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PEDIATRIC HEMATOLOGY

Inherited bone marrow failure in the pediatric patient

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Inherited bone marrow (BM) failure syndromes are a diverse group of disorders characterized by BM failure, usually in association with ≥1 extrahematopoietic abnormalities. BM failure, which can involve ≥1 cell lineages, often presents in the pediatric age group. Furthermore, some children initially labeled as having idiopathic aplastic anemia or myelodysplasia represent cryptic cases of inherited BM failure. Significant advances in the genetics of these syndromes have been made, identifying more than 100 disease genes, giving insights into normal hematopoiesis and how it is disrupted in patients with BM failure. They have also provided important information on fundamental biological pathways, including DNA repair: Fanconi anemia (FA) genes; telomere maintenance: dyskeratosis congenita (DC) genes; and ribosome biogenesis: Shwachman-Diamond syndrome and Diamond-Blackfan anemia genes. In addition, because these disorders are usually associated with extrahematopoietic abnormalities and increased risk of cancer, they have provided insights into human development and cancer. In the clinic, genetic tests stemming from the recent advances facilitate diagnosis, especially when clinical features are insufficient to accurately classify a disorder. Hematopoietic stem cell transplantation using fludarabine-based protocols has significantly improved outcomes, particularly in patients with FA or DC. Management of some other complications, such as cancer, remains a challenge. Recent studies have suggested the possibility of new and potentially more efficacious therapies, including a renewed focus on hematopoietic gene therapy and drugs [transforming growth factor-β inhibitors for FA and PAPD5, a human poly(A) polymerase, inhibitors for DC] that target disease-specific defects.

Introduction

Inherited bone marrow failure (BMF) syndromes are a diverse group of life-threatening disorders, usually presenting in the pediatric age group.[1] Although historically these disorders largely included syndromic categories, such as Fanconi anemia (FA), next-generation sequencing has added to the list an increasing number of new genetically defined entities, such as *ERCC6L2*-associated BMF. The genetic advances have also led to the recognition that some idiopathic cases of BMF/myelodysplasia (MDS) are cryptic forms of recognized syndromes, such as dyskeratosis congenita and FA. The genetic developments also raise an important question as to what should be considered an inherited BMF syndrome. This issue is complicated, because some germline genetic variants can produce very pleiotropic hematological and nonhematological phenotypes, and the associated phenotypes could be easily classified into more than 1 category. In this review, we included entities that are frequently associated with global BMF and/or constitutional cytopenia(s). A discussion of these entities, highlighting the genetic advances and management principles, is given herein. Tables 1-10 provide details on the marked heterogeneity with >100 currently identified disease genes. We also highlighted some newer entities associated with phenotypes varying from BMF to MDS and leukemia.

FA

FA was first described by Fanconi in 1927.[2] It is usually inherited as an autosomal recessive (AR) trait, but in a small subset of patients, it can be an X-linked recessive disorder. Patients with FA are clinically heterogeneous.[3] Typical features include BMF development and an increased predisposition to cancer. Affected individuals may also have ≥1 extrahematopoietic abnormalities, including dermatological (eg, cafe au lait spots), skeletal (eg, radial hypoplasia), genitourinary (eg, single kidney), gastrointestinal (eg, duodenal atresia), and neurological abnormalities (Table 2). Approximately one-third of patients have no overt extrahematopoietic abnormalities. Most patients are diagnosed at the end of the first decade of life; however, some patients are diagnosed in adulthood.

FA cells display hypersensitivity to DNA cross-linking agents, such as diepoxybutane (DEB) and mitomycin C (MMC). This FA cell hallmark led to the development of a diagnostic test several decades ago and has facilitated many advances, including elucidating the genetics with currently characterized 22 FA and FA-like disease subtypes/complementation groups.[3-20] The proteins encoded by the FA and FA-like genes (Table 3) participate in DNA repair.[21] Specifically, 8 of the FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and

Table 1. Characteristics of the inherited bone marrow failure syndromes

	FA	DC	SDS	DBA	CDA	CAMT	SCN	New*
Inheritance pattern	AR, XLR	XLR, AR AD	AR AD	AD XLR	AR AD	AR AD	AD AR	AR AD
Somatic abnormalities	Yes	Yes	Yes	Yes	Rare	Yes	Rare	Yes
Bone marrow failure	AA (90%)	AA (80%)	AA (20%)	RCA	Dysery	Meg	Neut	Yes
Short telomeres	Yes	Yes†	Yes	No	No	No		?
Cancer	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Chromosome instability	Yes	Yes	Yes	?	?	No	?	Yes‡
Genes identified	22	16	4	21	5	4	7	25+

CAMT, congenital amegakaryocytic thrombocytopenia and syndromic thrombocytopenia; Dysery, usually dyserythropoiesis; Meg, typically low megakaryocytes, but can progress to global bone marrow failure; Neut, usually low neutrophils; RCA, red cell aplasia, although some patients can develop global bone marrow failure; XLR, X-linked recessive.

*Includes new and overlapping syndromes.

†Yes, usually very short in DC and short in FA and SDS.

‡Yes, only some new subtypes are currently known to show chromosome instability.

FANCM) interact with one another to form a nuclear complex, the FA core complex. The FA core complex is necessary for activation of the FANCI-FANCD2 complex to a monoubiquitinated form (FANCI-FANCD2-Ub). FANCI-FANCD2-Ub then interacts with DNA repair proteins, such as BRCA2, BRCA1, and RAD51, leading to DNA damage repair. Patients with FA type D1 (FA-D1) and those with FA-S have biallelic variants in BRCA2 and BRCA1, respectively. These observations linked FA to the DNA damage-response pathway (Figure 1). BRCA2 is important for DNA damage repair by homologous recombination. Cells lacking BRCA2 inaccurately repair damaged DNA and are hypersensitive to DNA cross-linking agents. It has been established that FANCJ represents BRIP1 (partner of BRCA1) and FANCN represents PALB2 (partner of BRCA2) and that SLX4 is also an FA protein. These findings have strengthened the connection between FA and DNA repair; specifically, the FA network orchestrates incisions at cross-linked DNA sites.[22] Recent studies have suggested that the FA proteins are important in counteracting aldehyde-induced genotoxicity in hematopoietic stem cells.[23] FA proteins also have other functional roles, including cytokine regulation,[24] mitophagy, and ribosome biogenesis.[25] The multifunctional biological roles of FA and FA-like proteins are depicted in Figure 1.

Dyskeratosis congenita

Classic dyskeratosis congenita (DC), first described in 1910, is an inherited BMF syndrome characterized by the mucocutaneous triad of abnormal skin pigmentation, nail dystrophy, and mucosal leucoplakia.[26,27] These features frequently develop in children. Various other abnormalities have also been reported: dental (eg, severe caries), gastrointestinal (eg, esophageal stenosis), genitourinary (eg, phimosis), neurological (eg, cerebellar hypoplasia)], ophthalmic (eg, nasolacrimal duct narrowing), pulmonary (eg, pulmonary fibrosis), skeletal (eg, osteoporosis), and vascular; Table 2).[27,28] BMF is a major cause of mortality, and DC predisposes patients to cancer and pulmonary complications. X-linked recessive, autosomal dominant (AD), and AR subtypes of DC are recognized. Sixteen DC genes (*DKC1*, *TERC*, *TERT*, *NOP10*, *NHP2*, *TINF2*, *TCAB1*, *USB1*, *CTC1*, *RTEL1*, *ACD*, *PARN*, *NAF1*, *ZCCHC8*, *NPM1*, and *MDM4*)[29-43] have been identified (Table 4).

The gene mutated in X-linked DC (*DKC1*) was identified in 1998. It encodes a highly conserved nucleolar protein called dyskerin. Dyskerin associates with the H/ACA class of small nucleolar RNAs in small nucleolar ribonucleoprotein particles, which are important in guiding the conversion of uridine to pseudouridine during ribosomal RNA maturation (Figure 2). Dyskerin also associates with the RNA component of telomerase (TERC), where it stabilizes the telomerase complex, which is critical for telomere maintenance[44,45] (Figure 2). Heterozygous variants in *TERC* and *TERT* have been identified in patients with AD-DC[30-32] and in some patients with aplastic anemia (AA), MDS, acute leukemia, and pulmonary and liver fibrosis.[46-51] A subset of patients with the multisystem disorder Hoyeraal-Hreidarsson syndrome has *DKC1* variants.[52] Also, AR-DC is genetically heterogeneous with 9 subtypes because of biallelic variants in *NHP2*, *NOP10*, *TERT*, *TCAB1*, *USB1*, *CTC1*, *RTEL1*, *ACD*, and *PARN*. One AD-DC subtype is related to variants in *TINF2*, which encodes a component of the shelterin complex that protects telomeres and controls access of telomerase to a telomere. Subsequently, heterozygous variants in other genes (*RTEL1*, *PARN*, *NAF1*, *ZCCHC8*, *NPM1*, and *MDM4*) have been associated with some DC features.[38-43] Collectively, these observations have demonstrated that classic DC, Hoyeraal-Hreidarsson, and a subset of AA and MDS/acute myelogenous leukemia (AML) are principally related to a defect in telomere maintenance, and cells from these patients have very short and/or abnormal telomeres.[44,53] The multisystem abnormalities in these patients, including predisposition to cancer, have highlighted the critical role of telomeres and led to the recognition of a new category of human diseases called telomeropathies. Still, in different DC subtypes, the pathophysiology also includes nontelomere defects (Figure 2). For example, patients with *DKC1*, *NHP2*, and *PARN* variants also have ribosomal

Table 2. Features of syndromic inherited BMF syndromes

IBMF Subtype	Hematological	Extrahematological	Cancer
FA	Single cytopenia, global BMF, MDS, and AML.	Skin (eg, cafe au lait spots), skeletal (eg, radial hypoplasia, short stature, "Fanconi facies"), endocrine, genitourinary (eg, single kidney), gastrointestinal (eg, duodenal atresia), and neurological abnormalities.	Hematological (MDS, AML). Squamous cell carcinoma, especially of the head and neck and vulva. Other tumors (eg, liver) are also observed.
DC	Single cytopenia, global BMF, MDS, and AML.	The mucocutaneous triad of abnormal skin pigmentation, nail dystrophy, and mucosal leucoplakia. A variety of other abnormalities, including dental (eg, severe caries), gastrointestinal (eg, esophageal stenosis, cirrhosis), genitourinary (eg, phimosis), neurological (eg, cerebellar hypoplasia), ophthalmic (eg, nasolacrimal duct narrowing, retinopathy), pulmonary (eg, pulmonary fibrosis), skeletal (eg, osteoporosis), and vascular abnormalities.	Hematological (MDS, AML). Squamous cell carcinoma, especially of the head and neck and vulva. Other tumors (eg, liver) are also observed.
SDS	Single cytopenia (eg, neutropenia), global BMF, MDS, and AML.	Exocrine pancreatic insufficiency, skeletal (metaphyseal dysostosis, rib cage defects), failure to thrive, developmental delay, dental, and variable other abnormalities.	Hematological (MDS and AML).
DBA	Typically anemia, but can progress to global BMF, MDS, and AML.	Skeletal (triphalangeal thumb), short stature, craniofacial (eg, high arched palate),cardiac, and urogenital malformations.	Hematological (MDS, AML), rarely osteosarcoma and colon cancer.
CDA	Anemia with dyserythropoiesis.	Skeletal abnormalities and splenomegaly.	No
SCN	Neutropenia, frequently there are myeloid maturation arrest, MDS, and AML.	Usually, none in patients with *ELANE* variants. There may be extrahematopoietic abnormalities in non-*ELANE*–bearing patients.	Hematological (MDS and AML).
CAMT and other syndromic thrombocytopenias	Thrombocytopenia, BMF, MDS, and AML.	In typical CAMT, there are usually no other physical abnormalities. Patients with TAR have an absence of radius and sometimes other abnormalities. Those with a fusion of radius and ulna can also have skin, skeletal, and other extrahematopoietic defects.	Patients with classic CAMT can develop leukemia. Those with TAR usually have no cancer risk. Patients with radioulnar fusion due to *MECOM* variants can develop MDS and AML.

defects (Figure 2). The overall phenotype in any patient is therefore a summation of these different biological defects, environmental effects (eg, increased smoking-related risk of pulmonary complications), and age (eg, worsening of mucocutaneous features with aging). In addition, the clinical phenotype is influenced by the anticipation phenomenon, increasing disease severity in succeeding generations because of the inheritance of short telomeres through the germline. Collectively, these interacting factors make prognostic predictions and genetic counseling challenging.

Shwachman-Diamond syndrome

Shwachman-Diamond syndrome (SDS), first described in 1964, is usually an AR disorder characterized by exocrine pancreatic insufficiency, BMF, and extrahematopoietic abnormalities, particularly metaphyseal dysostosis (Table 2).[54,55] Pancreatic insufficiency becomes apparent early in infancy. Hematological abnormalities include neutropenia, AA (~20%), MDS, and leukemia (~25%). Most patients with SDS (>90%) have biallelic variants in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene[56] (Table 5). The *SBDS* gene product has an important role in 60S ribosomal subunit maturation and, therefore, in ribosome biogenesis.[57] Thus, SDS is principally a disorder of defective ribosome biogenesis.

Recently, it has been observed that biallelic variants in the *EFL1* and *DNAJC21* genes and heterozygous variants in the *SRP54* gene can produce an SDS-like disease.[57] Like SBDS, these proteins are also involved in ribosome biogenesis.

Diamond-Blackfan anemia

Diamond-Blackfan anemia (DBA), first described in 1934,[58] usually presents in early infancy with features of anemia.[59]

Table 3. FA genetic subtypes

Complementation group (gene)	Approximate % of patients with FA	Chromosome location	Gene product	Exons
AR				
A (*FANCA*)	65	16q24.3	FANCA	44
C (*FANCC*)	12	9q22.32	FANCC	22
G (*FANCG*)	12	9p13.3	FANCG/XRCC9	14
J (*FANCJ*)	<5	17q23.2	FANCJ/BRIP1	25
E (*FANCE*)	4	6p21.31	FANCE	10
F (*FANCF*)	4	11p14.3	FANCF	1
P (*FANCP*)	2	16p13.3	FANCP/SLX4	17
D1 (*FANCD1*)	<1	13q13.1	FANCD1/BRCA2	27
D2 (*FANCD2*)	<1	3p25.3	FANCD2	45
I (*FANCI*)	<1	15q26.1	FANCI	38
L (*FANCL*)	<1	2p16.1	FANCL	14
M (*FANCM*)*	<1	14q21.2	FANCM	25
N (*FANCN*)	<1	16p12.2	FANCN/PALB2	14
O (*FANCO*)*	<1	17q22	FANCO/RAD51C	12
Q (*FANCQ*)	<1	16p13.12	FANCQ/ERCC4	13
S (*FANCS*)*	<1	17q21.31	FANCS/BRCA1	24
T (*FANCT*)	<1	1q32.1	FANCT/UBE2T	7
U (*FANCU*)	<1	7q36.1	FANCU/XRCC2	3
V (*FANCV*)	<1	1p36.22	FANCV/REV7	10
W (*FANCW*)	<1	16q23.1	FANCW/RFWD3	18
X-linked recessive				
B (*FANCB*)	<1	Xp22.2	FANCB	17
AD				
R (FANCR)*	<1	15q15.1	FANCR/RAD51	13

FA subtypes (complementation groups) A, C, and G account for most patients with FA. As can be noted from the table, many FA genes encode proteins that had previously been known by other names and have important roles in DNA repair.

*Biallelic variants in *FANCM*, *FANCO*, and *FANCS* and heterozygous variants in *FANCR/RAD51* produce FA-like disease[3] (abnormalities overlap with those in patients with FA but are not sufficient to be classified as bona fide FA).

The hallmark of classic DBA is a selective decrease in erythroid precursors and normochromic macrocytic anemia associated with various extrahematopoietic abnormalities, such as craniofacial (eg, high arched palate), thumb, cardiac, and urogenital malformations (Table 2). MDS and AML have been reported in a few patients with DBA, suggesting an increased predisposition to cancer. There are also cases that have evolved into AA. Thus, although DBA is typically regarded as pure red cell aplasia, a global hematopoietic defect can be observed in some patients.

The first DBA gene (*RPS19*) was identified in 1999,[60] and it accounts for ~25% of patients with DBA in White populations. Subsequently, heterozygous variants of other genes encoding small (*RPS7*, *RPS10*, *RPS15*, *RPS17*, *RPS24*, *RPS26*, *RPS27*, *RPS28*, and *RPS29*) and large (*RPL5*, *RPL9*, *RPL11*, *RPL15*, *RPL18*, *RPL26*, *RPL27*, *RPL31*, *RPL35*, and *RPL35A*) ribosomal subunits proteins have been reported (Table 6). Collectively, the genetic basis in ~75% of patients with DBA can now be established.[61-68] These observations have also demonstrated that DBA is a ribosome biogenesis disorder.

Some genotype-phenotype correlations have emerged. For example, patients with variants in *RPL5* gene tend to have multiple physical abnormalities, including craniofacial, thumb, and heart anomalies, whereas isolated thumb malformations predominantly occur in patients with heterozygous *RPL11* variants. A subgroup of patients with DBA/DBA-like disease has been associated with variants in *GATA1* (encoding an erythroid transcriptional factor), *CECR1/DADA2*, *TSR2*, and *EPO*.[68,69]

In the Japanese population, *RPS19* variants account only for ~13% of patients with DBA, and there are also differences in the clinical phenotypes associated with different DBA genes compared with White populations. This result suggests ethnic differences in phenotypic expression, a feature that has been observed in other genetic diseases, including FA.

Congenital dyserythropoietic anemias

Congenital dyserythropoietic anemias (CDAs) comprise a heterogeneous group of disorders characterized by anemia, ineffective erythropoiesis, and morphological evidence of dyserythropoeisis.[70,71]

Table 4. DC genetic subtypes

DC Subtype	Approximate % of patients with DC	Chromosome location	Gene product	Exons
X-linked recessive	25	Xq28	DKC1 (dyskerin)	15
Autosomal dominant	12	14q12	TIN2	6
	5	3q26.2	TERC*	1
	3	5p15.33	TERT*	16
	<1	4q32.2	NAF1*	13
	<1	12q24.31	ZCCHC8*	17
	<1	5q35.1	NPM1	13
	<1	1q32.1	MDM4	13
Autosomal recessive	2	16q21	USB1	9
	2	20q13.3	RTEL1*	35
	1	16p13.12	PARN*	27
	<1	15q14	NOP10	2
	<1	5p15.33	TERT*	16
	<1	5q35.3	NHP2	4
	<1	17p13.1	WRAP5313	
	<1	17p13.1	CTC1	23
	<1	16q22.1	ACD/TPP1	13
Uncharacterized	>30	?	?	?

The major subtypes of DC are associated with variants in *DKC1*, *TINF2*, *TERC*, and *TERT*.

*Heterozygous variants in these genes have been associated with pulmonary disease in late adulthood. Most of the DC genes encode products that have a principal role in telomere maintenance; however, this is not the case for *USB1* and *NPM1*. Variants in some other genes (*GRHL2*, *DNAJC3*, *RECQL4*, and *LIG4*) can produce features that overlap with DC.

The first description of CDAs was published in 1966 by Crookston and colleagues.[72] In 1968, Heimpel and Wendt[73] classified CDAs into 3 types (I-III). Over the years, additional subtypes (IV-VII) have been added, often based on case reports.

Most patients with CDAI present with splenomegaly and anemia. In some patients, nonhematological features (eg, skeletal abnormalities) have been observed (Table 2). Ineffective erythropoiesis is evidenced by peripheral (anisocytosis) and BM (megaloblastic erythroid precursors, internuclear chromatin bridging, and binocularity affecting 3% to 7% of the erythroblasts) abnormalities and increased hemolysis markers. The defining feature is a "Swiss cheese" heterochromatin appearance in erythroblasts on electron microscopy. The first disease gene (*CDAN1*) was identified in 2002[74] (Table 7). Subsequently, *CDIN1* (CDAN1 interacting nuclease 1) was found to be responsible for some CDAI cases.[75]

CDAII is the most common CDA subtype and was described as hereditary erythroblastic multinuclearity with a positive acidified serum lysis test (HEMPAS) in 1969.[76] It is inherited as an AR trait. The anemia is variable (80-110 g/L), and ~10% of cases require regular blood transfusions. Clinical presentations include a variable degree of jaundice, hepatomegaly, splenomegaly, and liver cirrhosis. Peripheral blood morphology shows anisocytosis, and BM features include normoblastic erythroid hyperplasia with

Table 5. SDS genetic subtypes

SDS Subtype	Approximate % of patients with SDS	Chromosome location	Gene product	Exons
Classic				
Autosomal recessive	>90	7q11.21	SBDS	5
SDS-like				
Autosomal recessive	<2	5p13.2	DNAJC21	14
	<2	15q25.2	EFL1	22
Autosomal dominant				
	<2	14q13.2	SRP54	17

Table 6. DBA genetic subtypes

DBA subtype	Approximate % of patients with DBA	Chromosome location	Gene product	Exons
Autosomal dominant	25	19q13.2	RPS19	6
	10-20	Various*	—	—
	7	1p22.1	RPL5	8
	7	12q13.2	RPS26	4
	5	1p36.11	RPL11	6
	3	3q29	RPL35A	5
	3	6q21.31	RPS10	6
	2.4	10q22.3	RPS24	9
	1	15q25.2	RPS17	6
	<1	3p24.2	RPL15	5
	<1	2p25.3	RPS7	7
	<1	19p13.2	RPS28	4
	<1	14q21.3	RPS29	5
	<1	17p13.1	RPL26	4
	<1	19p13.3	RPS15	4
	<1	1q21.3	RPS27	4
	<1	4p14	RPL9	8
	<1	19q13.33	RPL18	7
	<1	17q21.31	RPL27	6
	<1	2q11.2	RPL31	5
X-linked recessive	<1	Xp11.23	GATA1	6
	<1	Xp11.22	TSR2	5
Uncharacterized	~25	?	?	?

*Refers to large deletions in different DBA genes. Variants in *EPO* and *CECR1/DADA2* can also produce DBA-like disease.

usually more than 10% binucleate erythroblasts. On electron microscopy, erythroid cells have a characteristic endoplasmic reticulum arrangement that gives them a double-membrane appearance. Red cells are hemolyzed by acidified sera, but not by the patient's own serum. In 2009, the gene encoding the secretory coat protein complex II component SEC23B has been shown to be responsible for CDAII.[77]

CDAIII is rare. In one of the largest (Swedish) families investigated, the disease was characterized by giant multinucleated erythroblasts. CDAIII exhibits AD inheritance and is caused by variants in *KIF23*.[78] *KIF23* encodes mitotic kinesin-like protein 1, which has a critical role in cytokinesis during cell division.

The precise role of the proteins encoded by *CDAN1*, *CDINI*, and *SEC23B* in disease pathology remains unknown. CDA-like disease related to variants in erythroid transcription factor genes (*GATA1* and *KLF1*)[79] have also been identified.

Severe congenital neutropenia

Severe congenital neutropenia (SCN), including Kostmann syndrome, is characterized by severe peripheral neutropenia ($<0.2 \times 10^9$/L).[80,81] These patients present with recurrent life-threatening infections in infancy. BM examination frequently shows maturation arrest in the myeloid lineage, and some patients can present with cyclical neutropenia. These patients

Table 7. CDA genetic subtypes

CDA Subtype	Approximate % of patients with CDA	Chromosome location	Gene product	Exons
Type I (AR)	Major subset	15q15.2	CDAN1	28
	Minor subset	15q14	CDIN1	18
Type II (AR)	Major subset	20p11.23	SEC23B	22
Type III (AD)	Rare	15q23	KIF23	25
Other subtypes	?	19p13.13	KLF1	3

Table 8. SCN genetic subtypes

Subtype	Approximate % of patients with SCN	Chromosome location	Gene product	Exons
Autosomal dominant	50-60	19p13.3	ELANE	6
	<2	1p22.1	GFI1	11
Autosomal recessive	15	1q21.3	HAX1	7
	5	17q21.31	G6PC3	8
	Rare	1q21.2	VPS45	18
	Rare	1p34.3	CSF3R	19
	?	3p25.3	JAGN1	2
Miscellaneous syndromes*	—	—	—	—

*A heterogeneous group that includes patients with neutropenia as part of a broader syndrome. Some of the genes and associated syndromes in this category are *WAS* (Wiskott-Aldrich syndrome protein), *SBDS* (Shwachman-Bodian-Diamond syndrome), *G6PC* (glycogen storage disease), *CXCR4* (WHIM syndrome), *TAZ* (Barth syndrome), *RBSN* (syndromic myelofibrosis and neutropenia), and *SMARCD2*.

can progress to MDS and leukemia, usually with an acquisition of secondary mutations in granulocyte colony-stimulating factor (G-CSF) receptor. In most patients, heterozygous variants in the neutrophil elastase gene (*ELANE*) have been identified.[82] These variants are thought to cause an accumulation of a nonfunctional protein, which, in turn, triggers an unfolded protein response, leading to a maturational arrest. The original family described by Kostmann had AR-SCN and was caused by biallelic variants in *HAX1*, [83] predicted to result in cell death defects. Variants in other genes (*GFI1*, *G6PC3*, *CSF3R*, *JAGN1*, and VPS45)[84-86] have also been associated with SCN (Table 8). Whereas *ELANE* variants typically produce isolated neutropenia, variants in some other genes are associated with extrahematological abnormalities. There are also several syndromes (reviewed by Hauck and Klein[81]) that involve neutropenia as part of a broader syndrome.

Congenital amegakaryocytic thrombocytopenia and other syndromic thrombocytopenias

Congenital amegakaryocytic thrombocytopenia (CAMT) usually presents in infancy and is characterized by isolated thrombocytopenia and a reduction or absence of megakaryocytes in the BM, usually without extrahematopoietic abnormalities. Approximately 50% of patients develop AA by the age of 5 years. The disease can evolve into MDS or leukemia. Patients with CAMT have biallelic variants in the gene (*MPL*) encoding thrombopoietin receptor (Table 9).[87]

Thrombocytopenia with absent radius (TAR) is usually diagnosed in infancy. TAR is caused by the compound inheritance of a low-frequency, noncoding, single-nucleotide polymorphism and a rare null allele in *RMB8A*. Thrombocytopenia associated with proximal radius and ulna fusion is a relatively new entity arising from heterozygous variants in *HOXA11* or *MECOM*. Although patients typically have thrombocytopenia, those with *MECOM* variants can exhibit very variable hematological phenotypes, including progression to MDS and leukemia.[88] Furthermore, some *MECOM* variants have been associated with hematological abnormalities, including global BMF in infancy, but no radioulnar fusion.[89]

New subtypes of inherited BMF and overlapping syndromes

There are familial BMF cases and/or those that have ≥1 extrahematopoietic abnormalities but do not fit into the entities discussed herein thus far. The availability of next-generation sequencing has enabled elucidation of the genetic basis of some of these disorders. Examples of these new entities include those associated with germline variants (Table 10) in *TPO*, *ERCC6L2*, *MYSM1*, *DUT*, *EXOC3L2*, *TP53*, and *SP1*[89,90] and the number of cases reported in each subtype varies.

Table 9. CAMT, syndromic thrombocytopenia, and other syndromic thrombocytopenias

Subtype	Approximate % of patients	Chromosome location	Gene product/locus	Exons
CAMT				
Autosomal recessive	Majority	1p34.2	MPL	11
TAR	Majority	1q21.1	RBM8A	6
Autosomal recessive				
Radioulnar synostosis	?	7p15.2 3q26.2	HOXA11	2 23
Autosomal dominant	—	—	MECOM*	—

MECOM (MDS1 and EVI1 Complex Locus) variants can be associated with variable hematological features ranging from isolated thrombocytopenia to global BM failure and leukemia.

Table 10. New BMF and overlapping syndromes

Subtype	Chromosome location	Gene product	Exons
Recently recognized BMF subtypes			
Autosomal recessive	9q22.32	ERCC6L2	27
	3q27.1	TPO/THPO	7
	1p32.1	MYSM1	23
	15q21.1	DUT	9
	19q13.32	EXOC3L2	10
	17p13.1	TP53	12
Autosomal dominant	7q21.3	SAMD9*	3
	7q21.2	SAMD9L*	6
	12q13.13	SP1	7
Familial MDS and leukemia			
Autosomal dominant	21q22.12	RUNX1	13
	19q13.11	CEBPA	1
	3q26.2	TERC*	1
	5p15.33	TERT*	16
	3q21.3	GATA2*	8
	4q12	SRP72	20
	10p12.1	ANKRD26	46
	16q22.1	ACD/TPP1	12
	12p13.2	ETV6	14
	5q35.3	DDX41	17
	20q13.33	RTEL1	35
	9p13.2	PAX5	11
	7q21.3	SAMD9*	3
	7q21.2	SAMD9L*	6
	3q26.2	MECOM*	23
	17p13.1	TP53	12
	12q13.2	ERBB3	28
	19q13.32	DHX34	21
Autosomal recessive	3q21.3	MBD4	8
	3q24	HLTF	25
	3p25.1	XPC/XPCC	18

*Variants in these genes can produce very diverse hematological features, including AA, MDS, and leukemia. They can also produce various extrahematopoietic abnormalities. For example, GATA2 deficiency can be associated with pulmonary alveolar proteinosis and primary lymphedema; *SAMD9* disease can be associated with adrenal insufficiency, intrauterine growth restriction, and genital abnormalities; and *SAMD9L* disease can be associated with neurologic/cerebellar, ophthalmic, and pulmonary complications.

There are also entities that are initially characterized in patients with MDS/leukemia or other syndromic diseases, but can also present with peripheral cytopenias. These entities include GATA2 deficiency and *SAMD9/SAMD9L*-related disease. In addition to MDS and leukemia, these patients can have a variable number of extrahematopoietic abnormalities. Germline variants in these genes are particularly prevalent in pediatric patients with MDS associated with monosomy 7. *GATA2* and *SAMD9/SAMD9L* are also included in the category of familial MDS/AML genes.[91] Other genes in this category include *RUNX1*, *CEBPA*, *TERC*, *TERT*, *SRP72*, *AKNRD26*, *ETV6*, *DDX41*, *RTEL1*, *PAX5*, *TP53*, *ACD*, *MECOM*, *HLTF*, *XPC*, and *DHX34* (Table 10). This highlights the overlapping nature of hematological (BMF, MDS, and AML) and extrahematological phenotypes produced by germline variants in the mentioned genes. It is likely that additional new entities of familial BMF/MDS will be characterized in the future.

Epidemiology

The true incidence and natural history of inherited BMF disorders remain uncertain. SCN and DBA are among the most prevalent of these disorders; for example, the estimated annual DBA birth incidence is 5 per 10^6. Tamary et al reported on a retrospective population-based registry of inherited BMF syndromes in Israel,[92] representing the first comprehensive population-based study to evaluate the incidence and complications of the different inherited BMF syndromes. A total of 127 patients

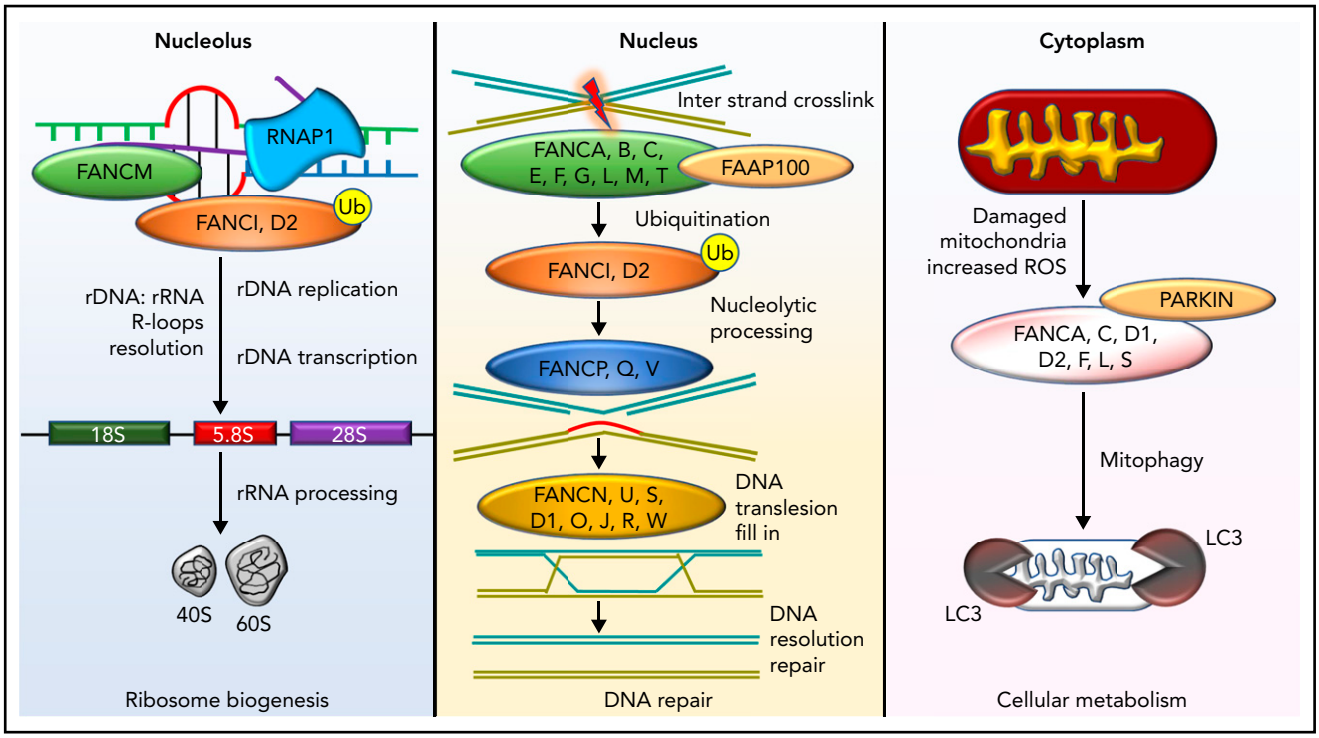

Figure 1. Diverse molecular functions of the FA pathway. DNA repair in the FA pathway predominantly restores DNA interstrand cross-links to ensure bona fide replication and transcription. However, this activity is also involved in the resolution of DNA: RNA hybrids known as R-loops occurring predominantly in the nucleolus due to ribosomal DNA transcription by RNA polymerase 1 (RNAP1). R-loop resolution by FANCM, FANCI, and FANCD2 proteins ensures ribosome biogenesis. FANC proteins also clear mitochondria damaged by excessive reactive oxygen species and, in conjunction with PARKIN, execute mitophagy. Ub, ubiquitin modification; FAAP100, FA core complex–associated protein 100.

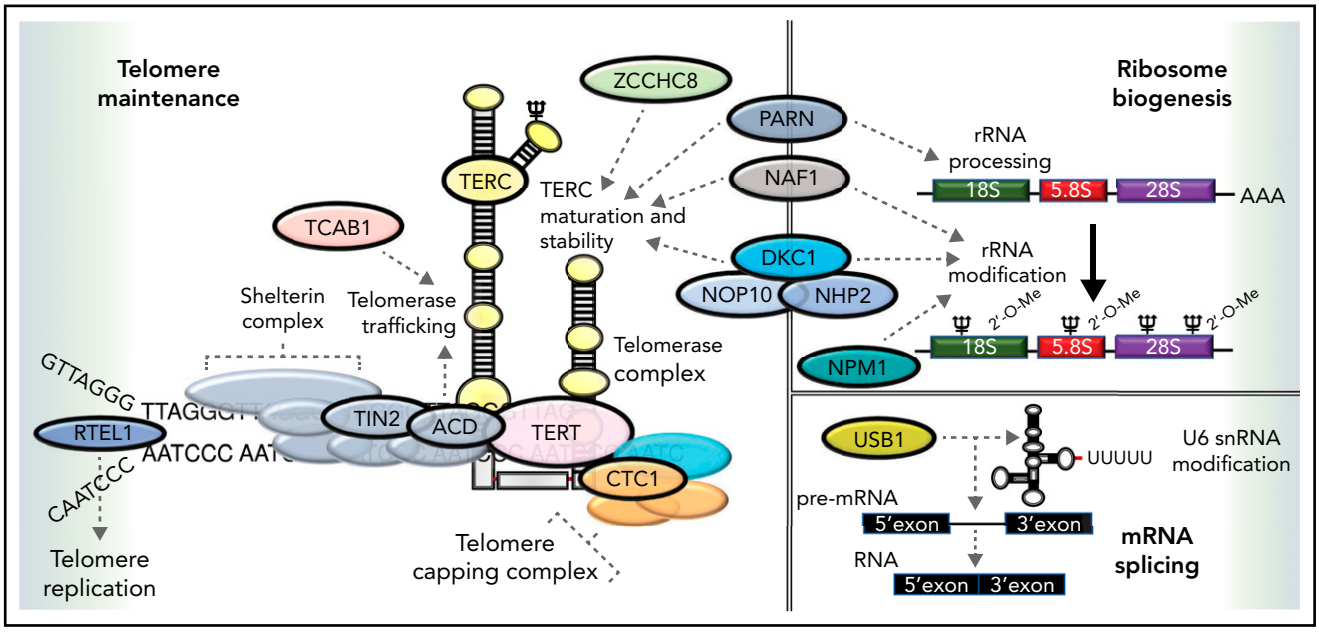

Figure 2. Functional overlap of DC genes involved in telomere maintenance and ribosome biogenesis. Proteins mutated in DC are indicated by named capsules and affect molecular functions, such as telomere replication (RTEL1), telomere protection (TIN2, ACD), telomerase (TERC, TERT, and DKC1), and telomerase maturation and stability (ZCCHC8, NAF1, PARN, DKC1, NOP10, and NHP2). Pseudouridylation of TERC and ribosomal RNA (rRNA) is performed by DKC1. The deadenylation function of PARN also regulates the maturation and processing of both TERC and rRNA. Recently, variants in NPM1 that regulate 2'O rRNA methylation have been reported in patients with DC. USB1 is an outlier, being involved in U6 spliceosomal RNA processing. Dashed arrows link different proteins to specific functions in which they are involved.

diagnosed from 1966 through 2007 were registered: 52% had FA, 17% had SCN, 14% had DBA, 6% had CAMT, 5% had DC, 2% had SDS, and 2% had TAR. The most common disease was FA, which also carried the worst prognosis, with severe BMF and development of cancer. These data are probably relevant only to Israel. For example, based on the data from this registry, the annual FA incidence was calculated to be ~2 per 100 000 live births, sevenfold higher than expected from the worldwide carrier frequency of 1 in 300 and probably reflecting a high consanguinity rate in Israel.

In a subsequent report from the Canadian registry,[93] the most common disease was DBA followed by FA, showing an FA incidence of ~11.4 cases per 10^6 births. It is likely that the true incidence/prevalence of these disorders varies in different regions of the world, reflecting such factors as consanguinity rates and environmental influences, such as infections. This variation has also been reported in a recent study by Bluteau et al from France.[89] Further studies on the epidemiology of these disorders are desirable.

General principles of diagnosis and management

A diagnosis of an inherited BMF should be considered in a pediatric patient when ≥1 BMF-associated extrahematopoietic features are identified clinically or by investigations. It should also be considered during differential diagnosis in children presenting with isolated AA, MDS, or leukemia. The specific extrahematopoietic abnormalities help diagnose a recognized syndrome, but this diagnosis is not always possible based on clinical features alone.

Chromosomal breakage analysis of blood lymphocytes after exposure to DEB or MMC remains a useful diagnostic test for FA. However, it may give unclear results if there is somatic mosaicism, and biallelic variants in the Nijmegen breakage syndrome gene can also cause increased chromosomal breakage with MMC or DEB. All children presenting with AA and MDS ideally should be tested for FA. Furthermore, children who present with leukemia and suggestive congenital abnormalities or who have monosomy 7, an additional chromosome 3, or complex karyotypes should be tested for FA. Genetic testing for FA genes is possible but not always straightforward. Telomere length, particularly using flow fluorescence in situ hybridization, can be a useful initial screening test in the diagnosis of DC or DC-like disease.[94] Patients with DC frequently, but not always, have chromosomes with very short telomeres. Genetic testing for DC genes can help substantiate the diagnosis. However, as in FA, this strategy is not straightforward, as many patients have certain variants that can be difficult to categorize, and the genetic basis will remain unknown even though approximately one-third of patients have been tested for currently known DC genes. In patients with global BMF, the other genes to consider are SDS genes and new entities, such as those, mentioned herein, including variants in *TPO*, *ERCC6L2*, *MYSM1*, *MECOM*, and *SAMD9/SAMD9L*. For patients presenting with isolated neutropenia, analysis of *ELANE* and *HAX1* may help substantiate the underlying diagnosis. For those with isolated anemia, an initial focus on *DBA* and *CDA* genes is warranted.

Because of the availability of next-generation sequencing, many clinicians now have access to targeted gene panels that can test for all BMF genes (>100) simultaneously (Tables 3-10). Furthermore, there is increasing access to whole-exome and whole-genome analyses. Similar to all tests, these approaches have advantages and disadvantages. For example, if a new variant(s) is identified even in a known disease gene, it is not always possible to be certain that the variant is responsible for the clinical phenotype. In such cases, studies of the segregation of the variant within families and functional analyses can provide useful additional information on the significance of the variant.

Once an inherited BMF diagnosis has been made, clinically and/or genetically, the chronic nature of these disorders should be explained to the patient and family. In general, patients need lifetime follow-up (ideally, in a special BMF clinic) and will need monitoring for hematological complications, including leukemia, immunological defects, and cancer. The frequency of monitoring investigations, such as blood tests, BM examinations, and pulmonary function tests, is difficult to precisely stipulate because of the considerable heterogeneity and the absence of randomized studies. However, regular follow-up is advisable, possibly annually, with more frequent monitoring being implemented as specific problems arise. Expert groups have developed consensus guidelines[95] that provide a useful framework for clinical practice.

Owing to the significant risk of cancer in many of these syndromes, particularly as patients enter adulthood, avoidance of smoking is advisable. They should also avoid sunbathing and minimize alcohol intake as they enter adulthood. Patients should be regularly screened for hematological and nonhematological cancer.[95] Treatment for cancer depends on the specific type, but the underlying genetic defect should be considered (ie, more supportive care and reduced drug doses).

Regarding pulmonary disease, patients should avoid smoking, particularly those with FA or DC. Medical treatment is usually difficult in severe lung disease, and lung transplant may be an option in some cases. Advice on skincare (eg, use of moisturizing creams) and sunlight avoidance are important. They should also avoid occupations that expose them to hazardous chemicals or repeated physical trauma. When doing domestic chores, such as cleaning, protective gloves should be used, particularly in DC. Avoiding extremes of temperature is desirable, as the skin is usually fragile compared with that in the normal population. Liver disease is more common in patients with FA or DC than in the population without these disorders; hence, all administered drugs require close monitoring. Drugs also should be used carefully, as patients with inherited BMF syndromes tend to be small and more sensitive to many drugs. This factor is particularly important in patients with FA or DC who undergo allogeneic hematopoietic stem cell transplantation (SCT).

Management of hematological complications

Major advances in supportive treatment have led to considerable improvements in the outcome of these patients. Red cell transfusions should be performed to maintain the hemoglobin at an asymptomatic level (typically, >80 g/L), and platelets should be maintained at >10 × 10^9/L. All patients with

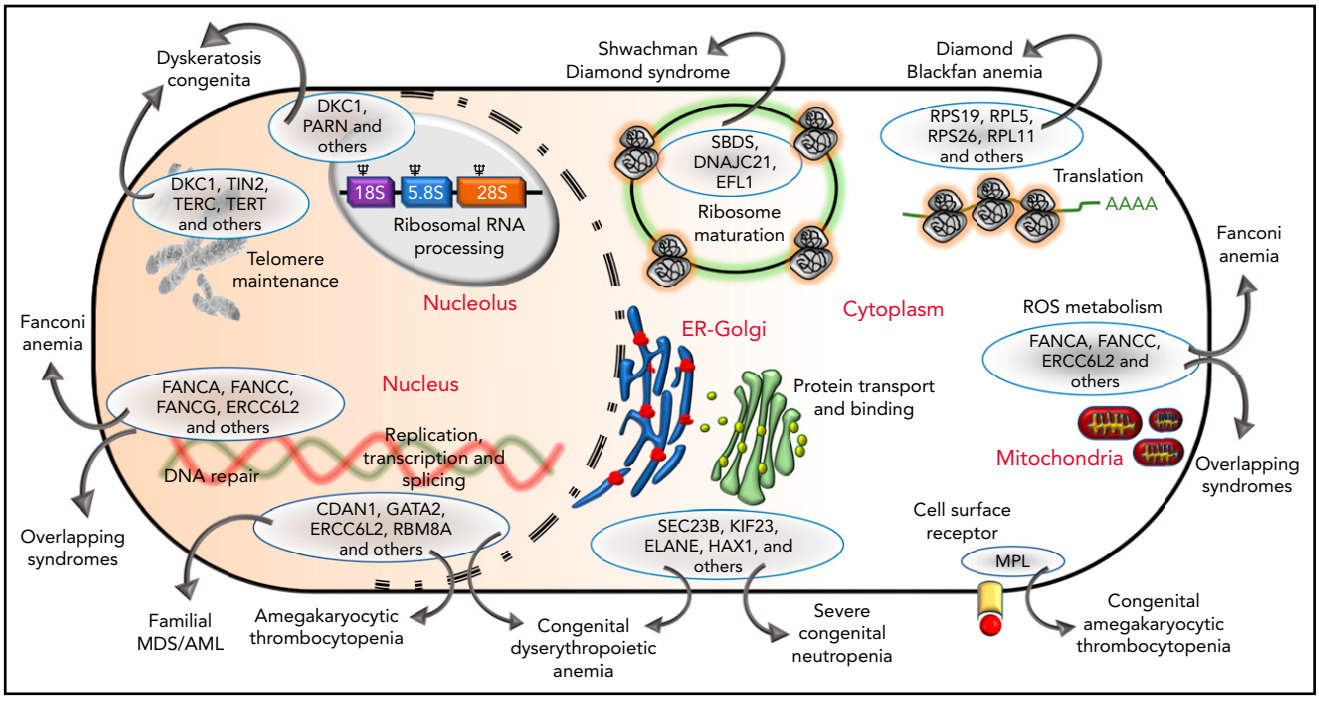

Figure 3. The genetic, subcellular, and molecular landscape of inherited BMF syndromes. The predominant molecular functions of the genes involved in the inherited BMF syndromes are illustrated in a cell diagram. The most clinically significant genes involved in the pathways are shown within the blue ellipses; for full listings of other genes, please refer to Tables 3-10. Outward-pointing arrows indicate different clinical subtypes and overlapping syndromes, as discussed in the text, which are caused by defects in the molecular pathways.

neutropenia must receive prompt therapy with broad-spectrum antibiotics if they develop an infection. Addition of G-CSF may be appropriate in these circumstances. Leukocyte-depleted and, where appropriate, cytomegalovirus-negative blood products should be chosen, to prevent the development of HLA antibodies and reduce the risk of cytomegalovirus.

Inherited BMF syndromes usually respond to specific interventions. In patients with FA and DC who have significant peripheral cytopenias (hemoglobin <80 g/L, neutrophils $<0.5 \times 09$/L, and platelets $<20 \times 10^9$/L), the first-line medical therapy in some countries is frequently oxymetholone started at 0.5 to 1.0 mg/kg per day and gradually increased, if necessary, to a maximum dose of 5 mg/kg per day. Patients with DC are usually more sensitive to oxymetholone than are patients with FA. There is also increasing experience in danazol use in these patients, and now, danazol is preferably used compared with oxymetholone.[96,97] Approximately 70% of patients with DC or FA will have a hematological response to danazol that can be durable for years in some patients. Patients with severe BMF and HLA-compatible donors can be cured of their hematological complications by SCT. In patients with severe BMF without significant comorbidities, it is reasonable to consider upfront SCT without prior androgen therapy. If family donors are to be used, ensuring that they have been adequately tested for the relevant genetic variant(s) is important. It has been established that patients with inherited BMF syndromes have greater efficacy and lower toxicity with low-intensity, fludarabine-based protocols. There is now considerable experience using such protocols in patients with FA or DC, but this is not the case with some rare entities.[98-102] The use of cord blood and haploidentical donors is also beneficial in specific circumstances. After many challenges, there has been some recent success with hematopoietic gene therapy in patients with FA subtype A.[103] In the future, therapeutic strategies that target disease-specific hematopoietic stem cell defects are likely to emerge. There have been exciting preclinical studies on the role of transforming growth factor-β inhibitors in FA[104] and PAPD5, a human poly(A) polymerase, inhibitors in DC.[105]

In patients with DBA, the first-line therapy remains prednisolone, as up to 80% of patients respond to this treatment. Prednisolone dose and frequency are titrated to the lowest number required to maintain reasonable hemoglobin and minimize side effects. In the minority of steroid-refractory patients or those who become refractory to prednisolone, treatment with regular blood transfusions is instituted and should be accompanied by a comprehensive iron-chelating program to prevent iron overload. At this stage, hematopoietic SCT may be appropriate and potentially curative for patients with DBA who have compatible sibling BM donors. The current emerging consensus is to recommend SCT before the age of 10 years (ideally, before 5 years) in every child requiring transfusion support with either a sibling or a fully matched, unrelated donor.[106]

Patients with CDA with mild anemia require no major interventions. Folate supplementation is prescribed to prevent folate deficiency. If regular transfusions are necessary, early attention to iron chelation is essential. Iron loading may also occur in nontransfused patients with CDA. Splenectomy may be beneficial in some patients (CDAII), and there are reports of successful hematopoietic SCT.[70,107] In CDAI, there are also case reports of improvement after treatment with

interferon-α.[70] The mechanism of this therapeutic benefit remains unclear.

The mainstay of neutropenia management in patients with SCN is G-CSF. More than 90% of patients respond to this treatment, and the dose is adjusted to maintain an absolute neutrophil count of 1.5 to 2.0 × 10^9/L. Other measures to prevent infection are also instituted, and any evidence of infection should be promptly treated. For patients with compatible donors, SCT may be appropriate if they have a poor response to G-CSF or there is evolution to MDS/leukemia.[108]

Concluding remarks

The major advances in the molecular basis of inherited BMF syndromes have provided insights into critical biological pathways, such as DNA repair (FA), telomere maintenance (DC), and ribosome biogenesis (SDS and DBA). They have also provided interesting links between inherited (eg, DBA and SDS) and acquired (eg, MDS and 5q− syndrome) hematological disorders.

Phenotypic similarities (BMF, extrahematopoietic abnormalities, and cancer) between these syndromes have been acknowledged for years (Tables 1 and 2). Not surprisingly, the overlap is also observed at the level of molecular pathology (Figure 3). For example, SDS and DBA are both disorders of ribosomal biogenesis, whereas FA, DC, and SDS all have short telomeres. Further overlapping and biological connections may emerge in the future.

In clinical practice, significant genetic advances have led to improved diagnosis, particularly for those with atypical presentations, and have enabled better personalized management. This includes the use of low-intensity, fludarabine-based conditioning protocols that have resulted in improvements in outcomes after hematopoietic SCT and the repurposing of drugs such as danazol. New therapies capable of correcting or ameliorating disease-specific defects of different syndromes are emerging in the laboratory setting, with potential for translation into the clinic.

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Footnote

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When to consider inherited marrow failure syndromes in adults

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The inherited bone marrow failure syndromes (IBMFS) are a heterogenous group of disorders caused by germline mutations in related genes and characterized by bone marrow failure (BMF), disease specific organ involvement, and, in most cases, predisposition to malignancy. Their distinction from immune marrow failure can often be challenging, particularly when presentations occur in adulthood or are atypical. A combination of functional (disease specific assays) and genetic testing is optimal in assessing all new BMF patients for an inherited etiology. However, genetic testing is costly and may not be available worldwide due to resource constraints; in such cases, clinical history, standard laboratory testing, and the use of algorithms can guide diagnosis. Interpretation of genetic results can be challenging and must reflect assessment of pathogenicity, inheritance pattern, clinical phenotype, and specimen type used. Due to the progressive use of genomics, new IBMFS continue to be identified, widening the spectrum of these disorders.

LEARNING OBJECTIVES

- Understand the clinical features and laboratory testing used to distinguish immune from inherited bone marrow failure (IBMFS) in adults
- Review when germline genetic testing for IBMFS is indicated and the common diagnostic challenges
- Learn why timely diagnosis of IBMFS is crucial for proper patient management

Introduction

Bone marrow failure (BMF), characterized by decreased production of 1 or more hematopoietic lineages, is classified as either inherited, due to germline variants, or acquired, most commonly immune mediated. Inherited bone marrow failure syndromes (IBMFSs) have been traditionally considered pediatric onset disorders; however, it is increasingly recognized that many present first in adulthood. Classical IBMFSs, including Fanconi anemia (FA), dyskeratosis congenita (DC), Shwachman Diamond syndrome (SDS), and Diamond Blackfan anemia (DBA) are mostly diagnosed in children but can present later in life, sometimes due to genetic somatic rescue or mosaicism. In adults, typical clinical findings may be missing, and diagnosis may be challenging without the use of specialized testing. Increased use of genetic testing has further characterized the broad spectrum of known IBMFSs and identified a wide range of new ones, the latter of which often present in adulthood and with predominant nonhematologic features.[1,2]

CASE 1

A 23-year-old male presented to the emergency room with pallor and bleeding and was pancytopenic: hemoglobin (Hb) 7.5 g/dL, absolute neutrophil count (ANC) 0.6×10^9/L, platelets 17×10^9/L, absolute monocyte count 0.08×10^9/L, and absolute reticulocyte count 50×10^9/L. No prior blood counts were available. No vitamin deficiencies or paroxysmal nocturnal hemoglobinuria clone were detected. Bone marrow examination showed hypocellularity of 30% with no dysplasia and a normal karyotype. Flow cytometry of the bone marrow showed a reduction in B-cells, B-cell precursors, and natural killer (NK) cells. Personal history was significant for recurrent pulmonary infections and warts. Family history was negative for malignancy or hematologic disorders. A diagnosis of acquired severe aplastic anemia (AA) was made.

Inherited versus immune marrow failure

Cytopenias and evidence of marrow hyperproliferation are the defining features of BMF but are present in both immune

and inherited forms.[3] Therefore, in evaluating BMF patients, careful consideration should first be given to the patient's disease history, concurrent medical comorbidities, and family history to assess for potential immune BMF versus IBMFS.[4] When positive, family history can aid in diagnosing IBMFSs, but varied clinical phenotype and disease heterogeneity even among family members make it less helpful when negative. Other causes of cytopenia, such as vitamin deficiency, viral infection, direct toxicity, autoimmune diseases, and medications, should be excluded. Patients with IBMFSs may have distinct clinical patterns of disease to guide diagnosis; for instance, limb and/or renal abnormalities in FA, lung and/or liver abnormalities in TBD, or recurrent atypical infections in GATA2 deficiency (Table 1).[5] More recently, disease-specific molecular profiles, including mechanisms of somatic genetic rescuing, have also been identified as potential markers of underlying IBMFS.[6]

Immune AA remains a diagnosis of exclusion; age of onset is bimodal with disease more common in younger and older patients. Clinical testing is focused toward excluding IBMFS; however, some features, such as the presence of glycosyl-phosphatidylinositol–negative paroxysmal nocturnal hemoglobinuria (PNH) clones and loss of heterozygosity in the chromosome 6 p arms (6pLOH), are reassuring markers of an immune etiology,[7,8] as is a clonal profile dominated by *PIGA*, *BCOR,* and *BCORL1*.[9,10] *BCOR* and *BCORL1* are the most common somatic mutations seen in AA and are often seen in isolation or co-occurring with *PIGA*.[10] While also present in myelodysplastic syndrome (MDS), they are not predominant and also tend to co-occur with other mutations.[11] T-cell large granulocytic leukemia clones are also more common in acquired than in inherited BMF but are less specific than PNH or 6pLOH (Figure 1).

Most specialized centers have routinely incorporated chromosome breakage studies and telomere length (TL) measurement by flow fluorescence *in situ* hybridization (FISH) in the clinical assessment of newly diagnosed AA. Other specialized testing, such as pancreatic dysfunction for SDS and erythroid adenosine deaminase activity for DBA, may be reserved for clinically suspected cases (Figure 2). Testing for primary immunodeficiency syndromes, using lymphocyte subsets and serum immunoglobulins, is pursued when there is a clinical history suggestive of recurrent and/or atypical infections, autoimmunity, or presence of severe lymphopenia.

CASE 1 (continued)

As the patient had no matched sibling donors, immunosuppressive therapy (IST) was promptly administered. Given his young age and history of warts and pulmonary infection, diagnostic genetic testing was performed. After 6 weeks, blood counts had not improved; ANC remained >0.5×10^9/L. Meanwhile the patient developed fever, progressive cough, and dyspnea. Computed tomography of the thorax showed patchy and nodular pulmonary infiltrates within the right middle, left, and lower lobes. Bronchoscopy samples grew mycobacterium avium complex (MAC). Immunosuppressive therapy was discontinued, and matched unrelated donor transplant was pursued. His germline genetic report returned and showed a pathogenic variant in the *GATA2* gene.

When to consider genetic testing

One of the most difficult considerations in work up of BMF is when to perform genetic testing. Most BMF is classified as immune (>90%), and genetic testing is costly and not always available. However, missing an IBMFS has significant clinical implications, and genetic testing is currently our best method of detection.[3,12,13] Increasingly, it is recognized that omission of genetic testing results in missed IBMFS diagnoses. A retrospective study including immune severe AA (SAA) patients using pre–hematopoietic stem cell transplant samples from the Center of International Blood and Marrow Transplant Research (CIBMTR) showed an undiagnosed IBMFS in ~7% of patients, one-third of whom were adults.[14] Most were nonclassical IBMFS (such as *RUNX1*, *MECOM*, *ANKRD26*, and *GATA2*) or TBD. Similarly in MDS, 7% of patients were found to have underlying germline predisposition, highest in the younger age group (33% for aged 11-20 years) but still significant in older patients (6%-8%).[15] One-quarter of germline genes mutated in MDS were implicated in IBMFSs.

Screening for IBMFSs should be also considered in young patients with atypical oncologic presentations or unexpectedly high toxicity to cytotoxic chemotherapy or hematopoietic stem cell transplant (HSCT).[16]

Recently, we developed a machine learning model to predict for immune versus inherited in adults with AA. By using patients' baseline clinical and laboratory characteristics, our model accuracy correctly predicted 88% of cases; TL, cutaneous findings, long-standing cytopenias, macrocytosis, and age/sex were top predictors.[17] Omission of TL dropped the model's accuracy, highlighting its importance. Adult patients with SAA without a positive family history or a clinical phenotype suggestive of inherited disease were rarely diagnosed with an IBMFS. Where genetic testing is not feasible, selection of patients for germline genetic screening should take into account age, clinical presentation, family history, and available laboratory and specialized test results.

Inheritance and penetrance as challenges for IBMFS diagnosis

IBMFSs can be linked to different inheritance patterns depending on the specific mutated gene being either autosomal recessive (AR) (2 mutated alleles required to cause disease), autosomal dominant (AD) (1 mutated allele required to cause disease), X-linked, or *de novo*. In general, AR disorders tend to have high penetrance and earlier disease onset while AD disorders have more variable penetrance and later onset, but there are exceptions to this.[18,19] *De novo* or AR variants represent a challenge for IBMFS diagnosis. *De novo* mutations first occur in the affected patient due to a mutation in the parental germ cell or during embryogenesis. In such cases, family history will be absent (as in case 1). Examples of IBMFSs that classically occur *de novo* are DBA (~50% of cases),[20] GATA2 deficiency, and the *TINF2* subset of TBD.[21] Family history may also be absent when consanguinity is present, family penetrance is incomplete, or due to AR inheritance. Therefore, one cannot omit specialized work up or genetic testing on the basis of family history alone.

Non-classical IBMFS

Newly described inherited monogenic diseases that may present as marrow failure have been defined, differing from the classical

Table 1. Inheritance and common clinical findings of inherited BMF syndromes

	Fanconi anemia[37]	Telomere biology disorders[1]	Shwachman Diamond syndrome[38]	Diamond Blackfan anemia[39]	GATA2	SAMD9/9L[28]	Platelet disorders[24]	MECOM-associated disorders[25]	ERCC6L2[2,40]	DADA2[41]
Affected genes	FANC genes, *BRCA2*	*DKC1, TERT, TERC, PARN, RTEL1, TINF2, CTC* + others	*SBDS, DNAJC21,* and others	RP genes, *TSR2, GATA1*	*GATA2*	*SAMD9/SAMD9L*	c-*MPL* (CAMT) *RBM8A* (TAR) *RUNX1, ETV6, ANKRD26,* and others	MECOM (MDS1 and EVI1 complex locus)	*ERCC6L2*	*ADA2*
Inheritance	AR except for FANCB (XLR) and FANCR (AD)	AD: *TERT, TERC, TINF2, RTEL1, PARN* AR: *CTC, RTEL1* XLR: *DKC1*	AR	AD, XLR or sporadic	AD	AD	AR: (CAMT, TAR) AD: *RUNX1, ETV6, ANKRD26*	AD	AR	AR
Common non hematologic clinic findings	- Limb abnormalities (absent radii/short thumbs) - Short stature - Renal anatomical defects - Café au lait spots - Microcephaly/ Microphthalmia	- DC triad: oral leukoplakia, dyskeratotic nails, reticulated skin - Pulmonary fibrosis - Liver disease (fibrosis, fatty liver) - AVM - Early grey hair - Immunodeficiency - Osteoporosis	- Failure to thrive/poor feeding - Steatorrhea - Recurrent infections - Skeletal abnormalities - Hepatomegaly - Intellectual disability - Congenital cardiac defects - Endocrinopathy	- Short stature/IUGR - Limb abnormalities (thumb) - Cardiac defects (VSD, ASD) - Cephalic malformation (microcephaly) - Developmental delay	- Immunodeficiency (atypical mycobacteria, recurrent warts from HPV) - Lymphedema - Thrombosis - Pulmonary alveolar proteinosis (dyspnea and cough)	- MIRAGE: myelodysplasia, infection, growth restriction, adrenal hypoplasia, genital problems, enteropathy - Ataxia Pancytopenia: cerebellar symptoms and pancytopenia - SAAD: nodular neutrophilic panniculitis, ILD, basal ganglia calcifications, cytopenia	- CAMT: some neurological associations, possibly related to ICH - TAR: skeletal defects (absent radii), cow's milk intolerance, renal tract abnormalities, cardiac defects) - RUNX1: platelet function defect	- Radioulnar stenosis - Clinodactyly - Hearing loss - Cardiac malformations - Renal malformations	- Microcephaly - Developmental delay	- Strokes - Vasculitis - Systemic inflammation - Hypogammaglobulinemia
Malignancy risk	- MDS/AML - SCC of skin, head/neck, anogenital	- MDS/AML - SCC skin, head/neck, anogenital - BCC skin	MDS/AML	- MDS/AML - Colon cancer - Osteogenic sarcoma	- MDS/AML - SCC skin, anogenital - BCC skin	MDS/AML	- RUNX1/ETV6/ ANKRD26: MDS/AML or ALL - ALL > ETV6, MDS/AML > RUNX1/ANKRD26	MDS/AML	MDS	None known

AD, autosomal dominant; AML, acute myeloid leukemia; AR, autosomal recessive; ASD, atrial septal defect; AVM, arteriovenous malformation; BCC, basal cell carcinoma; CAMT, congenital amegakaryocytic thrombocytopenia; DC, dyskeratosis congenita; HPV, human papilloma virus; ICH, intracranial hemorrhage; ILD, interstitial lung disease; MDS, myelodysplastic syndrome; RP, ribosomal protein; SAAD, SAMD9L-associated autoinflammatory disease; SCC, squamous cell carcinoma; TAR, thrombocytopenia absent radii; VSD, ventricular septal defect; XRL, X-linked recessive. References listed as superscript.

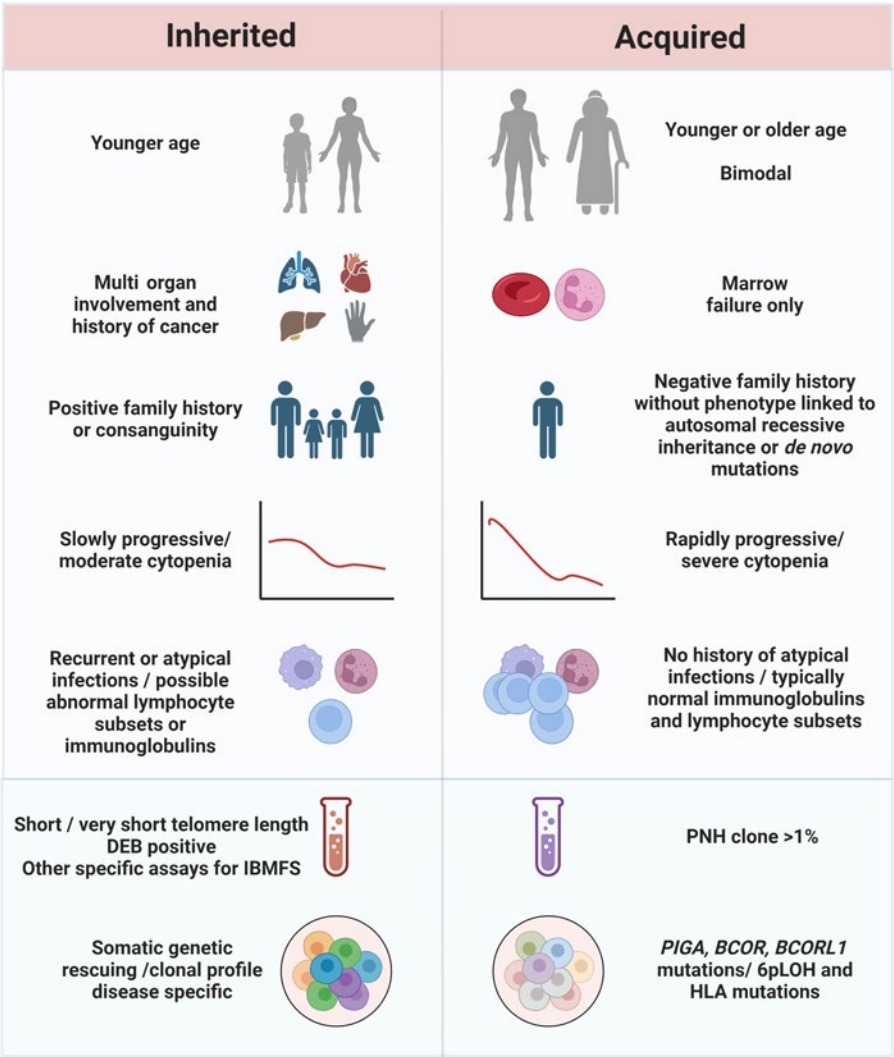

Figure 1. Considerations for inherited versus acquired bone marrow failure. Age, evidence of other organ involvement, and family history of cytopenia, hematologic malignancy, solid cancers, or other organ involvement (ie, familial pulmonary fibrosis in telomere biology disorders). Patients with IBMFSs may have had long-standing cytopenias for years; adult patients who present acutely with severe cytopenia more commonly have immune-mediated disease. Low immunoglobulins or lymphocyte subsets may point towards a primary immunodeficiency disorder; these are typically preserved in immune marrow failure. Paroxysmal nocturnal hemoglobinuria clones are commonly seen in immune bone marrow failure but very rare in IBMFS. Many IBMFS have disease specific clonal patterns and somatic genetic rescuing may occur. In immune BMF, ***PIGA*** (driver of PNH), ***BCOR/L1***, and human leukocyte antigen mutations predominate. DEB, diepoxybutane.

IBMFSs in terms of their typical age of onset, constellation of symptoms, and diagnostic testing. GATA2 deficiency was first described in 2010 as 4 different diseases based on the slightly different clinical observations. Patients can present in late adolescence or early adulthood, with a variable clinical phenotype, even among affected family members.[22] Patients with GATA2 deficiency may present with cytopenia and have a hypocellular marrow consistent with aplastic anemia. However, reduced numbers of B cells and precursors, NK cells, and monocytes are characteristic of GATA2.[23] Other manifestations include opportunistic infections, lymphedema, and predisposition to MDS and/or leukemia.[22]

Patients with isolated chronic thrombocytopenia, particularly with a pertinent family history, should be investigated for a familial platelet disorder. Congenital amegakaryocytic thrombocytopenia and thrombocytopenia with absent radii are usually apparent in early childhood. However, diseases related to RUNX1, ETV6, and ANKRD26 often present later in adolescence or adulthood; they are characterized by thrombocytopenia, variable bleeding phenotype, and predisposition to hematologic malignancy.[24] More recently identified, MECOM-associated syndromes (MDS1 and EVI1 complex locus) are typically pediatric and present with skeletal defects and amegakaryocytic thrombocytopenia.[25]

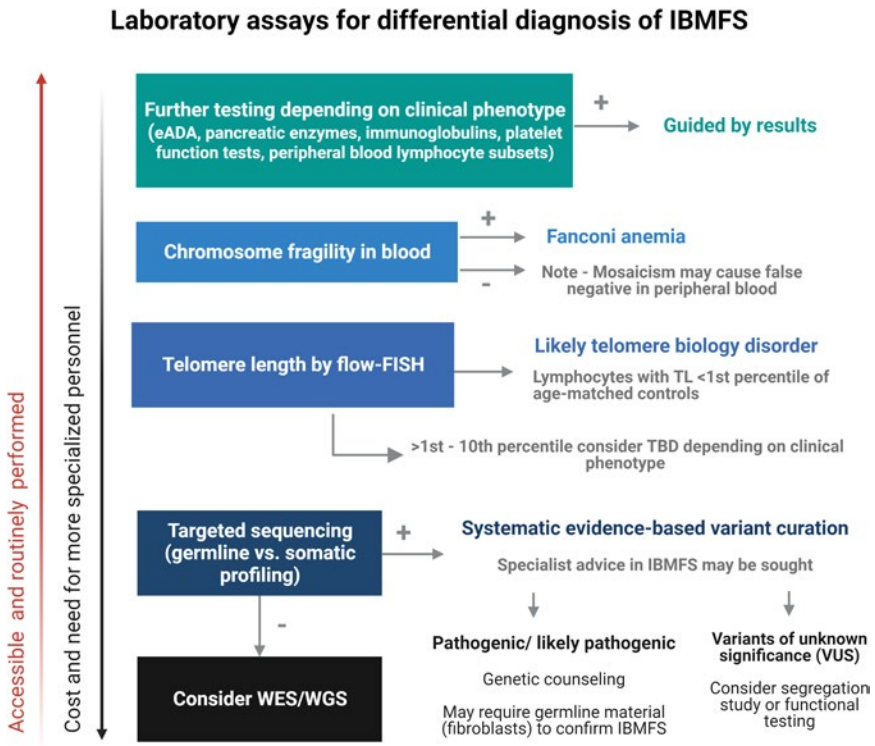

Figure 2. Approach to specialized work up of confirmed BMF. Specialized assays can be used for diagnosis of the IBMFS. Chromosome breakage studies for FA are performed by exposing cultured cells (usually peripheral blood lymphocytes) to diepoxybutane (DEB), a DNA cross-linking agents, and seeing how much chromosomal breakage is induced at a concentration of DEB that has little effect on normal cells.[42] Testing can be misleading or inconclusive for 2 reasons: 1) somatic reversion in the hematopoietic cells causes a false negative (this can be overcome by testing skin fibroblasts if you have a high clinical suspicion and negative peripheral blood DEB) or 2) recent chemotherapy administration (increase in baseline breakage). Telomere length is assessed in peripheral blood lymphocytes using flow-FISH and reported as a percentile for age. TL in lymphocytes <1st percentile is very sensitive and specific for TBD, ≥1st but <10th percentile is suggestive of possible TBD in the right clinical context, and ≥10th percentile is very unlikely to be TBD.[43] High erythroid adenosine deaminase (eADA) enzyme activity levels are found in cases of DBA. Targeted sequencing can identify both germline and somatic variants when peripheral blood is used; to confirm germline status, sequencing of a germline control tissue such as fibroblasts (skin biopsy) or testing of family members should be sought. Interpretation of genetic reports, particularly when VUS is reported, is challenging, and specialist input may be required. Testing for primary immunodeficiency syndromes is pursued when there is a clinical history suggestive of recurrent and/or atypical infections, autoimmunity, or presence of severe lymphopenia. Lymphocyte subsets and serum immunoglobulins are useful in this setting. FISH, fluorescence ***in situ*** hybridization; TBD, telomere biology disorder; WES, whole exome sequencing; WGS, whole genome sequencing.

Some inborn errors of immunity, characterized by immunodeficiency or other immune dysregulation, may also present as marrow failure, such as Toll-like receptor 8 gain of function mutations.[26] A careful history focused on infection and autoimmunity is required to identify such patients.

CASE 2

A 35-year-old female presented to the emergency department with dyspnea and pancytopenia: Hb 6.9 g/dL, ANC 1.2×10^9/L, platelets 6×10^9/L, and absolute reticulocyte count 55×10^9/L. Past medical history was significant for chronic immune thrombocytopenia and menorrhagia. Ferritin was 20 mcg/L with normal B12 and folate. Family history was significant for mother with chronic immune thrombocytopenia and iron deficiency and maternal aunt with leukemia. Bone marrow examination performed showed a mildly hypercellular marrow with megakaryocytic dysplasia (>10%), mild dysethryopoiesis, and dysgranulopoiesis. Blast count was 7% and karyotype was normal. Genetic testing identified variants in *RUNX1* (variant allele frequency [VAF] 55%) and *TET2* (VAF 35%). A diagnosis of MDS was made.

Clonal hematopoiesis vs germline predisposition in IBMFS

Most IBMFSs have an increased risk of myeloid malignancy, in particular MDS and acute myeloid leukemia.[16] Clonal hematopoiesis in myeloid-cancer genes may predict for clonal evolution in many IBMFSs, and distinct patterns of clonality can guide clinical suspicion for a particular disorder. In FA, malignancy has been linked to chromosome 1 q gain and cryptic *RUNX1/AML1* lesions;

in TBD, with *U2AF1*$^{S34/Q157}$ mutations; in SDS, with biallelic *TP53* mutations; and in *SAMD9/9L* disorders, with monosomy 7.[27-30] The genes and variants commonly found somatically mutated in typical MDS and acute myeloid leukemia are the same found in germline disorders, most commonly *RUNX1, ETV6, DDX41, TP53, GATA2, BRCA1, BRCA2,* and others.[31] Therefore, determining whether a variant is germline or somatic is crucial to distinguish *de novo* MDS from that secondary to IBMFS or another germline predisposition syndrome.

DNA sequencing assays covering genes related to hematologic disease often cannot distinguish whether variants are germline or somatic. In peripheral blood bulk DNA sequencing, germline variants are expected to be at allele frequencies of ~50% or ~100%, if heterozygous or homozygous, respectively. In ranges outside these limits (VAF <30% and >70%-85%), variants are often considered somatic.[32] However, revertant genetic rescuing can change the VAF of germline variants into somatic ranges, resulting in a false negative result; this should be considered when a suspicious variant outside the typical germline range is detected. When variants in germline predisposition genes are found at VAFs >30%, sequencing of germline tissues or affected relatives is important to distinguish between somatic and germline variants.[15,31,32] Cultured fibroblasts obtained by skin biopsies are considered optimal controls, but because their collection is difficult in many centers and extra time is required to culture the fibroblasts, results are delayed. Alternative sources of DNA are buccal swabs and hair follicles; buccal swabs are not recommended due to contamination with blood cells, and large numbers of hair follicles may be required to yield results. Co-occurrence of adaptive clonal hematopoiesis with germline variants related to IBMFS (mechanisms of somatic genetic rescuing) can be a natural proof-of-concept that a potential germline variant is pathogenic and disease causing. Examples of somatic markers of IBMFS include *PPM1D*, *POT1*, and the *TERT* promotor in TBD; *EIF6* mutations in SDS; transient monosomy 7 in SAMD9/9L disorders; and concurrent somatic *DDX41* mutations with germline *DDX41*.[33,34]

CASE 2 (continued)

The patient underwent a skin biopsy (with cultured fibroblasts) that identifies the same *RUNX1* variant but not the *TET2* in cultured tissue. The same *RUNX1* variant is confirmed in her mother. The patient is ultimately diagnosed with MDS with germline predisposition due to germline *RUNX1* and begins a work up for HSCT. The family history of thrombocytopenia, bleeding phenotype, and family history of leukemia are suspicious for a hereditary platelet disorder.

Difficulties in interpretation of genetic testing

A systematic evidence-based curation of variants and the incorporation of practical guides, often gene specific, for interpretation of genetic reports has been increasingly used in practice. The type of sample, timepoint of evaluation, and sequencing platform and depth are considered. Different methods with various sensitivities can detect types of genetic alterations: structural variations, small insertions and deletions (indels), single nucleotide variants, large copy-number variations (duplications and deletions), translocations, inversions, and aneuploidy. Large copy-number variations and small alterations in genes or intronic regions may not be covered by a targeted next generation sequencing panel.[6] Whole exome/genome sequencing as well as deletion/duplication analysis may identify uncharacterized genes linked to IBMFS.

Identification of variants of unknown significance (VUS) is a common challenge for interpretation of results, and negative reports may lead clinicians to often mistakenly rule out an IBMFS. Interpretation of a VUS should incorporate the patient's clinical phenotype, family history, frequency in the normal population, and prior reports in the literature of the variant's pathogenicity.[32] Segregation studies (assessment of affected and unaffected family members for the variant) may be useful. In cases with a suspicious family history and either negative germline testing or a VUS in a suspicious IBMFS gene, referral to specialist is recommended to guide further assessment.

Importance of detecting IBMFS for therapeutics

With high index of suspicion, early diagnosis of an IBMFS may improve outcomes. HSCT offers a cure for all marrow failure syndromes, inherited and acquired; however, IST is also standard for immune mediated marrow failure in adults and in older patients who lack a matched sibling donor.[3] Patients with IBMFSs do not respond to IST, leading to increased cytopenia-related complications, delay in appropriate care, and, potentially, suboptimal therapy if misdiagnosed. A recent study reported worse survival after HSCT in patients with unrecognized IBMFS compared to those with immune AA, most commonly due to organ failure.[14] When broken down by IBMFS subtype, patients with DNA damage response disorders (including FA) and TBD had significantly poorer overall survival while those with ribosome biology disorders (DBA and SDS) and hematopoiesis disorders (familial platelet disorders [RUNX1, MPL, ETV6, ANKRD26], MECOM, GATA2 deficiency and DADA2) had similar overall survival to those with immune AA.

HSCT is undertaken as a potentially curative option for the hematologic manifestations of a wide range of IBMFSs,[3,33] all with disease-specific HSCT considerations and outcomes. Differing genetic defects and associated clinical phenotypes make a uniform HSCT approach problematic. IBMFSs predispose patients to particular post-HSCT complications depending on underlying disease pathophysiology, including specific organ damage, increased graft-versus-host or graft-failure risk, or development of secondary malignancy. Choice of conditioning regimen is known to play an important role in improving outcomes in DNA damage response disorders and in TBD. In FA patients undergoing HSCT, secondary malignancies and endocrine complications predominate, which have been mitigated but not eliminated using radiation-free reduced-intensity conditioning.[35] TBD patients often have disease involving major organs, particularly the lung and liver, and vasculature, which may worsen with HSCT, resulting in increased morbidity and mortality; studies are ongoing to assess alkylator and radiation-free conditioning regimens (NCT01659606).[35,36] Therefore, at minimum, specialized testing for FA and TBD should be performed before HSCT in all BMF patients, even if a full genetic panel is not possible.

In cases in which HSCT is urgently indicated, the risks and benefits of waiting for genetic testing should be weighed against the likelihood of an IBMFS in an individual patient. Genetic

screening of familial donors in known IBMFS is also important and should occur prior to transplant, even in absence of clinical manifestations, to prevent unknowing use of an affected graft, although screening should also be balanced against the urgency of proceeding to HSCT.

Conclusions

Ideally, all new patients presenting with new onset BMF should undergo germline genetic screening regardless of age or clinical phenotype. Diagnostic work up of IBMFS can be challenging; in nonspecialist centers or poor-resource settings, specialized testing such as chromosome breakage studies, TL, or genetic testing may not be easily available. Interpretation of genetic results can be tricky and may require specialist input. Missing an IBMFS has important clinical consequences: such patients are at increased risk of other organ involvement and malignancy, and these patients require disease-specific monitoring. Identification of an IBMFS significantly impacts therapy; IST is typically ineffective, and HSCT may require modification from standard regimens or other special considerations.

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Inherited Telomere Biology Disorders: Pathophysiology, Clinical Presentation, Diagnostics, and Treatment

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Telomere biology disorders · Telomeropathies · Telomeres · Dyskeratosis congenita

Abstract
Background: Telomeres are the end-capping structures of all eukaryotic chromosomes thereby protecting the genome from damage and degradation. During the aging process, telomeres shorten continuously with each cell division until critically short telomeres prevent further proliferation whereby cells undergo terminal differentiation, senescence, or apoptosis. Premature aging due to critically short telomere length (TL) can also result from pathogenic germline variants in the telomerase complex or related genes that typically counteract replicative telomere shortening in germline and certain somatic cell populations, e.g., hematopoetic stem cells. Inherited diseases that result in altered telomere maintenance are summarized under the term telomere biology disorder (TBD). ***Summary:*** Since TL both reflects but more importantly restricts the replicative capacity of various human tissues, a sufficient telomere reserve is particularly important in cells with high proliferative activity (e.g., hematopoiesis, immune cells, intestinal cells, liver, lung, and skin). Consequently, altered telomere maintenance as observed in TBDs typically results in premature replicative cellular exhaustion in the respective organ systems eventually leading to life-threatening complications such as bone marrow failure (BMF), pulmonary fibrosis, and liver cirrhosis. ***Key Messages:*** The recognition of a potential congenital origin in approximately 10% of adult patients with clinical BMF is of utmost importance for the proper diagnosis, appropriate patient and family counseling, to prevent the use of inefficient treatment and to avoid therapy-related toxicities including appropriate donor selection when patients have to undergo stem cell transplantation from related donors. This review summarizes the current state of knowledge about TBDs with particular focus on the clinical manifestation patterns in children (termed early onset TBD) compared to adults (late-onset TBD) including typical treatment- and disease course-related complications as well as their prognosis and adequate therapy. Thereby, it aims to raise awareness for a disease group that is currently still highly underdiagnosed particularly when it first manifests itself in adulthood.

Introduction

The end structure of all chromosomes was identified in 1938 by Hermann Mueller as "telomeres" in flies and a little later by Barbara McClintock in corn [1, 2]. McClintock also recognized the importance of proper telomere maintenance

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for chromosomal stability by demonstrating that telomeres are required to prevent (otherwise free) chromosome ends from fusion with each other [1]. It took almost four decades until the underlying tandem repeat sequence of telomeres was first described by Elizabeth Blackburn in 1978 [3]. This noncoding, three-dimensional telomeric loop structure organizes and protects genetic information encoded in subtelomeric DNA [1, 4]. In the course of this review, we will (1) introduce the structure of the telomere complex including telomere-associated proteins as well as telomerase, (2) describe diseases that are thought to result from impaired telomere maintenance, (3) focus on the differential symptomology associated with early onset (classical "dyskeratosis congenita," DKC) as well as late-onset (so-called cryptic) telomere biology disorders (TBDs), and finally, (4) explain existing diagnostic and therapeutic options for patients with TBD.

Structure of Telomeres and Telomerase

The typical $(TTAGGG)^n$ telomere repeat sequences at the 192 ends of the 48 chromosomes of a human cell are organized by a sophisticated interplay between different proteins and nucleic acids that build various complexes essential for proper telomere maintenance and protection [4]. The most important complexes are the shelterin complex, telomerase and associated modifiers, the CST, cytoplasmic iron-sulfur assembly, and the Apollo complexes [4–8].

The shelterin complex contributes essentially to the formation of the T-loop end structure of the chromosomes and is comprised of proteins such as telomeric repeat binding factor 1 (TERF1), TERF2, TERF1 interacting nuclear factor 2 (TINF2) (=TIN2), repressor/activator protein 1 (RAP1), adrenocortical dysplasia protein homolog (TPP1), and protection of telomeres 1 (POT1) [6, 9]. The complex functionally constitutes a protective cap around the telomeres thereby protecting them from degradation and from being (mis)recognized as a double-strand break by the DNA repair machinery (shown in Fig. 1) [6, 9].

The telomerase complex consists of the proteins telomerase reverse transcriptase (TERT) with the corresponding telomerase RNA component (TERC), the protein dyskerin (DKC1), the H/ACA ribonucleoprotein complex subunit 2 (NHP2), the nucleolar protein 10 (NOP10), GAR1 ribonucleoprotein (GAR1), and the WD repeat containing antisense to TP53 (TCAB1) [4, 5]. The complex is of particular importance in germline and certain somatic (stem) cell populations and promotes the active lengthening or net stabilization of telomeres, e.g., in highly proliferative organs (shown in Fig. 1) [4, 5].

The CST complex includes the CST telomere replication complex component 1 (CTC1), the TEN1 subunit of CST complex (TEN1) and the STN1 subunit of CST complex (STN1) and is essential for telomere maintenance especially under conditions of replicative stress [6, 10]. There are also other complexes described as the cytoplasmic iron-sulfur assembly complex and the Apollo complex (contains the protein DNA cross-link repair 1B, DCLRE1B, = Apollo) [7, 11, 12]. Both complexes play a fundamental role in DNA repair and protection of telomeric DNA [7, 11, 12].

In general, many of the proteins involved in telomere regulation are important for DNA integrity, intracellular signaling cascades, and ribosomal function [8, 13, 14]. These proteins protect the telomeric region and regulate its tertiary and quaternary structure thereby avoiding genetic instability leading to end-to-end fusions, unbalanced translocations, and eventually loss of chromosomal DNA [8].

Telomeres and Telomerase Activity

In most somatic cells, telomeres shorten with each cell division due to the so-called "end replication problem" [1]. This physiological mechanism leads to age-related telomere length (TL) shortening thereby limiting the lifespan of cellular organisms [1, 15, 16]. Cells that reach critically short TL undergo replicative senescence and/or apoptosis [17]. Non-replication dependent reduction of TL can also occur due to oxidative damage [18, 19] or increased activity of the telomeric zinc finger-associated protein (TZAP) that can actively trim telomeres [20]. TL shortening can both act as an efficient regulator of cell proliferation as well as a tumor preventive mechanism by limiting the number of cell divisions a somatic cell can undergo under normal circumstances, a condition first described in 1961 as the so-called Hayflick limit. For most somatic cell types, the Hayflick limit is assumed to be reached after around 50–80 mitoses in somatic cells [21]. With increasingly critical shortness, telomeres can destabilize DNA leading to increased DNA degradation, increased fusion between different chromosomes, eventually resulting in dicentric chromosomes, chromosomal breakage, or aneuploidy [8].

The inverse correlation between an individual cell's TL and its lifespan was suggested to act in the sense of a biological clock [1, 17]. Nevertheless, some cellular compartments are capable to counteract replicative TL shortening via various mechanisms. In 1985, the telomerase complex that enables the elongation of the telomere repeat sequence was first discovered [22]. Telomerase is primarily recruited to the telomeric region by interacting with proteins of the shelterin complex [4, 8, 9]. Embryonic stem cells, sperm cells, but also some adult cell populations (e.g., hematopoietic stem cells [HSCs], epidermal cells, and activated (B-)lymphocytes) are able to (re)express and actively use the function of this protein [8]. Nevertheless, in steady-state, most somatic cells do not express telomerase which means that telomeres require special protective mechanisms [4]. Apart from telomerase activity, other

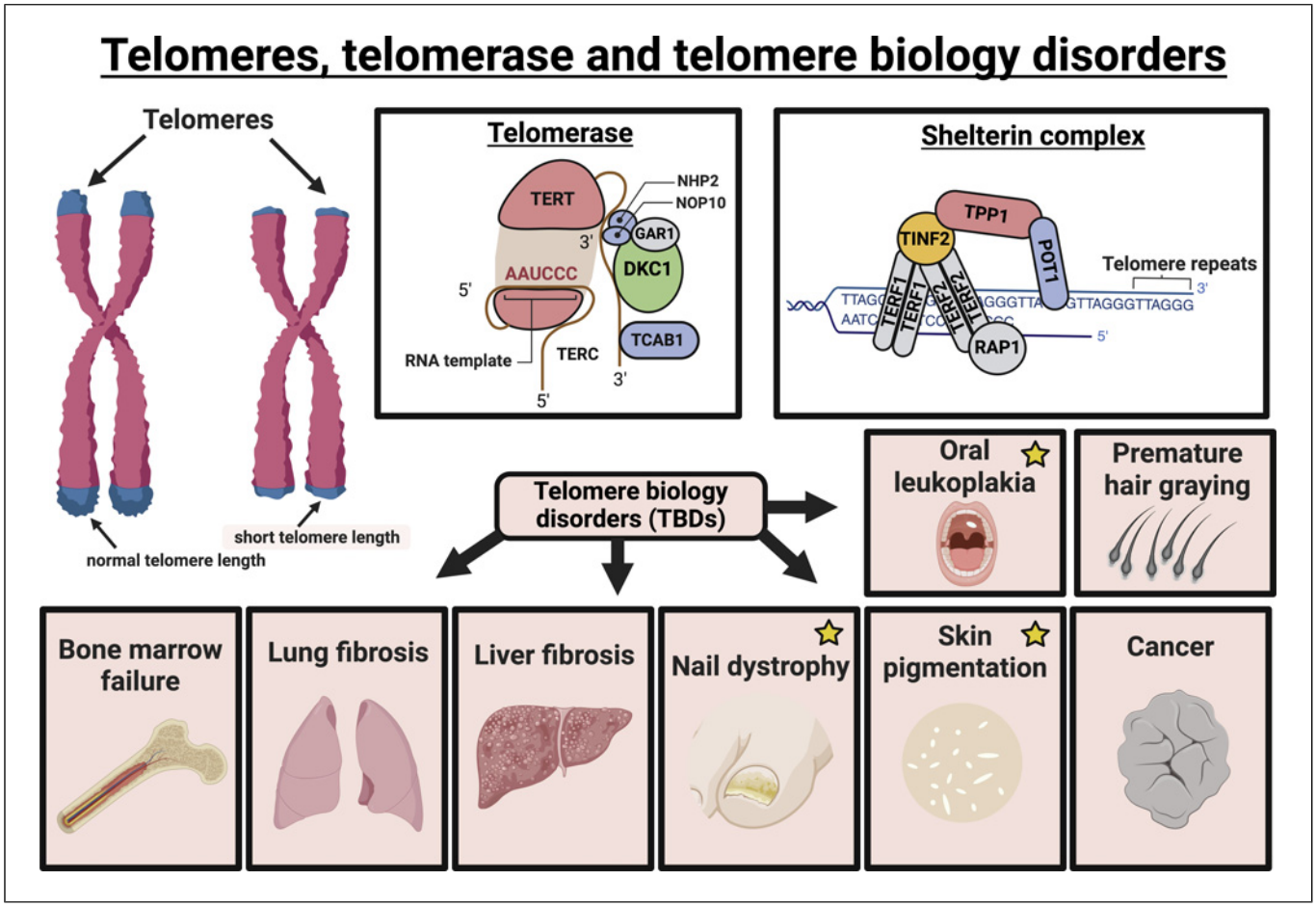

Fig. 1. Telomeres, telomerase, and TBDs. The figure shows parts of the telomerase and shelterin complex and their interaction with the telomere repeat region. The components are shown in different colors depending on the described disease-causing heritability. Red indicates components that are inherited in an autosomal dominant (and/or autosomal recessive) manner. Blue indicates components that are mostly inherited in an autosomal recessive manner. Green indicates the x-linked inheritance of *DKC1*, orange shows *TINF2* (often de novo), and gray indicates components for which the current literature is sparse or not present. Potential symptoms of telomere biology disorders (TBDs) are indicated in the bottom part of the figure. The classical triad of dyskeratosis congenita (DKC) is marked with a yellow star. Adapted from "Telomeres and Telomerase," by BioRender.com (2024). Retrieved from "https://app.BioRender.com/biorender-templates".

mechanisms known to be able to sustain and increase TL are summarized under the term "alternative lengthening of telomeres" (ALT) [23].

Brief Introduction into TBDs

Historically, DKC was first described on the basis of skin manifestations such as leukoplakia and nail dystrophy by the dermatologist Zinsser [24], as well as Engman [25] and Cole [26]. One of the first systematic reviews of dyskeratosis congenita was published by Costello and Buncke [27] in 1956 and later reports expanded the spectrum of clinical symptoms related to dysfunction of other organ systems such as bone marrow failure (BMF) [28, 29]. Only in 1998, the connection between a defective telomerase component leading to prematurely shortened telomeres was discovered, thereby proving the causal link between a pathogenic variant in a single gene and the multisystem pathophysiology of DKC [30, 31]. In the following years, techniques to routinely measure TL in the peripheral blood of normal individuals and patients with HSC-associated disorders were developed [32–35] enabling the functional screening of patients for an underlying TBD as practiced today [36, 37].

Whereas accelerated telomere shortening had first been described in blood cells from patients with acquired aplastic anemia in 1998 [38], this was initially suspected to be a secondary phenomenon, i.e., to result from increased stem cell turnover of residual HSCs as a response to continuous autoimmune-mediated damage to the stem cell compartment [39]. Subsequently, in 2005 it was

Table 1. Genes that have been proven to cause telomere biology disorders (TBDs)

Gene	Protein	Function	Potential consequence of pathogenic variants	Estimated proportion of all TBDs, *n* (%)	Year reported (clinical condition)	Nature of inheritance
Adrenocortical dysplasia protein homolog (*ACD*) (=*TPP1*) [46, 47]	TPP1 (not (!) tripeptidyl peptidase 1)	• Part of the shelterin complex (Capping/ Bridging shelterin) • Bridging component • Recruits POT1 and telomerase • Binds to the POT1 protein, TINF2, and the ataxia telangiectasia and Rad3-related protein (ATR) • Regulates TL and supports stability • Regulates DNA repair	• Decreased recruitment of the telomerase	1.5	2014	Autosomal dominant or autosomal recessive
CST telomere replication complex component 1 (*CTC1*) [48, 49]	CTC1	• Part of the CST complex • Replication of the telomere repeats • Stimulation of DNA polymerase α-primase	• Instable telomeres • Impaired replication of the telomere repeats • Sometimes less prominent/no change in TL	3	2012	Autosomal recessive
DNA cross-link repair 1B (*DCLRE1B*) (=*SNM1B*) (=*Apollo*) [11, 50]	Apollo	• Interacts with TERF2 • Protects telomeres during and after replication • Function in DNA repair • Overhang processing	• Genetic instability • Often with normal TL (in some cases very short telomeres were only detected by using more sensitive methods)	<1	2010	Autosomal recessive
Dyskerin pseudouridine synthase 1 (*DKC1*) [30]	Dyskerin	• Part of the telomerase complex • Stabilization of TERC	• Reduced telomerase activity • Instability of TERC	25	1998	X-linked
MDM4 regulator of p53 (*MDM4*) [51]	MDM4	• Regulates and degrades TP53	• Increased TP53 activity • Short TL	<1	2020	Autosomal dominant
Nuclear assembly factor 1 ribonucleoprotein (*NAF1*) [52]	NAF1	• Regulation of telomerase • Enables protein binding and binding of TERC • Stabilization of TERC and H/ACA small ribonucleoproteins (chaperon function) • Important factor for the biosynthesis of ribosomes	• Reduced telomerase activity • Instability of TERC	<1	2016	Autosomal dominant

Table 1 (continued)

Gene	Protein	Function	Potential consequence of pathogenic variants	Estimated proportion of all TBDs, *n* (%)	Year reported (clinical condition)	Nature of inheritance
H/ACA ribonucleoprotein complex subunit 2 (*NHP2*) (=*NOLA2*)	NHP2	• Part of the telomerase/dyskerin complex • (Non-catalytic) component of H/ACA small nucleolar ribonucleoproteins • Biogenesis of telomerase • Stabilization of TERC • Essential for the biogenesis of ribosomes	• Reduced telomerase activity • Instability of TERC	<1	2008	Autosomal recessive
Nucleolar protein 10 (*NOP10) (=NOLA3)* [53, 54]	NOP10	• Part of the telomerase/dyskerin complex • Biogenesis of telomerase • Stabilization of TERC	• Reduced telomerase activity • Instability of TERC	<1	2007	Autosomal recessive
Nucleophosmin 1 (*NPM1*) [55]	NPM1	• Modifying rRNA (for example regulating ribosomal RNA 2′-O-methylations (2′-O-Me)) • Interacts with NHP2 and NOP10	• Impaired ribosome function by aberrant 2′-O-Me on rRNA residues	<1	2019	Autosomal dominant
Poly(A)-specific ribonuclease (*PARN*) [56–60]	PARN	• Processing of TERC • Stabilization of TERC	• Reduced telomerase activity • Instability of TERC • Sometimes only with subtle change in TL (close to the 10% percentile)	>1% (especially in patients with idiopathic PF)	2015	Autosomal dominant or autosomal recessive
Protection of telomeres 1 (*POT1*) [61, 62]	POT1	• Part of the shelterin complex • Binds to telomeres (3′ overhang) and interacts with the CST complex • Binds to TPP1 • Binds to ATR and inhibits ATR-mediated DNA damage response • Inhibits end-to-end fusion/protects the G-strand	• Impaired replication of the telomere repeats • Dysfunctional regulation of the telomerase • Defective binding to the telomere overhang	<1	2016	Autosomal recessive
Replication protein A1 (*RPA1*) [63]	RPA1	• Important for DNA replication and telomere maintenance • Functions in DNA repair • Binds single-stranded DNA and protect its structure	• Increased amount of unfolded telomeres • Increased binding affinity to single-stranded and telomeric DNA (gain of function)	<1	2022	Autosomal dominant

Table 1 (continued)

Gene	Protein	Function	Potential consequence of pathogenic variants	Estimated proportion of all TBDs, *n* (%)	Year reported (clinical condition)	Nature of inheritance
Regulator of telomere elongation helicase 1 (*RTEL1*) [64–68]	RTEL1	• Replication of the telomeres • Dissociation and stability of T-loops • Target of the cytosolic iron-sulfur protein assembly (CIA) complex • Prevents loss of telomeres	• Decreased/ impaired telomere replication • Decreased telomere stability	10	2013	Autosomal dominant or autosomal recessive
STN1 subunit of CST complex (*STN1*) [69]	STN1	• Part of the CST complex • Important for telomere replication	• Decreased/ impaired telomere replication	<1	2016	Autosomal recessive
Telomerase RNA component (*TERC*) [70]	TERC	• Part of the telomerase complex • Elongation of telomeres	• Reduced telomerase activity	10	2001	Autosomal dominant
Telomerase reverse transcriptase (*TERT*) [40, 41]	TERT	• Part of the telomerase complex • Recruitment of telomerase • Elongation of telomeres • Impacts the Wnt signaling pathway • Might promote pluripotency and mobilization of stem cells	• Reduced telomerase activity • Impaired telomerase recruitment	7	2005	Autosomal dominant or autosomal recessive
TERF1 interacting nuclear factor 2 (*TINF2*) (=*TIN2*) [71, 72]	TIN2	• Part of the shelterin complex (bridging shelterin) • Stabilizes the shelterin complex • Protects telomeres and regulates TL • Recruits and regulates telomerase • Binds to TPP1, TERF1, and TERF2. Stabilizes TERF1/ TERF2 interaction • Inhibits PARsylation of TERF1	• Impaired telomere maintenance	20	2008	Autosomal dominant (often as a de novo mutation)

Table 1 (continued)

Gene	Protein	Function	Potential consequence of pathogenic variants	Estimated proportion of all TBDs, *n* (%)	Year reported (clinical condition)	Nature of inheritance
WD repeat containing antisense to TP53 (*WRAP53*) (=*TCAB1*) [73, 74]	TCAB1	• Associated with the telomerase complex • Facilitates (physiological) protein-protein and protein-RNA interactions • Interacts with Dyskerin, TERT, and TERC • Recruits telomerase to the telomeres • Recruits proteins to the sides of Cajal bodies and sides of DNA damage • Transcript regulates levels of *TP53* RNA	• Impaired trafficking and recruitment of telomerase	<1	2011	Autosomal recessive
Zinc finger CCHC-type containing 8 (*ZCCHC8*) [75]	ZCCHC8	• TERC processing and maturation	• Decreased/ impaired telomerase function • Accumulation of extended TERC/ decreased maturation of TERC	<1	2019	Autosomal dominant
Unknown [17, 45]				≈20		

The table summarizes genes with the respective protein name and function that might harbor pathogenic variants that lead to impaired telomere maintenance and clinical manifestations. Potential consequences of pathogenic variants, the estimated proportion of pathogenic variants in this gene among all telomere biology disorders (TBDs), the year of the first clinical description and the suspected inheritance pattern are shown [43, 45, 76, 77].

shown that pathogenic germline variants in the telomerase reverse transcriptase (*TERT*) gene can be detected in adult patients with aplastic anemia thereby defining the so-called late-onset, adult TBDs [40, 41]. Due to the rapid development of sequencing techniques, a larger number of other genes with pathogenic variants were detected in juvenile and adult TBD patients in the following years, such as variants in the regulator of telomere elongation helicase 1 (*RTEL1*) or the poly(A)-specific ribonuclease (*PARN*) in patients with interstitial lung disease [42].

At the moment, there is sufficiently strong evidence that certain variants of the following genes might cause a clinically relevant TBD phenotype (see Table 1): adrenocortical dysplasia protein homolog (*ACD*) (=*TPP1*), CST telomere replication complex component 1 (*CTC1*), DNA cross-link repair 1B (*DCLRE1B; =SNM1B*; = *Apollo*), dyskerin pseudouridine synthase 1 (*DKC1*), MDM4 regulator of p53 (*MDM4*), nuclear assembly factor 1 ribonucleoprotein (*NAF1*), H/ACA ribonucleoprotein complex subunit 2 (*NHP2*) (=*NOLA2*), nucleolar protein 10 (*NOP10*) (=*NOLA3*), nucleophosmin 1 (*NPM1*), *PARN*, protection of telomeres 1 (*POT1*), replication protein A1 (*RPA1*), *RTEL1*, STN1 subunit of CST complex (*STN1*), telomerase RNA component (*TERC*), *TERT*, TERF1 interacting nuclear factor 2 (*TINF2*) (=*TIN2*), WD repeat containing antisense to TP53 (*WRAP53*) (=*TCAB1*), and zinc finger CCHC-type containing 8 (*ZCCHC8*) [17, 43–45].

While initially DKC was considered an appropriate term for all telomere-related diseases, it later became clear that on the one hand side, certain pediatric forms such as the Revesz syndrome, the Hoyeraal-Hreidarsson syndrome, or the Coats plus syndrome are characterized by a strikingly more severe phenotype and earlier disease onset clearly distinct from classical DKC [48, 64, 78]. On the other hand, adult-onset forms of TBDs sometimes only manifest themselves beyond 40 years of age, often lacking a skin phenotype and displaying distinct and highly variable organ system manifestations from classical DKC [36, 45].

Consequently, in parallel to the identification of a growing number of disease-causing genes, there was also a change in nomenclature [7, 44]. For instance, some patients with a Coats plus syndrome harbor a pathogenic variant in a gene important for telomere maintenance and show a TBD phenotype, but do not have shorter TL [43, 48, 61]. This led to the change in terminology away from the term telomeropathy or telomere disease to the currently used term TBD [17, 43].

The main clinical symptoms and diagnostic of TBDs are shown below. We proceed chronologically according to age of first disease manifestation. TBDs that will be discussed include early pediatric forms, and classical adolescence DKC, but the focus of this review is on different forms and manifestations of late-onset (previously called "cryptic") TBDs.

Diagnostics

In general, there are several techniques available to measure TL [33, 36, 37]. The current standard for clinical detection of shortened TL is the combination of a quantitatively telomere-binding FISH probe in combination with flow cytometry (flow-FISH) [32, 33]. This method was developed for the analysis of clinical specimen, i.e., to allow for high cell numbers (see telomere Q-FISH for comparison [79]) in high throughput and to be able to analyze at the same time different blood cell subpopulations involved in disease pathophysiology [34, 39, 80–82]. Interestingly, lymphocyte TL measured via flow-FISH is sensitive and highly specific and was empirically shown to be superior for diagnosis of inherited TBD over granulocyte TL probably because the latter population is more directly involved in the disease course of AA itself (see above) while lymphocyte TL more specifically reflects the hereditary "telomere genotype" [15, 43, 83]. In analogy to percentile-based growth curves that are used to track physical development in children, TBDs can be identified on the basis of differences in TL from age-adapted percentiles [36, 83]. Thereby, TL in the peripheral blood shows a negative correlation with age and it follows an at least bi-phasic kinetic [33]. The most frequently used diagnostic threshold for TBDs is currently a TL value below the 1% percentile of normal individuals measured by flow-FISH in lymphocytes. Due to the substantially larger "diagnostic window" in children, conventional methods for TL measurement like PCR with lower sensitivity are still being used in pediatric patients [37]. In borderline cases or in scenarios with a high degree of clinical suspicion for an underlying TBD (e.g., due to family history, clinical phenotype or other aspects), detection of TL in lymphocyte subsets might also help increase sensitivity and specificity [15, 43, 83]. Although overall lymphocyte TL is the most established marker to diagnose TBDs, more recent publications indicate that the simultaneous detection of lymphocyte and granulocyte TL should be preferred [43, 84]. In case of short TL below the 1% percentile, subsequent genetic testing is recommended to test for disease causing germline variants in so-far known TBD-related genes [36, 37, 83]. However, based on current knowledge a pathogenic variant cannot be detected in all clinical TBD cases primarily suggesting that not all gene alterations involved in telomere maintenance are yet known and secondly, highlighting the diagnostic value of TL measurements as the yet only functional test available for primary screening of TBDs [17, 45].

Early Severe Pediatric Forms of TBDs

Early severe pediatric forms of TBDs with often characteristic underlying genetic variants are the Revesz syndrome, the Hoyeraal-Hreidarsson syndrome, and the Coats plus syndrome [45, 64, 69, 78]. Revesz and Hoyeraal-Hreidarsson syndrome are characterized by a severe disease course, very short telomeres (considerably below the 1% percentile), and a disease onset in the early childhood [45, 65, 78]. In comparison, the Coats plus syndrome does not necessarily have to be accompanied by short TL (in this rare case of a TBD leading to a limited diagnostic value of TL screening) but otherwise shows classical signs of TBD [61, 69, 76]. All three forms overlap clinically with classical DKC and are associated with significantly reduced survival [45, 85].

Revesz syndrome typically features bilateral exudative retinopathy, intracranial calcification, cerebellar hypoplasia thereby causing ataxia, (intrauterine) growth restriction, general developmental delay, and fine hairs. Affected patients have a high risk for BMF. It is often caused by variants in the *TINF2* gene [78, 77].

Hoyeraal-Hreidarsson syndrome, on the other hand, shares some signs of Revesz syndrome such as cerebellar hypoplasia and intrauterine growth restriction, but can also present with progressive immunodeficiency, microcephaly, and moderate to severe mental retardation. Also, progressive pancytopenia (early onset) is frequently observed. In many cases, pathogenic germline variants in the *DKC1* gene are detected as the underlying cause of the syndrome [11, 64, 65, 86, 73].

Characteristic findings of Coats plus syndrome are exudative retinopathy, cerebroretinal microangiopathy with concomitant brain calcifications, brain cysts, loss of the white matter (leukodystrophy), gastrointestinal bleedings, and bone abnormalities (e.g., osteopenia). Abnormalities of the skeletal system are often accompanied by impaired bone healing. The leukodystrophy can lead to a progressive cognitive decline [48]. Also, anemia and thrombocytopenia (with or without BMF) are common symptoms. Variants in the genes *CTC1*, *STN1* and *POT1* are frequently found in patients with Coat plus syndrome [48, 61, 69].

Table 2. (Inherited) telomere biology disorders (TBDs) together with accompanying symptoms

Disease	Genes that might harbor pathogenic variants	Clinical manifestations
(Classical) Dyskeratosis congenita (DKC) (=Zinsser-Engman-Cole syndrome) [30, 49, 53, 55, 56, 66, 67, 70, 71, 74] (Manifestation: childhood or (early) adolescence)	*DKC1*, *TERT*, *TERC*, *TINF2*, *RTEL1*, *NOP10*, *NHP2*, *CTC1*, *ACD* (=*TPP1*), *PARN*, *RPA1*, *WRAP53* (=*TCBA1*), *DCLRE1B* (*Apollo*), *NPM1*	• Dystrophic nails* • Skin pigmentations* (e.g., hyper/hypopigmented areas, often with a reticular pattern) • Oral leukoplakia*(potential precancerous) • BMF • Gray hairs and alopecia • Palmoplantar hyperkeratosis and hyperhidrosis • Short stature and osteoporosis • Avascular necrosis (e.g., hip or shoulder) • Lung fibrosis and emphysema • (Pulmonary) arteriovenous malformations • Liver fibrosis, liver cirrhosis, and various other liver diseases • (Gastro)intestinal diseases • Neurological symptoms • Visual disorders • Hypogonadism • Increased watering of the eyes due to lacrimal duct atresia • Dental caries • Esophageal strictures • Stenosis of the urethra • Increased cancer and leukemia risk
Hoyeraal-Hreidarsson syndrome [11, 64, 65, 73, 86] (severe phenotypic variant of DKC) (Manifestation: early childhood)	*DKC1*, *TERT* (autosomal recessive), *TINF2*, *PARN*, *WRAP53* (=*TCBA1*), *ACD* (=*TPP1*), *RTEL1*, *NHP2*, *DCLRE1B* (*Apollo*)	• Possible overlap with most characteristics of DKC • Cerebellar hypoplasia and microcephaly • Immunodeficiency (progressive) • (Intrauterine) growth restriction • Mental retardation • BMF (early onset and progressive) • Mucocutaneous lesions (e.g., hyper/hypopigmentation, nail dystrophy, and premalignant leukoplakia [oral + gastrointestinal]) • Higher risk of cancer and PF
Revesz syndrome [71] (severe phenotypic variant of DKC) (Manifestation: early childhood)	*TINF2*	• Bilateral exudative retinopathy • Skin hyper/hypopigmentation • Nail dystrophy • Oral leukoplakia • Fine and sparse hairs • Intracranial calcifications • Balance problems/ataxia • (Intrauterine) growth restriction • High risk of BMF • Risk of liver and lung fibrosis • High risk of cancer (e.g., leukemia)
Coats plus syndrome [48, 61, 69] (Manifestation: (early) childhood or (early) adolescence)	*CTC1*, *STN1*, *POT1*	• Dilatation of blood vessels in the retina (exudative retinopathy/cerebroretinal microangiopathy) • (Brain) calcifications • (Brain) cysts • Gastrointestinal bleedings • Bone abnormalities (e.g., osteopenia) • Impaired bone healing • Leukodystrophy/leukoencephalopathy

Table 2 (continued)

Disease	Genes that might harbor pathogenic variants	Clinical manifestations
		• Ataxia, seizures, spasticity, and cognitive decline • Anemia and thrombocytopenia • Changed skin pigmentations (café-au-lait spots) • Malformations of the nails • Prematurely gray hairs • Portal hypertension • Often without short TL
"**Cryptic**" **DKCs/TBDs** (**Manifestation**: **adolescence or (late) adulthood**)		
BMF/aplastic anemia [41, 46, 50, 51, 55, 58, 63, 72]	*DKC1*, *TERT*, *TERC*, *NOP10*, *NHP2*, *RTEL1*, *ACD* (=*TPP1*), *DCLRE1B* (*Apollo*), *TINF2*, *NPM1*, *MDM4*, *RPA1*	• Pancytopenia (anemia, thrombocytopenia, neutropenia) • Hypocellular BM • Increased bleeding tendency • Increased susceptibility to infections
Idiopathic PF, interstitial lung diseases, emphysema [42, 47, 52, 54, 59, 62, 63, 75, 86, 89, 90]	*DKC1*, *TERT*, *TERC*, *NOP10*, *NHP2*, *ACD* (=*TPP1*), *RTEL1*, *PARN*, *RPA1*, *NAF1*, *TINF2*, *ZCCHC8*, *POT1*	• (Progressive) shortness of breath • Shallow and fast breathing • (Dry) cough – increased fatigue • (Unintended) weight loss • Clubbing of the finger tips (and toes)
Idiopathic liver cirrhosis/nodular regenerative hyperplasia/liver disease [68, 91, 92]	*TERT*, *TERC*, *RTEL1*, *TINF2*, *DKC1*, *NHP2*, *NOP10*, *NAF1*, *WRAP53* (=*TCBA1*)	• Increased fatigue • Increased bleeding tendency/bruising • Nausea and decreased appetite • (Unintended) weight loss • Edema • Jaundice of the skin and eyes • Itchy skin
Head and neck cancer (HNSCC), squamous cell carcinoma (e.g., anogenital cancer, skin, or esophagus) and other cancers [51, 93–100]	*TERT* (but also *TERT* promoter**), *TERC*, *CTC1*, *MDM4*, *NAF1*, *POT1****	• Cancer at a young age • Absence of risk factors • Family history of cancer or symptoms typical of TBD • Increased therapy-related toxicity
Others: MDS/hematopoietic diseases (e.g., *TERT*, *TERC*, *RPA1*, *NAF1*) [52, 63, 101, 102], immunodeficiencies/(auto)inflammation (e.g., *TERT*, *TERC*, *DKC1*, *RTEL1*) [84, 103, 104], and inflammatory bowel diseases/gastrointestinal manifestations [105, 106]		

Shown are different manifestations of telomere biology disorders (TBDs) and clinical symptoms which fall within the spectrum of a TBD. The table demonstrates potential clinical manifestations as well as genes that were predominantly described in the literature for different variants of TBD. The table shows diseases that often manifest in the early childhood (Revesz and Hoyeraal-Hreidarsson syndrome), childhood and early adolescence (DKC and Coats plus syndrome) and during late adulthood (cryptic TBDs). Important clinical features are printed in bold. *Classical DKC triad. **Associated with longer TL. ***Might also cause longer telomeres.

Adolescence or Young Adults: Classical DKC

The paradigmatic TBD is DKC or Zinsser-Engman-Cole syndrome. Classical DKC can be caused by different genetic variants in genes affecting telomerase function, telomere maintenance, and telomere interaction. Classical DKC is often diagnosed during childhood or early adolescence. In classical DKC pathogenic variants are found in genes such as *DKC1*, *TERT*, *TERC*, *TINF2*, *RTEL1*, *NOP10*, *NHP2*, *CTC1*, *ACD* (=*TPP1*), *PARN* and *WRAP53* (=*TCBA1*) [30, 49, 53, 56, 66, 67, 70, 71, 74]. Most variants lead to reduced telomerase activity and increased telomere erosion [15, 83]. More recent reports indicate that variants in *RPA1*, *STN1*, *DCLRE1B* (*Apollo*), and *NPM1* can also cause (classical) DKC [50, 55, 63].

Classical DKC shows severe manifestations early in life and the typical mucocutaneous triad with leukoplakia, reticulated skin pigmentations and nail dystrophy (classical DKC, shown in Fig. 1) [44, 83, 85]. Apart from the mucocutaneous triad, the characteristic clinical disease course includes an increased risk for BMF and cancer development [43, 44]. Additional symptoms reflecting the multi-systemic nature of TBDs include early hair

graying and alopecia, palmoplantar hyperkeratosis and hyperhidrosis, short stature and osteoporosis, avascular bone necrosis, lung fibrosis and lung emphysema, liver disease including liver fibrosis and liver cirrhosis, arteriovenous malformations, hypogonadism, different gastrointestinal diseases, neurological symptoms, visual disorders, atresia of the lacrimal duct, severe dental caries, esophageal strictures, and stenosis of the urethra (shown in Fig. 1; Table 2) [15, 44, 72, 83, 85, 87, 88].

About 80% of all cases show signs of BMF leading to significantly increased mortality. Especially patients with hematopoietic insufficiency experience a substantially increased risk of developing secondary hematopoietic malignancies such as myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) [85]. The cumulative risk of developing a secondary malignancy by the age of 50 years is about 40–50% [85]. As a result, median overall survival for patients with DKC was shown to be reduced to about 42 years in two independent cohorts [85].

Late-Onset Adult TBDs or Cryptic TBDs

In addition to TBDs first manifesting themselves in childhood or adolescence, there is nowadays increasing awareness about so-called late-onset TBDs also referred to as cryptic TBDs or adult-onset TBDs [36, 91]. Despite of this increasing perception as an important clinical subgroup, e.g., of patients with aplastic anemia (and/or liver cirrhosis and/or lung fibrosis), adult-onset hereditary syndromes in general and TBDs in particular are still severely underdiagnosed presumably because non-pediatricians are simply not as sensitized for a hereditary disease manifesting itself for the first time in adulthood [45]. What also contributes to this underdiagnosis is the fact that clinical symptoms are often subtle and highly variable between individuals as well as heterogeneously distributed over the organ systems involved [36, 43, 45]. The age of first manifestation depends very much on the respective genetic alteration and its respective impact on telomere biology (with or without the influence of additional external factors) [43, 76, 77]. Furthermore, TBDs are characterized by disease anticipation, i.e., age of critical telomere shortening and as a consequence, clinical onset of symptoms tends to decrease with consecutive generations [43, 44]. First manifestations often take place in middle-aged adulthood [36, 91]. The spectrum of cryptic TBDs might also include individuals who remain oligo- to even asymptomatic for most of their lives. Symptoms are often unspecific, making it very challenging to accurately diagnose a TBD [36, 91]. Cryptic TBD patients can present with only a single manifestation typically affecting lung, bone marrow, liver, or other TBD-associated symptoms [36, 45, 57, 91]. The four quantitatively most important clinical manifestations are given below.

BMF or MDS as a Manifestation of a Cryptic TBD

Due to the high cell turnover of the hematopoietic system with daily blood cell productions exceeding 10^{12} cells in steady-state [107], blood and bone marrow cells are particularly susceptible toward defects in telomere maintenance genes [17, 45]. It is assumed that critically short telomeres functionally impact the individual bone marrow replicative capacity until eventually, the bone marrow function exhausts due to HSCs depletion [17]. TL can therefore be used as a (bio-)marker to approximate the degree of replicative exhaustion of the HSC compartment (very short TL indicates long-lasting and intensive compensatory proliferation of the residual HSC compartment) and/or severity of the underlying genetic defect. Pathogenic variants in *DKC1*, *TERT*, *TERC*, *NOP10*, *NHP2*, RTEL1, *ACD* (=*TPP1*), *DCLRE1B* (*Apollo*), *TINF2*, *RTEL1*, *NPM1*, *MDM4*, and *RPA1* have been described in patients showing signs of BMF or aplastic anemia [41, 46, 50, 51, 55, 58, 63, 72]. Furthermore, pathogenic variants in *TERT*, *TERC*, *RPA1*, and *NAF1* have been recently associated with MDS-typical changes in the bone marrow [52, 63, 101, 102]. Ineffective hematopoiesis and an increased risk of malignant transformation can therefore be considered a consequence of telomere-mediated genetic instability [41, 108].

Due to the fact that many TBD-associated diseases may lead to cytopenia and BMF, exclusion of a TBD is part of the comprehensive diagnostic assessment in every newly diagnosed case of BMF [43, 109]. In particular, the clarification of the family history, examination of the patient for TBD-typical stigmata (see Fig. 1) and TL measurement are indispensable (see Fig. 2) [43]. The assessment of TL represents a relatively cheap and essential (pre)screening tool which should be followed (in case of significantly shortened TL) by a further work-up with a next-generation sequencing approach that focuses on pathogenic variants in genes that are involved in telomere maintenance (Fig. 2).

Correct identification of TBD patients is of utmost importance as patients with TBDs typically show no relevant clinical response to standard treatment for aplastic anemia like antithymocyte globulin, cyclosporine A, or eltrombopag [110]. In addition, TBDs are characterized by a significantly poorer outcome after allogeneic stem cell transplantation (allo-SCT) [111, 112]. Especially, the choice of a potentially affected family donor with the same genetic aberration should be avoided due to an increased risk of complications after allo-SCT (graft-failure, accelerated telomere shortening, and graft-versus-host disease) [112, 113]. A very careful donor selection, an adapted therapy

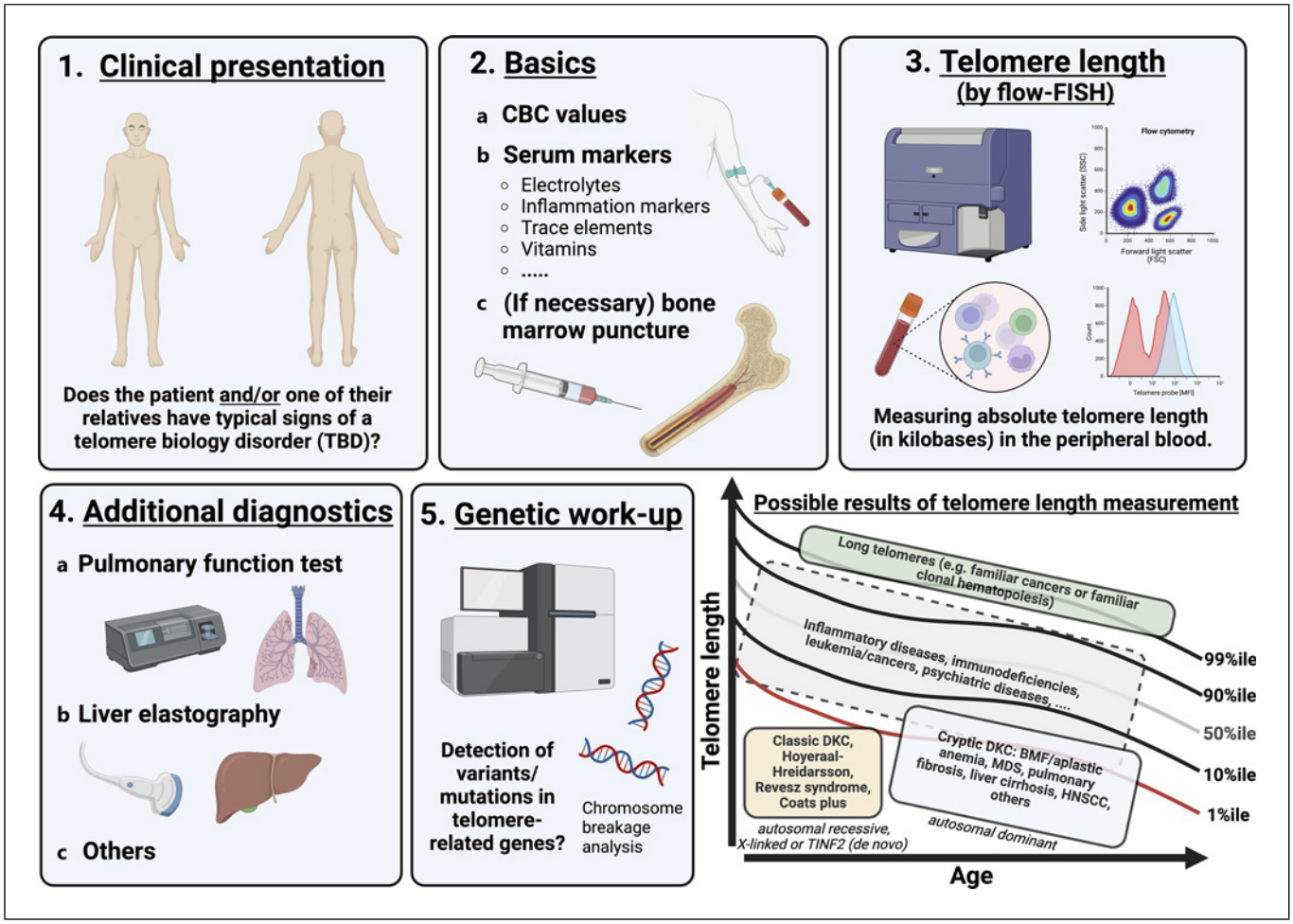

Fig. 2. Diagnostic procedures to confirm a suspected telomere biology disorder (TBD). The step-by-step diagnostic algorithm for proper identification of a telomere biology disorders (TBD) is shown as follows: (1) clinical presentation + basic diagnostics: the clinical appearance is often the first and sometimes the only indicator for a present TBD; (2) basic diagnostics such as a routine laboratory work-up mainly serve to exclude other causes that explain the patient's phenotype; (3) flow-FISH diagnostic: to measure the absolute telomere length (TL) in peripheral blood lymphocytes and granulocytes; (4) advanced diagnostics: variable and highly dependent on the clinical presentation of the patient. Complications such as PF or liver fibrosis/liver cirrhosis can be diagnosed and quantified; (5) genetic work-up: NGS diagnostic, whole exome or whole-genome sequencing to detect a genetic cause of TBD. A chromosome breakage analysis should be performed to rule out other differential diagnoses that might lead to chromosomal instability and (secondary) telomere shortening (especially Fanconi anemia). Graph: possible results of telomere measurements and typical results for various diseases. Created with "BioRender.com."

regime and (if possible) avoidance of radiotherapy due to the increased risk of secondary malignancy as well as the development of fatal interstitial lung diseases are preferable for TBD patients [111, 113, 114]. In addition, special check-ups as well as follow-ups after treatment are recommended in order to recognize common secondary diseases (e.g., secondary malignancies) [44]. Moreover, the introduction of affected patients to a specialized center for a second opinion, study inclusion, and inclusion in central registries should be standard of care. There are a few registries worldwide that systematically collect patient data, document the disease course of TBD patients and systematically archive patient material (see Acknowledgment).

Pulmonary Fibrosis as a Manifestation of a Cryptic TBD

Pulmonary fibrosis (PF) is a disease characterized by progressive scar formation and thickening of the tissue around the alveoli in the lung thereby impairing the vital process of gas exchange [115]. The disease can be caused by external factors like air pollution, or previous medical treatments as radiation therapy or drugs with pulmonary toxicity (e.g., bleomycin, busulfan, and several other medications) [111, 116]. Some cases remain etiologically unclear and are therefore described as idiopathic [59, 115]. Usually, PF occurs in middle-aged and older

individuals so that young patients with a family history raise a strong suspicion that the disease might be caused by pathogenic germline variants [115]. However, in analogy to inherited BMF, there are also reports indicating that even older patients suffering from PF might harbor pathogenic variants in TBD-associated genes that are inherited in an autosomal dominant manner [89]. Several studies showed that idiopathic PF can be caused by pathogenic variants in TBD-associated genes such as *DKC1*, *TERT*, *TERC*, *NOP10*, *NHP2*, *ACD* (=*TPP1*), *RTEL1*, *PARN*, *RPA1*, *NAF1*, *TINF2*, *ZCCHC8*, *POT1*, *RPA1* [42, 47, 52, 54, 59, 62, 63, 75, 86]. It has also been shown that genetically impaired telomere maintenance is associated with several other interstitial lung diseases [59]. Symptom severity and type of manifestation differed greatly between patients with TBD-associated PF, but for all patients a continuously progressive disease course was observed [59].

Cryptic Liver Cirrhosis as a Manifestation of a Cryptic TBD

Liver diseases are an increasing problem in the Western world with a significantly rising number of cases with non-alcoholic fatty liver disease and nonalcoholic steatohepatitis [117, 118]. In around 5% of all cases with liver cirrhosis, the underlying etiology cannot be identified, leading to the fact that these cases being classified as cryptogenic cirrhosis [118]. In TBD patients, a hepatic involvement is common (in some cohorts 40% of all TBD patients [119]), difficult to specifically detect [91] and contributes to mortality to a highly variable degree [91, 119]. Interestingly, the presence of a TBD-causing pathogenic variant was a risk factor for the progression from liver fibrosis to liver cirrhosis [120]. Studies in patients with liver cirrhosis who undergo liver transplantation have shown that cryptic TBD can be the cause of isolated liver fibrosis/liver cirrhosis [68, 92, 120]. It was demonstrated that aberrations in telomere maintenance genes such as *TERT*, *TERC*, *RTEL1*, *TINF2*, *DKC1*, *NHP2*, *NOP10*, *NAF1*, and *WRAP53* (=*TCBA1*) were able to cause severe hepatic manifestations [68, 91, 92, 121].

Solid Cancers as a Manifestation of a Cryptic TBD

Even though the risk for many cancers including head and neck squamous cell carcinoma (HNSCC) are associated with somatic *TERT* promoter and *POT1* variants leading to longer TL [93, 94, 122], there are also indications that pathogenic germline variants in TBD-associated genes promote cancer development [17, 45]. Many tumor types that occur in the context of TBDs also show a higher incidence in the presence of (very) short telomeres in the general population [85, 108, 123–125]. For example, variants in *TERT*, *TERC*, *CTC1*, and *MDM4* lead to a higher risk for HNSCC and other squamous cell carcinomas (SCCs) (unpublished data) [51, 95, 126].

Patients with DKC have hundreds-fold greater risk of developing HNSCCs, SCCs of the skin and the anogenital region [85, 108]. 30% of the macroscopic oral leukoplakia in TBD patients might transform to HNSCC [108, 127]. Also, a higher risk of stomach cancer, lung, esophagus, and colon cancer is described in DKC patients [85]. Even the risk for AML development, Hodgkin's disease as well as non-Hodgkin lymphoma was found to be increased [108]. Future studies are needed to address the question whether the increased susceptibility toward carcinogenesis in TBDs is explained by increased genetic instability only and/or by impaired tumor surveillance by the immune system [103]. Importantly, treatment of TBD-associated malignancies is expected to result in increased treatment-related toxicities due to a higher risk of functional organ damages, secondary BMF and increased mortality [87, 111, 116, 128]. For this reason, adapted therapy regimes (and components) including potential dose reductions in TBD patients suffering from cancer should be discussed individually with specialized TBD centers.

Treatment of TBDs

Androgen derivatives as cyclostragenol, oxymetholone, and danazol are molecules that may increase telomerase activity [129–131]. The first case of a patient with AA who continuously showed in vivo multi-lineage telomere elongation in peripheral blood cells associated with transfusion independence following androgen treatment was published in 2012 [132]. For various androgen derivatives such as oxymetholone and danazol, elongation of TL in the peripheral blood was shown for TBD patients [109, 132–136]. About 69% of all patients had a hematologic response to oxymetholone treatment [135], but side effects such as liver toxicity and virilization were frequent and were primarily observed in female patients [109, 135]. For danazol, on the other hand, side-effects were less frequent and about 50–100% of all TBD patients had at least a short-term response in the sense of an increase in blood counts [109, 135, 136]. Previous data showed that androgens are intracellularly converted to estrogens acting on the estrogen sensitive promotor of the telomerase gene resulting in an increased telomerase activity [129]. In line with this mechanism, an increased telomerase activity was observed after androgen treatment [129, 135]. Another study demonstrated that 11/12 patients under danazol treatment gained TL (mean increase of 386 bp) and 83% showed a hematologic response after 24 months [134].

Even though allo-SCT can reconstitute cytopenias in the hematopoietic system in TBD-associated BMF, long-term survival remains low in TBD patients with only 23% after 10 years [112]. One frequent long-term complication for TBD patients after allo-SCT was progressive PF [111, 112]. Due to the fact that increased (therapy-related) toxicity in TBD patients has been described [112], it was shown that a non-myeloablative protocol with reduced intensity and the avoidance of radiation were able to improve overall outcome [113, 114]. There are some indications that allo-SCT should not be enforced in patients with pre-existing organ damages, whereby patients with progressive BMF more likely benefit from the procedure [111, 113].

In the future, telomerase gene therapy represents a highly promising potential therapeutic strategy for patients with TBDs especially in patients with early BMF. Even though this concept is still experimental, first studies have already been carried out (NCT04211714). In line with this, in telomere-deficient mice that recapitulated the phenotype of BMF/aplastic anemia, cytopenias were successfully treated by using a *Tert* gene therapy system that was based on an adeno-associated virus serotype 9 (AAV9) vector [137]. Also, as another promising therapeutic approach, it was shown that PAPD5 inhibitors are able to restore telomerase activity in stem cells thereby implying that these drugs might be useful to restore the replicative capability of stem cells in TBD patients [138].

Conclusion

TBDs are characterized by impaired telomere maintenance that is often caused by pathogenic variants in various telomere-associated genes. Most patients with TBDs are prone to develop premature and eventually functional telomere shortening. Even though classical TBDs are very rare, the number of unreported cases of adult-onset TBDs is expected to be higher and every physician and particularly hematologist, hepatologist and pulmonologist should be aware of the classical symptom complexes and particularly, the heterogeneity of this hereditary disease group. TL screening by flow-FISH represents a sensitive and cost-effective method to functionally identify patients with suspected TBDs. This is of high importance as TBD patients require specific and individual treatment decisions, are prone to (often immediately life-threatening) complications and careful follow-up care concepts are needed to detect secondary (particularly malignant) disorders at the earliest possible time point (e.g., where they are still locally resectable). Furthermore, adequate counseling is of utmost importance in affected families. Inclusion into innovative clinical trials and registries including proper biobanking as well as connection of the patients with specialized patient support groups (see Acknowledgment) should be offered, e.g., with the help of a specialized tertiary hematology center.

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Author Contributions

B.R. and T.H.B. conceptualized the project, designed and wrote the manuscript. B.R. has created the figures by using "BioRender.com." M.T., M.K., R.M., and F.B. provided scientific input, revised, and updated the manuscript, figures, and tables accordingly. All authors approved the final version of the manuscript including figures and tables.

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