Germline Genetic Predisposition to Hematologic Malignancy

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ABSTRACT

Development of hematologic malignancies is driven by mutations that may be somatic or germline. Availability of next-generation DNA sequencing technologies has facilitated the development of individualized diagnostic evaluations and tailored treatment strategies. Until now, such personalized medical approaches have largely centered on prognostic stratification and treatment strategies informed by acquired somatic mutations. The role of germline mutations in children and adults with hematologic malignancies was previously underappreciated. Diagnosis of an inherited predisposition to hematologic malignancy informs choice of therapy, risk of treatment-related complications, donor selection for hematopoietic stem cell transplantation, evaluation of comorbidities, and surveillance strategies to improve clinical outcomes. The recognition that patients with inherited hematologic malignancy syndromes may present without classic clinical stigmata or suspicious family history has led to increased reliance on genetic testing, which, in turn, has raised new diagnostic challenges. Genomic testing is a rapidly evolving field with an increasing number of choices for testing for the practicing clinician to navigate. This review will discuss general approaches to diagnosis and management of patients with germline predisposition to hematology malignancies and will consider applications and limitations of genomic testing in clinical practice.

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INTRODUCTION

Li and Fraumeni^{1,2} described families with increased cancer frequency in 1969 and subsequently identified mutations in the tumor suppressor *p53*. Since that time, novel mutations that lead to cancer predisposition have continued to be discovered. These germline mutations are inherited in all of the patient's cells and may be shared between multiple family members. This is in contrast to somatic mutations, which may drive development of cancer and acquisition of subsequent mutations but are only present in specific cells. Somatic versus germline mutations, therefore, carry distinct clinical implications for the patient and family.

WHY TEST FOR GENETIC PREDISPOSITION TO HEMATOLOGIC MALIGNANCIES?

Timely identification of cancer predisposition syndromes informs clinical care, and germline testing can be a useful part of clinical care (Fig 1). Interpretation of marrow pathology is also informed by diagnosis as many of these disorders are characterized by baseline mild dysplasias that

may lead to the erroneous diagnosis of myelodysplastic syndrome (MDS). The clinical implications of clonal cytogenetic abnormalities or somatic mutations with clonal hematopoiesis may differ in the context of these syndromes, and additional studies are needed.^{3,4} In addition, many syndromes are associated with bone marrow failure (BMF), which responds poorly to standard treatment with antithymocyte globulin and cyclosporine and, thus, would require a different treatment approach compared with the patient with acquired aplastic anemia. Similarly, some germline disorders conditioning regimens may require reduced-intensity conditioning approaches for hematopoietic stem cell transplant.

Many of these disorders are associated with an increased risk of severe toxicity with standard doses of chemotherapy or radiation. Such toxicities may be manifested by protracted or irreversible cytopenias, end organ damage, or severe mucositis. Early identification of such patients allows tailored therapy with reduced-intensity regimens—for example, patients with Fanconi anemia require reduced-intensity conditioning regimens for transplantation.

Surveillance and diagnostic approaches may change as well. For example, patients with ataxia telangiectasia are exquisitely sensitive to radiation

Fig 1. Diagnostic evaluation. ADA, adenosine deaminase; BMF, bone marrow failure; DEB, diepoxybutane; FISH, fluorescence in situ hybridization; GU, genitourinary; MMC, mitomycin C

and, therefore, imaging for workup, surveillance, or follow-up should prioritize modalities that avoid ionizing radiation whenever possible. Of importance, the currently poor clinical outcomes of hematologic malignancy in many of these disorders provides a compelling rationale for surveillance strategies to initiate transplantation before development of leukemia or lymphoma.

An additional important consideration is that, although HLA matched sibling donors are generally considered the donor of choice for allogeneic stem cell transplants, siblings may carry the same mutation as the proband; therefore, screening is recommended even if the sibling seems to be clinically unaffected. For example, it is necessary to screen potential sibling donors for a patient with a GATA2 disorder to avoid transplantation of the same genetic mutation. Timely testing of siblings and family

members also allows family counseling and family planning, and some parents opt to pursue preimplantation genetic testing both for diagnosis of the mutation and for the possibility of an HLA matched sibling bone marrow transplant.

WHO SHOULD BE TESTED FOR AN INHERITED PREDISPOSITION TO HEMATOLOGIC MALIGNANCIES?

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 analyzed whole genome and whole exome sequencing (WES) data as well as a targeted panel of 565 genes in

1,120 patients with pediatric cancer to investigate the frequency of germline mutations in known cancer predisposition genes. Germline mutations were identified in 8.5% of patients, including in 4.4% of leukemia patients, yet strikingly only 23% of patients with an identified mutation had a family history that was suggestive of cancer predisposition.⁵ Similarly, a study that used a targeted panel of 28 hematology malignancy genes identified germline mutations in 18% of adult patients referred to the Hereditary Hematologic Malignancy Clinic in Houston.⁶ Likewise, a screening panel of 12 genes implicated in familial acute myeloid leukemia (AML) and MDS were able to identify pathogenic germline variants in 29% of patients with familial MDS/AML.³

Clinical stigmata and family history provide important clues to an underlying genetic predisposition to hematologic malignancy and can alert the astute physician to a possible diagnosis (Table 1 provides a partial list of common phenotypes that are associated with the listed syndromes). Recent clinical summaries of clinical features familial MDS and acute leukemia syndromes provide useful references to the practicing clinician and will not be reviewed here.⁷⁻¹⁰ Physical anomalies, unexpectedly short stature, failure to thrive, endocrinopathies, immune deficiencies, or other characteristic features of these syndromes in a patient who presents with cytopenias, unexplained red cell macrocytosis, or malignancies should raise concern of an inherited condition. A personal or family history of excessive toxicities with chemotherapy or radiation, including protracted or nonrecovering cytopenias, or a history of poor stem cell yield during mobilization may also hint at an underlying disorder.¹¹ A family history of first- or second-degree relatives with malignancy—particularly cancers presenting at a young age—cytopenias, unexplained macrocytosis, congenital anomalies, or characteristic features associated with inherited

cancer syndromes should also raise suspicion. Please refer to Table 2 for a list of major genetic syndromes associated with hematologic malignancies together with references for additional information. Although frequently considered to be a pediatric concern, genetic predisposition to hematologic malignancies may manifest later in life.⁴⁹

Of importance, absence of characteristic clinical features or a negative family history does not exclude the presence of germline mutations. The mutation may arise *de novo* in the proband or may result from parental gonadal mosaicism by which the mutation is acquired in the parental germ cells (oocytes or sperm). Many genes have variable penetrance and, therefore, the phenotype may be silent or attenuated. Phenotypic expression is highly variable among affected individuals even within the same family. A complete family medical history is often unknown, incomplete, or unavailable. Obtaining a detailed three-generation pedigree is a practiced skill and a time-consuming process. The patient or parents may need to clarify the family history with extended family members, or additional time may be needed for symptoms to manifest in affected family members.

Cancer predisposition disorders may present with bone marrow failure, hematologic malignancy, MDS, or nonhematologic manifestations, many of which overlap and can complicate diagnoses (Tables 1 and 3). In pediatric patients, primary MDS is typically hypocellular and, thus, the distinction between BMF and hypocellular MDS may be challenging in the absence of clonal cytogenetic abnormalities. Whereas traditionally, genetic BMF syndromes had been associated with classic syndromic phenotypes, reliance on clinical evaluation and family history misses a significant subset of patients. Approximately one third of patients with Fanconi anemia lack apparent clinical stigmata.^{55,56} Many patients with mutations in

Table 1. Comparison of Clinical Features

Clinical Feature	FA	DC	SDS	DBA	RUNX1	CEBPA	ETV6	ELANE	GATA2	DDX41	Noonan	Bloom	AT	NBS	CMMRD
Hematologic															
Anemia	X	X	X	X			(X)		X	X					
Neutropenia	X	X	X	(X)			(X)	X	X	X					
Thrombocytopenia	X	X	X	(X)	X		X		X	X					
Platelet dysfunction					X		X				X				
Immune															
Immunodeficiency		X	X						X			X	X	X	X
Other															
Limb	X			X											
Renal	X	X		X											
Cardiac	X	X	X	X							X				
Pulmonary		X													
Skin	X	X	X					X				X			X
Exocrine pancreas			X												
Mucosa		X													
Neurologic	X	X	X								X		X	X	X
Growth	X	X	X	X				X			X	X	X	X	
Facial	X			X							X				
Vascular		X										X	X		
Dental	X	X	X												
Hair		X													
Skeletal	X	X	X	X											

NOTE. Variability exists between individuals with the same genetic cancer predisposition syndromes. This chart is a simplified reference and is not comprehensive. Please refer to disease-specific reviews for further details of clinical phenotype. Many patients will lack several, if not all, of the hallmark features and, similarly, additional accompanying features may be present that have not yet been described.

Abbreviations: AT, ataxia telangiectasia; CMMRD, constitutional mismatch repair disorder; DBA, Diamond Blackfan anemia; DC, dyskeratosis congenita; FA, Fanconi anemia; NBS, Nijmegen breakage syndrome; SDS, Shwachman Diamond Syndrome.

Table 2. Genes Involved in Predisposition to Hematologic Malignancies

Pathway	Syndrome	Gene	Inheritance	Hematologic Malignancy*	Other Malignancy	Associated Abnormalities	References
DNA repair	Fanconi anemia	<i>FANCA</i>	AR	MDS, AML	Squamous cell carcinoma: oral, GI, vulvar	Short stature, hyper/hypopigmentation, facial dysmorphologies, skeletal, digits, hypogonadism, cardiopulmonary, GI, CNS, bone marrow failure	Alter ¹² ; Rosenberg et al ¹³
		<i>FANCB</i>	X-linked				
		<i>FANCC</i>	AR				
		<i>FANCD1/BRCA2</i>	AR				
		<i>FANCD2</i>	AR				
		<i>FANCE</i>	AR				
		<i>FANCF</i>	AR				
		<i>FANCG</i>	AR				
		<i>FANCI</i>	AR				
		<i>FANCI/BRIP1/BACH1</i>	AR				
		<i>FANCL</i>	AR				
		<i>FANCM</i>	AR				
		<i>FANCN/PALB2</i>	AR				
	Ataxia telangiectasia	<i>FANCO/RAD51C</i>	AR				
		<i>FANCP/SLX4</i>	AR				
		<i>FANCO/ERCC4</i>	AR				
		<i>FANCR/RAD51</i>	AD				
		<i>FANCS/BRCA1</i>	AR				
		<i>FANCT/UBE2T</i>	AR				
		<i>FANCU/XRCC2</i>	AR				
	Nijmegen breakage	<i>FANCV/REV7</i>	AR	Lymphoma, ALL (particularly T-ALL)		Neurologic dysfunction, telangiectasia, immunodeficiency, premature graying, gonadal dysfunction	Gatti and Perlman ¹⁴
		<i>ATM</i>	AR				
		<i>NBN</i>	AR	Non-Hodgkin lymphoma and ALL (primarily T cell)	Rare reports of brain tumors, rhabdomyosarcoma	Short stature, microcephaly, facial dysmorphism, immunodeficiency	De Miranda et al ¹⁵ ; Gładkowska-Dura et al ¹⁶
	Bloom syndrome	<i>BLM</i>	AR	Lymphoma, ALL, MDS, AML	Carcinomas	Short stature, sun-sensitive rash, pulmonary disease, immunodeficiency	Sanz et al ¹⁷
		<i>MLH1</i>	AD	Lymphoma, ALL, MDS, AML	GI (colon), ovarian, uterine, CNS, other		Ripperger and Schlegelberger ¹⁸ ; Vasen et al ¹⁹
	Constitutional mismatch repair deficiency syndrome (Lynch syndrome)	<i>MSH2</i>	AD				
		<i>MSH6</i>	AD				
		<i>PMS2</i>	AD				
	Ligase IV syndrome	<i>EPCAM</i>	AD				
		<i>LIG IV</i>	AR	Lymphoma, ALL		Short stature, microcephaly, facial dysmorphism, immunodeficiency, developmental delay	De Miranda et al ¹⁵

(continued on following page)

Table 2. Genes Involved in Predisposition to Hematologic Malignancies (continued)

Pathway	Syndrome	Gene	Inheritance	Hematologic Malignancy*	Other Malignancy	Associated Abnormalities	References
Transcription factor	Familial platelet disorder with propensity to myeloid malignancy	<i>RUNX1</i>	AD	MDS, AML			Bannon and DiNardo ²⁰ , Ganly et al ²¹
		<i>CEBPa</i>	AD	AML		Eosinophilia	Smith et al ²²
	Familial AML	<i>GATA2</i>	AD	MDS, AML, CMML		Mono-MAC syndrome	Hahn et al ²³ ; Ostergaard et al ²⁴ ; Hsu et al ²⁵
						Enberger syndrome, immunodeficiencies, hearing loss, warts, atypical mycobacterial infections, lymphedema, monocytopenia, marrow failure	Spinner et al ²⁶
Ribosomal abnormalities	Susceptibility to ALL 3	<i>PAX5</i>	AD	B-ALL			Shah et al ²⁷
		<i>ETV6</i>	AD	ALL, MDS, AML, CMML		Bleeding	Zhang et al ²⁸
	Diamond Blackfan Anemia	<i>GATA1</i>	X-linked	MDS, AML	Osteosarcoma, soft tissue sarcomas	Short stature, facial dysmorphism, radial ray anomalies, cardiac, renal, marrow failure	Vlachos and Muir ²⁹ , Vlachos et al ³⁰
		<i>RPL5</i>	AD				
Shwachman Diamond Syndrome		<i>RPL11</i>	AD				
		<i>RPL15</i>	AD				
		<i>RPL23</i>	AD				
		<i>RPL26</i>	AD				
		<i>RPL27</i>	AD				
		<i>RPL31</i>	AD				
		<i>RPL35a</i>	AD				
		<i>RPL36</i>	AD				
		<i>RPS7</i>	AD				
		<i>RPS10</i>	AD				
		<i>RPS15</i>	AD				
		<i>RPS17</i>	AD				
		<i>RPS19</i>	AD				
		<i>RPS24</i>	AD				
		<i>RPS26</i>	AD				
		<i>RPS27</i>	AD				
		<i>RPS27A</i>	AD				
		<i>RPS28</i>	AD				
		<i>RPS29</i>	AD				
		<i>(TSR)21†</i>	X-linked				
		<i>SBDS</i>	AR	MDS, AML		Short stature, exocrine pancreatic dysfunction, skeletal dysplasias, immunodeficiencies, marrow failure	Dror et al ³¹ ; Myers et al ³²

(continued on following page)

Table 2. Genes Involved in Predisposition to Hematologic Malignancies (continued)

Pathway	Syndrome	Gene	Inheritance	Hematologic Malignancy*	Other Malignancy	Associated Abnormalities	References
Telomere maintenance	Dyskeratosis congenita	<i>DKC1</i>	X-linked	MDS, AML	Oral and GI squamous cell carcinoma	Nail dystrophy, skin pigmentation abnormalities, oral leukoplakia, idiopathic pulmonary fibrosis, liver disease, short telomeres, marrow failure	Savage and Bertuch ³³ ; Savage and Cook ³⁴ ; Simon et al ³⁵ ; Stanley et al ³⁶
		<i>TERC</i>	AD				
		<i>TERT</i>	AD, AR				
		<i>NOLA3/NOP10</i>	AR				
		<i>NOLA2/NHP2</i>	AR				
		<i>TINF2</i>	AD				
		<i>WRAP53/TCAB1</i>	AR				
		<i>CTC1</i>	AR				
		<i>RTEL1</i>	AD, AR				
		<i>ACD/TPP1</i>	AD, AR				
		<i>PARN</i>	AD, AR				
		<i>NAF1</i>	AD				
		<i>STN1</i>	AD				
Checkpoint regulation	Li Fraumeni	<i>TP53</i>	AD	MDS, AML, ALL	Breast cancer, osteosarcoma, soft tissue sarcomas, brain tumors, adrenocortical carcinoma		Malin ³⁷ ; Similuk et al ³⁸
RAS pathway	Noonan syndrome	<i>PTPN11</i>	AD	JMML, ALL	Solid tumors	Facial dysmorphism, short stature, cardiac, broad neck, thoracic, cryptorchidism, coagulopathy	Kratz et al ³⁹
	Noonan-like	<i>CBL</i>	AD	JMML			Muraoka et al ⁴⁰ ; Martinelli et al ⁴¹
Other	Severe congenital neutropenia	<i>ELANE</i>	AD	MDS, AML			Van der Werff Ten Bosch and Van den Akker ⁴² ; Carlsson et al ⁴³ ; Rosenberg et al ⁴⁴ ; Bannan and DiNardo ²⁰ ; Boztug and Klein ⁴⁵
	Thrombocytopenia 2	<i>ANKRD26</i>	AD	MDS, AML, CMML		Platelet dysfunction	Thrasher ⁴⁶ ; Sullivan et al ⁴⁷
	X-linked neutropenia	<i>WAS</i>	X-linked	MDS, AML			Carlsson et al ⁴³ ; Lewinsohn et al ⁴⁸ ; Polprasert et al ⁴⁹ ; Bannan and DiNardo ²⁰ ; Chen et al ⁵⁰
	Wiskott Adrich Syndrome	<i>WAS</i>	X-linked	Lymphoma, ALL		Thrombocytopenia, eczema, immunodeficiency	Thrasher ⁴⁶ ; Sullivan et al ⁴⁷
	SCN3	<i>HAX1</i>	AR	MDS, AML	Seizures		Carlsson et al ⁴³
		<i>DDX41</i>	AD	MDS, AML, CML			Lewinsohn et al ⁴⁸ ; Polprasert et al ⁴⁹ ; Bannan and DiNardo ²⁰ ; Chen et al ⁵⁰
	Ataxia Pancytopenia Syndrome	<i>SAMD9L</i>	AD	MDS, AML	Ataxia		Kirwan et al ⁵¹
	Neurofibromatosis	<i>SRP72</i>	AD	MDS		Bone marrow failure	Sminog and Goldacre ⁵² ; Malkin et al ⁵³
		<i>NF1</i>	AD	JMML AML	Optic glioma, malignant peripheral nerve sheath tumor	Café-au-lait macules, axillary and inguinal freckling, neurofibromas, Lisch nodules, skeletal, vasculopathy	
	Down's syndrome	Trisomy 21	NA	ALL, AML		Mental retardation, facial, cardiac, GI, transient myeloproliferative disorder	Izraeli et al ⁵⁴

NOTE: Genes with strong associations to hematologic malignancies are listed; therefore, not all genes that are associated with these disorders are listed. Many of the primary immunodeficiencies are also associated with lymphoid malignancies, which are not covered in depth.
Abbreviations: AD, Alzheimer's disease; AR, autosomal recessive; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; MDS, myelodysplastic syndrome; NA, not applicable.
*Common associated hematologic malignancies.
†Leukemia risk not well defined.

Table 3. Comparison of Hematologic Malignancies Commonly Associated With Cancer Predisposition Syndromes

Syndrome	Marrow Failure	MDS	AML	ALL	Lymphoma
GATA2 spectrum disorders (<i>GATA2</i>)	X	X	X		
Fanconi Anemia	X	X	X		
Dyskeratosis congenita	X	X	X		
Shwachman Diamond Syndrome (<i>SBDS</i>)	X	X	X		
Severe congenital neutropenia (<i>ELANE</i>)	X (neutropenia)	X	X		
Diamond Blackfan anemia	X (anemia)	X	X		
Familial platelet disorder with propensity to myeloid malignancy (<i>RUNX1</i>)		X	X		
Familial AML with Mutated DDX41 (<i>DDX41</i>)		X	X		
Li Fraumeni (<i>TP53</i>)		X	X	X	
Familial acute myeloid leukemia (<i>CEBPA</i>)			X		
Thrombocytopenia 5 (<i>ETV6</i>)		X	X	X	
Constitutional mismatch repair deficiency syndrome		X	X	X	X
Bloom Syndrome (<i>BLM</i>)		X	X	X	X
Ataxia Telangiectasia (<i>ATM</i>)				X	X
Nijmegen Breakage Syndrome (<i>NBN</i>)				X	X
Wiskott Aldrich Syndrome (<i>WAS</i>)				X	X
Susceptibility to ALL 3 (<i>PAX5</i>)				X	

NOTE. Genes are noted above if there is a predominant gene for the predisposition syndrome. Genes for syndromes with multiple genes are listed in Table 2. Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

genes that affect telomere maintenance lack the classic clinical triad that had been originally relied upon to diagnose dyskeratosis congenita, and these clinical features may not be apparent at a younger age.³³

One recent study identified pathogenic germline mutations in 11.2% of 85 pediatric and young adult patients with idiopathic BMF or MDS who lacked a genetic diagnosis after standard clinical evaluation and clinically directed genetic testing.⁵⁷ By using a similar targeted panel of BMF and MDS genes, a retrospective study of pediatric or young adult patients who had undergone stem cell transplantation for aplastic anemia or MDS identified pathogenic mutations in 5.1% of patients with aplastic anemia (5 of 98 patients) and in 13.6% of patients with MDS (15 of 110 patients).⁵⁸ All but one of these mutations were germline in origin. A next-generation sequencing panel of 72 inherited BMF syndrome genes identified a causative gene in 18% of 83 patients who presented with BMF in the Canadian Inherited Marrow Failure Registry.⁵⁹ These studies underscore the importance of screening patients with BMF patients, as the majority of patients did not have a positive family history of cancer or physical anomalies. Patients with genetic cancer predisposition disorders are at risk for malignancy regardless of whether associated syndromic features are present or absent.

Patients and families should receive counseling regarding the indication for sending for genetic testing, limitations of genetic tests, potential results that could be returned, and implications for clinical management. Although genetic testing may inform treatment decisions, some patients present with a florid hematologic malignancy or severe cytopenias that require urgent initiation of therapy that cannot be deferred several weeks to await genetic test results. Conventional single-gene Sanger sequencing typically requires a minimum of 2 weeks, whereas gene panels or WES require additional time for completion of analysis. For patients who are in urgent need of treatment but for whom suspicion is high of an inherited cancer predisposition syndrome on the basis of clinical information or family history, consultation with an expert in these disorders may be helpful.

WHAT TYPE OF TESTING IS AVAILABLE FOR IDENTIFYING HEMATOLOGIC MALIGNANCY SYNDROMES?

Genetic Testing Platforms

Sanger sequencing for mutation(s) in a single gene or a sequential series of genes is a reasonable strategy when a clear clinical phenotype strongly suggests a likely diagnosis. When a mutation(s) has been previously identified in a proband, testing of additional family members to screen only for the previously identified mutation is effective. However, testing can be complicated, as many families do not have a previously diagnosed cancer-predisposing mutation identified, 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. When diagnosis is unclear or when initial clinically directed genetic testing is negative, serial testing of all possible genes is both expensive and time-consuming. The recent availability of next-generation sequencing in the clinical laboratory allows cost-effective, multiplexed targeted analysis of multiple cancer predisposition genes of interest.

The growing number of targeted gene panels differ in genes sets included for a given disease category and vary with respect to inclusion of specific coding regions or pathogenic noncoding regions. Of note, some hematologic malignancy gene panels are designed to target specific gene regions known to be somatically mutated and may omit regions of the gene that need to be included in the diagnostic evaluation of germline hematologic malignancy disorders. Therefore, targeted gene panels that are designed for somatic mutation analysis should not be relied upon for diagnostic evaluation of germline mutations. Consultation may be sought with an expert in genetic hematologic malignancy syndromes.

WES is a powerful tool for evaluation of patients in whom mutations have not been identified in the known diagnostic genes,

and the breadth of this coverage can be helpful especially when traditional targeted panels have not identified a causative gene. Identification of new, previously unrecognized genetic mutations via WES has increased our understanding of the pathogenesis of many disease types; however, some regions of the genome are poorly covered by WES and regions of interest may be missed. Coverage may vary between different genomic regions, sequence features, sequencing run, sample preparation batch, and sequencing platform. WES may fail to cover pathologic noncoding regulatory regions, such as promotor mutations or enhancer mutations. Analysis of WES results may be complicated by pseudogenes or segmental duplications. The sheer amount of data and variants of unclear significance can complicate analysis.

Targeted capture sequencing approaches added to WES allow optimization of probe design to maximize coverage of genomic regions of interest, including noncoding regulatory sequences and can improve detection of copy number variants. Supplementation with other methodologies to detect copy number variants, such as array comparative genomic hybridization or multiplexed ligation-dependent probe amplification, may also be useful. Table 4 lists additional testing considerations.

Interpretation of Genetic Results

Genetic test reports classify variants according to the likelihood that they are pathogenic. Some mutations in known genes have been previously described as associated with hematologic malignancy (Table 2). For recessive disorders, it is critical to confirm that variants affect both alleles in *trans* because a single allele carrying two variants—mutations in *cis*—would still leave an intact second allele. One way to confirm this is to test the parents of the proband. If one parent also carries both mutations, then the mutations must be in *cis* rather than in *trans*. As noted above, functional testing of patient cells may also be helpful to confirm a recessive disorder.

A range of criteria are used to classify variants, and available data may evolve over time to result in reclassification. Demonstration that the variant tracks with the clinical disorder in multiple unrelated families provides strong genetic evidence that the variant is pathogenic. Presence of the variant in multiple affected single individuals, together with absence of the variant in healthy population genetic databases, also provides evidence of pathogenicity; however, variants with incomplete penetrance or delayed onset of the clinical phenotype may result in misleading interpretations. Asymptomatic heterozygous carriers of variants that cause

Table 4. Genetic Testing Approaches

Table 4. Genetic Testing Approaches		
Method	Use	Limitation
Germline		
Single Gene Analysis	Consider when clinical suspicion for a specific gene is high Example: GATA2 gene testing in a patient with Emberger or Monomac syndrome	Some tests only look for a specific mutation or limited region within in a single gene Sanger sequencing can miss copy number variants Sequential testing of single genes is expensive and time-consuming
Targeted multigene panel	Consider for comprehensive evaluation of germline predisposition to hematologic malignancy Can optimize coverage of genes and regions of interest	Wide variation between panels regarding which genes are included and whether pathogenic noncoding regulatory regions are included Some genetic panels are not designed to screen for copy number variants Variable analytic pipelines between gene panels; coverage of certain genes may be poor
Whole exome sequencing	Useful for gene discovery, particularly if lacking mutations in known genes Often performed on a research basis if targeted panels are negative but there is a strong suspicion of germline mutation	Variable coverage across the genome and variations between analytic pipelines May miss mutations in known genes if poor coverage Misses pathogenic noncoding regulatory regions, such as promoters and enhancers
Whole genome sequencing	May consider if whole exome sequencing is negative or if there is strong suspicion that a mutation may be in a noncoding region Typically performed on a research basis	Analysis is challenging
Somatic		
Single gene sequencing	May use single-gene sequencing, multigene panel, whole exome sequencing, or whole genome sequencing to assess for somatic mutations in cancer cells	Need source of germline DNA to determine if mutation is somatic or germline
Multigene panel		Panels or analytic pipelines designed for somatic mutation analysis may omit regions that are important for diagnosis of germline cancer predisposition
Whole exome sequencing		
Whole genome sequencing		
Functional		
Chromosomal breakage (MMC/DEB)	Useful diagnostically when clinically available Useful to assess novel variants or variants of unknown clinical significance	Functional testing is only available for a subset of genes Availability often limited to research laboratories
Telomere length		Assay must be relevant to disease pathogenesis

recessive disorders may result in the presence of pathogenic variants in healthy control populations. Ethnicity may affect the prevalence of different polymorphisms. Programs that are designed to predict whether variants are likely to be deleterious on the basis of evolutionary conservation or predicted structural effects may be helpful but must be interpreted with caution.

An understanding of the molecular function of the gene of interest may guide interpretation of a variant. For example, variants that affect amino acid residues or domains that are critical to protein function may be more likely to be deleterious. Integration of genetic testing with functional testing and clinical assessment together inform the diagnostic evaluation. For genes of known function, assays to test the effect of the particular variant may be helpful, though such assays are typically only available in research laboratories. Examples of functional tests include chromosomal breakage testing for Fanconi anemia and telomere length analysis for telomeropathies. These types of tests may be helpful in testing variants of unknown significance. For example, a variant in a Fanconi anemia gene that confers increased chromosomal breakage with mitomycin C or diepoxybutane offers compelling evidence for pathogenicity. Similarly, demonstration of shortened telomere length raises confidence that a variant in a telomere maintenance gene is pathogenic. Therefore, functional testing may be used as a primary screen for inherited BMF syndromes and may provide useful confirmatory information.

To further complicate matters, hematologic malignancy genes may be mutated somatically in hematopoietic cells or constitutionally in the germline. Examples of such genes include *RUNX1*, *TP53*, *CEBP α* , *GATA2*, and genes in the *RAS* pathway. In some cases, somatic mosaicism, wherein a pathogenic variant has undergone reversion to a functional allele, may confound diagnostic testing. Somatic reversion in lymphocytes has been documented for Fanconi anemia and dyskeratosis congenita.^{60,61} In such cases, diagnosis may be confirmed by testing an alternative tissue, such as skin fibroblasts.

Although a high variant allele frequency may be suggestive of a germline disorder, germline versus somatic mutations are best distinguished by sequencing a nonhematopoietic tissue, such as a fibroblast cell line grown from a skin punch biopsy. Other commonly used samples, such as saliva or a buccal swab, may be contaminated with blood cells, particularly lymphocytes. Skin fibroblasts offer the disadvantage that a punch biopsy entails an additional invasive procedure, and they also require several weeks in culture, thus time to diagnosis may be delayed. Whereas somatic mutations arise in individual hematopoietic cells, germline mutations are found in all hematologic cells as well as other tissues, which may result in additional hematologic phenotypes and extra-hematologic comorbidities. As detailed in the following section, germline variants require genetic counseling considerations that differ from those required for somatic variants.

associated with cancer predisposition syndromes may be particularly difficult to treat. Some syndromes are associated with exquisite patient sensitivity to chemotherapy or radiation, which results in morbidity or mortality from end organ toxicity or from complications of prolonged pancytopenia. For some genetic disorders, patients may need appropriate referrals to additional subspecialists for evaluation of associated nonhematologic complications, such as immunodeficiency in *GATA2* syndromes or pulmonary complications with telomere biology disorders, among others (Table 2).

Identification of leukemia predisposition before development of hematologic malignancy informs the need for malignancy surveillance. Suggested screening approaches for predisposition to myeloid leukemias include regular CBCs and may also include periodic bone marrow exams, including morphology, cytogenetics and fluorescence in situ hybridization for surveillance of marrow dysplasia, clonal evolution, and development of leukemia in certain patient groups with genetic predisposition. Published recommendations have been outlined by expert panels for many of the inherited BMF syndromes.^{30,31,34} General screening recommendations to monitor for development of hematologic malignancy have been proposed to include regular peripheral blood counts one to three times per year and bone marrow evaluation, including morphology, cytogenetics, and fluorescence in situ hybridization at diagnosis. Frequency of monitoring bone marrow exams is informed by the cancer risks associated with the specific disorder, and additional marrow evaluations should be performed in response to unexplained persistent or progressive changes in blood counts or for symptoms that are suggestive of an underlying malignancy. Additional studies are needed to establish screening recommendations. Potential related family donors should be tested for the pathogenic mutation(s) before transplant.

Of note, a significant subset of patients with a family history that is suggestive of inherited cancer predisposition remain genetically undefined, which suggests that there may be additional genes to be discovered.³ Patients and families benefit from genetic counseling when returning genetic test results to review implications of positive tests for the patient and caveats of negative tests. Patients with undefined familial cancer disorders may benefit from surveillance. Selection of related donors for hematopoietic stem cell transplant is challenging when the causative gene has not been identified in cases of familial cancer predisposition. In some cases, when a mutation is not identified but the suspicion for familial germline cancer predisposition is high, the decision to use an HLA matched family donor may be challenging. In these cases, a careful related donor evaluation may include blood counts and a bone marrow exam to minimize the risk of choosing an affected donor. Risks of inadvertently selecting an affected related donor must be weighed against risks associated with the available unrelated donor pool.

MANAGEMENT OF INHERITED HEMATOLOGIC MALIGNANCY SYNDROMES

The rationale for surveillance is that early detection of clonal evolution allows treatment with a hematopoietic stem cell transplant before development of leukemia. Leukemias that are

FUTURE DIRECTIONS

We are currently in an exciting time with increasingly powerful tools for genetic testing and rapid advances in our understanding of genetic predisposition to malignancies. Diagnosis of an inherited predisposition to hematologic malignancy allows

individualized, tailored therapy and management. The paucity of comprehensive, easily accessible databases of gene variants with expert annotation remains a major challenge for the interpretation of genetic testing. Patients may benefit from consultation with specialized centers that are experienced in evaluation and treatment of these disorders. Future studies are needed to develop optimal diagnostic strategies and clinical management for these patients, including surveillance for malignancies, treatment regimens, transplant conditioning regimens, and supportive care to improve patient survival and outcomes. Genetic testing analyzed in the context of clinical and laboratory findings, together with a mechanistic understanding of gene function, provides previously unprecedented opportunities for individually tailored medical management.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Germline Genetic Predisposition to Hematologic Malignancy

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