell ALL: *ETV6-RUNX1* in 22%; MDS: 10%–15%; AML: 7%–9% including *RUNX1-RUNX1T1* in 5%	B cell ALL: favorable prognosis with *ETV6-RUNX1* translocation; MDS: adverse; AML: favorable with *RUNX1-RUNX1T1* translocation; inconclusive with other *RUNX1* abnormalities	1, 4, 95–98, 118–120, 186, 187, 195
ETV6	Embryonic lethal; lack of all hematopoietic lineages if absent in bone marrow	Missenses most common with majority in ETS domain and 1 recurrent mutation at p.P214L in linker domain; truncations throughout the gene	Childhood ALL: 0.8%; inherited thrombocytopenia: 2.6%	B cell ALL: translocations most common; MDS: missenses, truncating; AML: translocations, missenses, and truncating	B cell ALL: *ETV6-RUNX1* in 22%; MDS: 3%; AML: 1%	B cell ALL: favorable prognosis with *ETV6-RUNX1* translocation; MDS: adverse	1, 136–140, 149–155, 185–188, 195
IKZF1	B cell, T cell, erythroid, and myeloid deficits	Missenses throughout gene (majority); truncating throughout gene; large deletions	Childhood B cell ALL: 0.9%	B cell ALL: deletions most common (isoforms with exons 4–7del have dominant-negative activity whereas larger deletions are haploinsufficient); truncations and missenses less common	T cell ALL: 4%; B cell ALL: 20%–30% (includes 3% of *ETV6-RUNX1* translocated cases and 70% of *BCR-ABL* or *BCR-ABL*–like ALL)	Adverse	189–193
PAX5	B cells arrested at pro–B cell stage	p.G183S	2 Families	B cell ALL: deletions most common; missenses (including p.G183S), truncations throughout the gene, and rearrangements less common	B cell ALL: 30%	No clear impact on outcome	192, 194–197

Transcript numbers: CEBPA, NM_004364; GATA-2, NM_032638; RUNX1, NM_001754; ETV6, NM_001987; IKZF1, NM_006060; PAX5, NM_016734.

emergence and function (Figure 1). In support of this model, GATA-2 (106, 107) and RUNX1 (108, 109) control megakaryopoiesis, and disrupted megakaryopoiesis and atypical megakaryocytes are hallmarks of GATA-2– and RUNX1-linked pathologies (4, 70).

Conditional *Runx1* deletion studies identified PU.1, another ETS-family transcription factor, as a major component of the RUNX1-regulated genetic network (110). Restoration of PU.1 expression in *Runx1*-knockout mice or mice with mutations in PU.1 upstream regulatory elements where RUNX1 binds can partially or fully rescue hematopoietic defects in either system. Expression profiling of patient platelets containing a heterozygous *RUNX1* mutation revealed the downregulation of direct RUNX1

target genes including 12-lipoxygenase (*ALOX12*), platelet myosin light chain (*MYL9*), pallidin (*PLDN*), and thrombopoietin receptor (*MPL*) (111). These genes are involved in diverse platelet pathways, mirroring human FPDMM platelet abnormalities, including decreased platelet aggregation and ATP secretion in response to agonists, dense and α-granule deficiencies, and decreased platelet thrombopoietin receptors (112).

RUNX1 occupies target sites in the 5′-UTR of *ANKRD26*, which encodes an ankyrin repeat protein that associates with the inner cell membrane and is expressed in many tissues, including brain, liver, and adipose tissue, and the hematopoietic system (113). Homozygous mutation of murine *Ankrd26* causes massive obesity, insulin resistance, and large body size. In humans, mutations that cluster in a 22-nucleotide region of the 5′-UTR of *ANKRD26* cause THC2 syndrome, a familial thrombocytopenia and hematologic malignancy syndrome that is strikingly similar to FPDMM and lacks the murine obesity phenotype (114–116). Studies in human megakaryocytes revealed that these 5′-UTR mutations occur at RUNX1 binding sites, where RUNX1 and the ETS factor FLI1 function to suppress *ANKRD26* expression. RUNX1/FLI1 failure to bind these sites led to increased thrombopoietin, MAPK and ERK signaling, and defective proplatelet formation (117). As expected, *ANKRD26* expression is elevated in FPDMM patient platelets with *RUNX1* mutations, implicating this network in the thrombocytopenia of both disorders.

RUNX1 dysfunction in human pathologies. Heterozygous *RUNX1* germline mutations cause FPDMM, featuring platelet dysfunction, thrombocytopenia, and hematopoietic malignancy predisposition (Table 3) (4). As with GATA-2 deficiency syndrome, even thrombocytopenia in FPDMM is incompletely penetrant, with a subset of affected individuals displaying normal platelet counts throughout their lifespan (118, 119). This suggests that a single *RUNX1* allele can support thrombopoiesis, and other factors contribute to this disease phenotype and MDS/AML progression.

In FPDMM, truncating mutations, including nonsense, frameshift, splice-site, as well as large deletions, occur throughout *RUNX1* and account for the majority (70%) of reported mutations (Table 4). *RUNX1* loss due to whole gene deletions alone or as part of larger, multigenic abnormalities on chromosome 21q in the context of additional syndromic features such as intellectual disability are also seen (118). Missense mutations clustering in the RUNT domain, especially at sites p.R201 and p.R204, are also common. Whereas the nonsense and deletion mutations decrease protein levels, some missense mutations generate mutant proteins that retain CBF-β dimerization, but are DNA binding–defective (93, 120) and dominant-negative inhibitors in vitro (120). An association between carrying a mutation that generates dominant-negative activity in vitro and a higher proportion of individuals developing leukemia has been described (120); however, larger cohorts need to be analyzed.

As with *GATA2*, whether diverse insults or a predominant genetic or environmental insult triggers the transition to MDS/AML in FPDMM remains unresolved. Analyses of somatic events occurring before MDS/AML development in FPDMM have begun to yield insights. In a small FPDMM cohort, 67% of asymptomatic patients younger than 50 years old displayed clonal hematopoiesis in PB (77). This is remarkably higher than the less than 1% frequency expected in the general population (121–123), suggesting a baseline increase in mutagenic events in FPDMM HSPCs. Although the mechanistic underpinnings of this observation are unknown, the RUNX1 link to DNA repair pathways (e.g., RUNX1 interacts with the homologous recombination pathway component FANCD2; ref. 124) necessitates future investigation.

In FPDMM, the age of onset of hematologic malignancy development is a median 33 years but ranges widely (5–79 years) (Table 3). MDS and AML occur most frequently, although T cell acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma, and other lymphoid malignancies have been observed (125). At the onset of MDS/AML, loss of the normal *RUNX1* allele and acquisition of various chromosomal abnormalities have been described (126). Somatic mutations in the cell cycle–regulatory phosphatase *CDC25C* were detected in 53% of a small Japanese *RUNX1* mutant cohort with FPDMM (127), but these were not detected in French or American studies (77, 128). Acquired mutations in other genes, e.g., *PDS5B*, *TET2*, *PHF6*, and *DNMT3A*, have been detected in individual cases (77, 129). It will be important to elucidate global RUNX1-dependent genetic networks and functional circuits, as the deleterious consequences of target gene alterations often remain elusive when the focus is on individual genes. As GATA-2 activates *Runx1* transcription, and at least certain GATA-2 and RUNX1 target genes overlap, the integrated GATA-2/RUNX1 network constitutes an invaluable resource for establishing whether pathogenic network perturbations are highly specific, or whether any deviation from the physiological network promotes the transition of benign hematologic disorders to malignancy.

Thrombocytopenia 5 (THC5)

Mechanistic foundations. *ETV6*, formerly termed TEL (translocation-ETS-leukemia) (130), is an ETS transcription factor (131) identified from its frequent involvement in leukemogenic translocations that yield fusion proteins. *RUNX1* (132) is one of numerous *ETV6* fusion partners (130, 133–135). Targeted deletion of murine *Etv6* is embryonic lethal and disrupts yolk sac angiogenesis (136). While analysis of ETV6 function in adult mice via embryonic stem cell aggregation chimeras revealed it to be dispensable for yolk sac and fetal liver hematopoiesis (137), it was essential for BM hematopoiesis, providing an example of a transcription factor required in one hematopoietic compartment and not others. Conditional deletion studies confirmed an ETV6 requirement for BM HSC survival (138).

Contrasting with other ETS transcription factors that activate genes (131), ETV6 has been reported to function predominantly as a transcriptional repressor (139–141). ETV6 harbors an N-terminal helix-loop-helix domain, also deemed a "pointed" or sterile α motif (SAM) domain, that mediates dimerization and repression (142–145). This domain distinguishes ETV6 from most ETS factors, which lack this domain, and serves as a corepressor docking site (141, 142). An autoinhibitory domain ("linker region"), residing between the SAM domain and the C-terminal DNA-binding domain (146), restricts ETV6 DNA binding. This autoinhibitory mechanism is opposed by ETV6 self-association, which facilitates DNA binding to sites containing multiple ETS motifs (146–148).

ETV6 dysfunction in human pathologies. The majority of the heterozygous germline *ETV6* mutations reported are mis-

sense mutations within the C-terminal DNA-binding domain (Table 4) (149–153). One linker region mutation, p.P214L, is also recurrent (152, 154). Rare truncating or missense mutations outside the DNA-binding and linker domains have been reported. These mutations decrease ETV6 nuclear localization, DNA binding, and/or transcriptional repression (149, 155), and certain mutants exhibit dominant-negative activity in vitro. All of the mutations disrupt ETV6-mediated repression and ETV6-dependent genetic networks.

Nearly 100% of affected individuals with THC5 described to date have thrombocytopenia, with a mean observed platelet count of 86 (149–155), suggesting that a single copy of *ETV6* is insufficient for normal platelet development. In vitro cultures of patient-derived *ETV6* mutant megakaryocytes revealed proplatelet maturation deficits and decreased polyploidization (Table 3) (155), supporting this hypothesis. In contrast with the diverse platelet pathologies of FPDMM, THC5 platelets exhibit only mild, inconsistent defects in platelet aggregation studies in vitro, and granule deficiencies have not been reported (155), suggesting quantitative and/or qualitative differences in the consequences of ETV6 and RUNX1 mutations in megakaryocytes.

As with FPDMM and GATA-2 deficiency syndrome, hematologic malignancies occur only in a subset, suggesting that the germline *ETV6* mutation alone is insufficient for transformation to malignancy and likely requires additional genomic or environmental insults (Table 3). Individuals with THC5 can have an increased number of circulating $CD34^+$ hematopoietic progenitors in PB (153), suggesting a potential role for ETV6 in HSC/BM niche interactions in this pathophysiology. Among the 25%–39% of THC5 mutation carriers reported who progressed to develop a hematologic malignancy (149–151, 153–155), childhood-onset ALL has been the most frequently occurring hematopoietic malignancy. Subsequently, rare, germline *ETV6* variants were identified in 1% (31 of 4,405) of unselected children with ALL and were associated with an older age at ALL diagnosis (10 vs. 5 years, $P = 0.02$) and a hyperdiploid karyotype (64% vs. 27%, $P < 0.01$) (152), suggesting a larger role for *ETV6* germline variation in ALL risk. Biphenotypic acute leukemia, MDS, AML, and polycythemia vera have also been observed in THC5, reflecting ETV6 activity to regulate myeloid development as well (149–151). Considering ETV6 function in BM hematopoiesis, it is unclear why heterozygous *ETV6* dysregulation predominantly predisposes to ALL. Furthermore, the cooperating genomic lesions that cause ALL versus myeloid malignancy progression are not established.

Familial AML due to germline *CEBPA* mutation

Mechanistic foundations. C/EBPα is a basic leucine zipper (bZip) transcription factor that has been studied extensively in diverse systems. C/EBPα contains a leucine zipper that forms an amphipathic α helix that combines with the leucine zipper on a partner protein to mediate dimerization (156–158). The C/EBPα basic region contains DNA-binding specificity and affinity determinants (159). Since C/EBPα binds DNA as a homo- or heterodimer, by mediating dimerization, the leucine zipper domain indirectly confers DNA binding. This concept is exemplified by a leucine zipper swap in which leucine zippers from proteins with distinct DNA-binding specificities (e.g., C/EBPα and GCN4) are exchanged, preserving dimerization and the DNA-binding specificity imparted by the respective basic region (160).

Targeted ablation of murine *Cebpa* is lethal several hours after birth and is characterized by severe dysregulation of liver metabolic processes (161). From a hematopoietic perspective, *Cebpa* homozygous mutant mice are defective in granulopoiesis, but not other hematopoietic processes (162), highlighting its lineage specificity. RUNX1 induces C/EBPα expression, which inhibits myeloproliferation and promotes granulocytic differentiation. Lack of C/EBPα expression is an important component of the myeloproliferative phenotype of *Runx1* mutant mice (163).

CEBPA dysfunction in human pathologies. Acquired *CEBPA* mutations occur in 6%–9% of adult-onset AML cases (Table 4) (1, 164) and generate mutant proteins with or without dominant-negative activity (165–169). Approximately 5%–11% of these patients carry one of the detected mutations in the germline (170). In acquired and germline settings, the majority of causative *CEBPA* mutations are frameshifts occurring 5′ to a second transcriptional start site encoding a shorter isoform (p30) (171–173). These mutations reduce expression of full-length C/EBPα (p42 isoform) and increase expression of a dominant-negative isoform (p30), diminishing p42 availability to promote differentiation and cell cycle arrest (169, 174). Among germline cases at the time of AML development and AML cases with acquired biallelic mutations, nearly all cases have acquired a second *CEBPA* mutation on the previously normal allele, commonly disrupting the C/EBPα C-terminal leucine zipper. Rare families with germline missense mutations disrupting the leucine zipper have been reported (175, 176).

Germline upstream frameshift versus leucine zipper missense mutations appear to differ in expected penetrance, highlighting differences in biologic mechanisms. Whereas approximately 100% of those carrying an upstream frameshift are expected to develop AML, a lower proportion of those with distal leucine zipper mutations develop AML (estimated at 45%) (171, 176). These differences closely parallel findings in model systems. Whereas mice with a heterozygous upstream frameshift do not develop overt AML, mice with biallelic frameshifts develop AML with 100% penetrance and more rapidly than mice carrying biallelic distal leucine zipper mutations (174, 177). Mice with both an upstream frameshift and a distal leucine zipper mutant allele develop AML the fastest, suggesting a synergism that explains the observed pattern in germline and sporadic *CEBPA*-mutated cases described above (164, 178, 179).

As with the other MDS/AML predisposition syndromes described, the single germline *CEBPA* mutation alone appears to be insufficient to induce AML. Individuals in the dozen or so pedigrees reported with a germline *CEBPA* mutation are clinically normal until the emergence of AML (180). The AML phenotype is uniform across pedigrees, usually featuring a French-American-British M1, M2, or M4 morphology with abnormal eosinophils and a normal karyotype (171). In addition to the uniform acquisition of a second *CEBPA* mutation, *GATA2*, *WT1*, and *EZH2* mutations are the most frequent co-occurring mutations acquired at the time of AML (171). This uniform AML phenotype differs from the diverse hematologic malignancies seen in GATA-2 deficiency syndrome, FPDMM, and THC5, and parallels the more lineage-restricted phenotypes of C/

EBPα dysregulation, rather than the broader spectrum of hematopoietic-regulatory activities of GATA-2, RUNX1, or ETV6.

AML prognosis in these cases is favorable and similar to that of sporadic AML featuring biallelic acquired *CEBPA* mutations (171). However, later relapses involving leukemic clones molecularly independent of the initial leukemic presentation and more favorable postrelapse survival characterize familial in comparison with sporadic cases (171), suggesting that these are actually de novo AML episodes arising from a leukemia-prone HSC pool in germline *CEBPA* mutation carriers. The exact mechanisms favoring acquisition of the second *CEBPA* mutation or other cooperating genomic lesions remain unknown, and elucidating these mechanisms is critical to facilitate progress in preventing AML in individuals with this syndrome.

Summary

Next-generation sequencing has revealed that germline mutations predisposing to MDS/AML are considerably more common than previously thought. Analyses of these mutations in genes encoding transcription factors continue to unveil mechanistic insights that may provide new avenues for innovating much-sought-after molecularly targeted therapies. The transcription factors described herein exhibit varying degrees of mechanistic overlap. A factor can regulate expression of the other, and multiple factors expressed at the same time and in the same cell can function collectively in heteromeric complexes at target genes. Genetic networks established and maintained by these factors are still being discovered. While it is relatively straightforward to conduct transcriptional profiling to tabulate gene expression changes resulting from a given transcription factor perturbation, now even at the single-cell level, it is highly challenging to integrate this rudimentary information with other -omic data sets to yield a lucid view of the regulatory networks. Furthermore, new approaches are required to decipher functionally critical circuits within the networks and elucidate how altering the expression and activity of components within these circuits impacts cell function. It would not be surprising if MDS/AML resulting from germline mutations of transcription factor–encoding genes involves a multitude of perturbations of network components to yield a spectrum of disease phenotypes with implications for precision medicine therapeutics.

Multidisciplinary studies with large patient cohorts and diverse models are required to forge principles to understand the complex path from germline mutation to benign pathology to MDS/AML. In contrast to the opportunity for large-scale clinical studies with somatic mutants, germline mutations are considerably less frequent, and international collaboration is vital to achieve analytical power. The propensity for germline mutations to generate a predisposition or to induce disease may vary in different populations owing to additional genetic and/or environmental parameters. It will be crucial to forge new systems that enable the discovery of disease triggers, as well as disease suppressors. Identifying aberrant networks, triggers, and suppressors may catalyze the development of therapeutic alternatives to HSC transplantation. The momentum in gene-editing technologies may ultimately benefit those with germline mutation–linked disease. Though efforts have begun to correct genetic defects in patient-derived induced-pluripotent cells (181, 182), the path to rigorously gauge utility, safety, and broad applicability of this strategy will require intense efforts and considerable perseverance. Leveraging mechanistic insights involving aberrant networks and circuits to develop normalization therapies, to repress mutant allele expression, or to elevate wild-type allele expression constitutes a high-priority line of investigation that will translate into transformative clinical advances.

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