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REVIEW

Inherited aplastic anaemias/bone marrow failure syndromes

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Ribosome biogenesis;
Shwachman-Diamond syndrome;
Telomerase;
Telomeres

Summary The inherited aplastic anaemias/bone marrow (BM) failure syndromes are a heterogeneous group of disorders characterized by BM failure usually in association with one or more somatic abnormality. The BM failure often presents in childhood but this may not be until adulthood in some cases highlighting the need for the adult haematologist to be aware of these disorders. Indeed some patients initially labelled as ''idiopathic aplastic anaemia'' are cryptic presentations of these genetic syndromes. Since 1992, when the first Fanconi anaemia (FA) gene was cloned there have been considerable advances in the genetics of these syndromes. These advances are beginning to provide a better understanding of normal haemopoiesis and how this might be disrupted in patients with BM failure. They have also provided important insights into some fundamental biological pathways: DNA repair-FA/BRCA pathway; telomere maintenance- dyskeratosis congenita related genes; ribosome biogenesis-Shwachman Diamond syndrome and Diamond-Blackfan anaemia genes. Additionally, as these disorders are usually associated with developmental abnormalities and an increased risk of cancer they are providing new insights into human development and the genesis of cancer. These advances have led to improved diagnosis of patients with these disorders. They may now also provide the platform for developing new treatments.

Introduction

Bone marrow failure syndromes/aplastic anaemias are a diverse group of disorders characterized by the inability of the bone marrow (BM) to produce an adequate number of blood cells. They are associated with significant premature mortality. The majority (~70%) of these cases are categorized as idiopathic because their primary aetiology is unknown (Table 1a). In a subset (~10–15% of cases), a drug or infection can be identified that precipitates the BM failure/aplastic anaemia (AA), although it is not clear why only some individuals are susceptible. In approximately 15–20% of

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Table 1 The bone marrow failure syndromes.

(a): ''Classical'' classification

Idiopathic	(~ 70%[a])	Primary pathology unknown	
Inherited	(~15–20%[a])	Dyskeratosis congenita	
		Fanconi anaemia	
		Shwachman-Diamond syndrome	
		Others	
Secondary	(~10–15%)	Radiation	Predictable[b] e.g. Total body irradiation
		Drugs	Predictable[b] e.g. Busulphan
			Idiosyncratic[c] e.g.Chloramphenicol Non-steroidals
		Viruses	Idiosyncratic[c] e.g Hepatitis viruses
		Immune	e.g. as part of SLE

(b): ''Classical'' inherited bone marrow failure syndromes

A. Usually associated with global haemopoietic defect
Fanconi anaemia (FA)
Dyskeratosis congenita (DC)
Shwachman-Diamond syndrome (SDS)
Pearson syndrome (PS)
Familial aplastic anaemia (autosomal and X-linked forms)

B. Usually associated with single lineage haemopoietic defect
(i) Anaemia
Diamond-Blackfan anaemia (DBA)
(ii) Neutropenia
Severe congenital neutropenia (SCN) including Kostman syndrome
(iii) Thrombocytopenia
Congenital amegakaryocytic thrombocytopenia (CAMT)
Amegakaryocytic thrombocytopenia with absent radii (TAR)

[a] This figure is changing as some patients initially presenting as idiopathic AA are subsequently found to have a ''cryptic'' inherited bone marrow (BM) failure syndrome.
[b] These agents produce BM failure in all patients if used at a sufficiently high dose.
[c] The BM failure only develops in some individuals following exposure to the agent, suggesting that there may be a genetic predisposition to the development of BM failure. SLE = systemic lupus erythematosus.

patients (the principal focus of this review) the disease is constitutional/inherited, where the disease is familial and/or presents with one or more other somatic abnormalities[1] (Table 1b). The features of some of the classical genetic syndromes are summarized in Table 2. The precise incidence/prevalence of these remains unclear. The BM failure (which can involve all or a single lineage) usually presents in childhood but the age at presentation is very variable and includes clinical presentation

Table 2 Characteristics of the inherited bone marrow failure syndromes compared to idiopathic aplastic anaemia.

	FA	DC	SDS	DBA	CAMT	AA
Inheritance pattern	AR, XLR	XLR, AR, AD	AR	AD	AR	?
Somatic abnormalities	Yes	Yes	Yes	Yes	Yes	?
Bone marrow failure	AA (>90%)	AA (~80%)	AA (20%)	RCA[a]	Meg[b]	AA
Short telomeres	Yes	Yes	Yes	Yes	?	Yes
Cancer	Yes	Yes	Yes	Yes	Yes	Yes
Chromosome instability	Yes	Yes	Yes	?	?	Yes
Genes identified	13	4	1	3	1	*

FA = Fanconi anaemia; DC = dyskeratosis congenita; SDS = Shwachman-Diamond syndrome. DBA = Diamond-Blackfan anaemia; CAMT = congenital amegakaryocytic thrombocytopenia; AA = idiopathic aplastic anaemia; AD = autosomal dominant; AR = autosomal recessive; XLR = X-linked recessive.
[a] RCA = Red cell aplasia although some patients can develop global BM failure.
[b] Meg = low megakaryocytes which can progress to global BM failure.
* Heterozygous mutations in TERC and TERT are risk factors for some cases of idiopathic AA.

in adulthood in some cases. Scientifically they constitute an important group of disorders since recent advances in the genetics of these is not only beginning to unravel their pathophysiology but is also providing important insights into normal haemopoiesis as well as important biological pathways such as those responsible for genomic stability, telomere maintenance and ribosome biogenesis. These advances have also led to the availability of new genetic tests which now facilitates diagnosis when patients present with atypical clinical features; this includes the recognition that some patients initially labelled as idiopathic AA are ''cryptic forms'' of these BM failure syndromes.

This review focuses on FA, DC, SDS, DBA and CAMT because all of these 5 syndromes can be associated with global BM failure.

Fanconi anaemia (FA)

Clinical features

FA is usually inherited as an autosomal recessive trait but rarely it can be an X-linked recessive disorder. FA patients display progressive BM failure and an increased predisposition to malignancy.[1–3] Affected individuals may also have one or more somatic abnormalities including dermatological (e.g. café au lait spots), skeletal (e.g. hypoplastic thumbs, scoliosis), genitourinary (e.g. horseshoe kidney), gastrointestinal (e.g. duodenal atresia), cardiac and neurological abnormalities. A subset of FA patients (approximately a third) have no overt physical/somatic abnormalities. The majority of patients present towards the end of the first decade of life. However, increasingly some patients with atypical presentations (e.g. myelodysplasia) are being first diagnosed in adulthood and many patients diagnosed in childhood are surviving into adulthood due to improved medical care.

Molecular and cell biology

FA cells characteristically show an abnormally high frequency of spontaneous chromosomal breakage and hypersensitivity to DNA cross-linking agents such as diepoxybutane (DEB) and mitomycin-C (MMC). A diagnostic test, based on the increased chromosomal breakage seen in FA cells compared to normal controls after exposure to DEB or MMC (*DEB/MMC stress test*) has been available for over 20 yrs. This has facilitated many advances in our understanding of FA. Other features of the FA cell phenotype include hypersensitivity to oxygen, abnormal cell cycle kinetics (prolonged G2 phase), increased apoptosis and accelerated telomere shortening.

There is considerable genetic heterogeneity in FA with 13 subtypes/complementation groups (FA-A, FA-B, FA-C, FA-D1, FA-D2, FA-E, FA-F, FA-G, FA-I, FA-J, FA-L, FA-M and FA-N) currently recognized.[4] The genes responsible for these subtypes (*FANCA*, *FANCB*, *FANCC*,*FANCD1*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCJ*, *FANCL*, *FANCM* and *FANCN*,respectively) have all been identified. It has also been recognised that although FA is usually an autosomal recessive disorder in a small minority of FA patients it is X-linked (FANC-B subgroup). Table 3 shows the approximate prevalence of the different FA subgroups.

Studies from many research groups over the last 15 yrs have demonstrated that the FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM proteins interact with each other and form a nuclear complex called the ''FA core complex'' (Fig. 1). The identification of the *FANCD2* gene provided an important link between the FA proteins and DNA repair.[5] The FA core complex is required for the activation of the FANCD2 protein to a monoubiquitinated isoform (FANCD2-Ub). In normal (non-FA) cells, FANCD2 is monoubiquitinated in response to DNA damage and is targeted to chromatin containing the DNA damage (e.g. DNA cross-link). FANCD2-Ub then interacts with DNA repair proteins (including BRCA2, BRCA1, RAD51) leading to repair of the DNA damage. In cells from FA-A, FA-B, FA-C, FA-E, FA-F, FA-G, FA-L or FA-M patients FANCD2 monoubiquitination is not observed. It has been shown that cell lines derived from FA-D1 patients have biallelic mutations in BRCA2.[6] This observation has linked the FA proteins (FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, and FANCM) with BRCA1 and BRCA2 in a common pathway. The BRCA2 protein is important in the repair of DNA damage by homologous recombination (HR). Cells lacking BRCA2 inaccurately repair damaged DNA and are hypersensitive to DNA cross-linking agents. These recent findings therefore suggest that BRCA2 and other FA proteins cooperate in a common DNA damage response pathway, the ''FA/BRCA pathway''. It has also been observed that FANCJ is BRIP1 (partner of BRCA1) and that FANCN[7] is PALB2 (partner of BRCA2). These findings further strengthen the connection between the FA and BRCA proteins and DNA repair.[8] Disruption of this FA/BRCA pathway results in the cellular and clinical phenotype common to all FA subtypes.

There is new data to suggest that the FA/BRCA pathway is activated in response to DNA damage (e.g. replication fork arrest) by ATR (Ataxia–Telangiectasia and RAD3 related protein) (Fig. 1). The

Table 3 Inherited BM failure genetic subtypes.

(a) FA complementation groups/genetic subtypes				
Complementation group/gene	Approximate % of FA patients	Chromosome location	Protein (amino acids)	Exons
A (*FANCA*)	65	16q24.3	1455	43
B (*FANCB*[a])	<1	Xp22.2	859	10
C (*FANCC*)	12	9q22.3	558	14
D1 (*FANCD1*[b])	<1	13q12.3	3418	27
D2 (*FANCD2*)	<1	3p25.3	1451	44
E (*FANCE*)	4	6p21.3	536	10
F (*FANCF*)	4	11p15	374	1
G (*FANCG*)	12	9p13	622	14
I (*FANCI*)	<1	15q26.1	1328	35
J (*FANCJ/BRIP1*[c])	<5	17q23.1	1249	20
L (*FANCL*)	<1	2p16.1	375	14
M (*FANCM*)	<1	14q21.3	2048	23
N (*FANCN/PALB2*[d])	<1	16p12.1	1186	13
(b): DC genetic subtypes				
DC Subtype	Approximate % of DC patients	Chromosome location	RNA/protein product	Exons
X-linked recessive	30	Xq28	dyskerin	15
Autosomal dominant	<5	3q26	TERC	1
	<5	5p15	TERT	16
Autosomal recessive	<1	15q14	NOP10	2
	<1	5p15	TERT	16
Uncharacterized[*]	>50	?	?	?
(c): SDS genetic subtypes				
SDS Subtype	Approximate % of SDS patients	Chromosome location	RNA/protein product	Exons
Autosomal recessive	>90	7q11	SBDS	5
Uncharacterized	<10	?	?	?
(d): DBA genetic subtypes				
DBA Subtype	Approximate % of DBA patients	Chromosome location	RNA/protein product	Exons
Autosomal dominant	25	19q13.2	RPS19	6
	2	10q22—23	RPS24	7
	1	15q25.2	RPS17	5
Uncharacterized[**]	>70	?	?	?
(e): CAMT genetic subtypes				
CAMT Subtype	Approximate % of CAMT patients	Chromosome location	RNA/protein product	Exons
Autosomal recessive	?	1p34	MPL	12
Uncharacterized	?	?	?	?

[a] *FANCB* is on the X-chromosome.
[b] *FANCD1* is *BRCA2*.
[c] FANCJ is BRIP1 (partner of BRCA1).
[d] FANCN is PALB2 (partner of BRCA2).
[*] These are likely to represent more than one genetic locus and include the genetically heterogeneous AR DC.
[**] These are likely to represent more than one genetic locus.

pathway is inactivated by the de-ubiquitinating enzyme, USP1. ATR appears to directly regulate the FA pathway as it is required for the monoubiquitination of FANCD2 and phosphorylates FANCD2 at several sites. Furthermore it has been recently established that FANCI[9] (the protein mutated in FA-I subtype) is a paralogue of FANCD2. FANCI associates with FANCD2 as the FANCI-FANCD2 (ID) complex. Like FANCD2, FANCI is also monoubiquitinated and it too is a substrate for ATR.

ATR is mutated in a sub-set of patients with Seckel syndrome, a disease exhibiting some pheno-

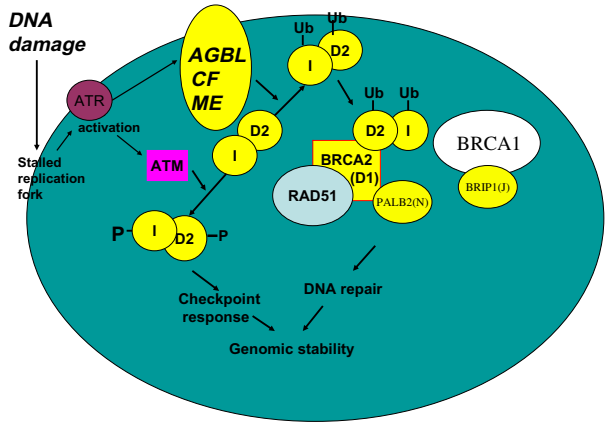

Figure 1 Schematic representation of the ''FA/BRCA pathway''. Following DNA damage when a replication fork encounters a DNA cross-link, ATR (Ataxia Telangiectasia and RAD3-related protein) is activated. This leads to the activation of the FA pathway as well as cell cycle checkpoint activation via the ATM (Ataxia Telangiectasia Mutated) protein. Activation of the FA pathway leads to the formation of the ''FA core complex'' (consisting of the FA proteins A, B, C, E, F, G, L and M). This activated FA core complex leads to the monoubiquitination of FANCD2 (FANCD2-Ub) and FANCI (I-Ub). The I-Ub/FANCD2-Ub complex is then targeted to the chromatin containing the cross link where it interacts with BRCA2 and possibly other DNA repair proteins (e.g. RAD51, J, N) leading to the repair of the DNA damage. Proteins mutated in the different FA sub-types are shown in yellow.

typic similarity to FA. ATR and ATM (Ataxia Telangiectasia Mutated) are known to phosphorylate FANCD2 and FANCI. ATR-Seckel cells also exhibit defects in FANCD2-monobiquitation. Furthermore, Nijmegen breakage syndrome (NBS) cells (mutated in *NBS*) show defects in FANCD2-monobiquitination. This suggests that as well as clinical overlap between FA, NBS and Seckel patients there is also overlap in the biological defects observed in cells from these patients. Thus although many of the steps involved in the FA/BRCA pathway have now been elucidated gaps still remain in our understanding of the precise sequence of physical interactions between the different molecules involved in this matrix of pathways.

It is noteworthy, the phenotypes associated with biallelic *BRCA2* (FA-D1) and *PALB2* (FA-N) mutations are markedly similar to each other but different from the other Fanconi anaemia genes. Specifically, FA-D1 and FA-N are associated with high risks of solid childhood malignancies (e.g. Wilm's tumour and medullobalstoma) which are not usually seen in the other FA subtypes. Furthermore heterozygous mutations in *BRCA2* (FA-D1), *PALB2* (FA-N) and *BRIP1* (FA-J) confer an elevated risk of breast cancer yet this is not the case for the other FA-genes. These differences highlight that the relationship between the FA proteins and their interactions with other molecules is complex at both the clinical and molecular levels.

Treatment

The major cause of mortality in FA patients is the development of BM failure. Anabolic steroids such as oxymetholone can produce useful trilineage (erythroid, myeloid and megakaryocytic lineages) haematological responses in 50–70% of patients but the majority will become refractory after a variable period. Oxymetholone is a good holding treatment. Definitive treatment for the BM failure is with haemopoietic stem cell transplantation (SCT). From the *in-vitro* and *in-vivo* studies it has become clear that cells from FA patients are hypersensitive to agents such as cyclophosphamide and irradiation compared to non-FA patients. Following the poor results of early protocols SCT conditioning regimens have been modified by reducing the dose of cyclophosphamide and radiation. Using the low dose cyclophosphamide (20 mg/kg) and 4.5–6Gy of total body irradiation the actuarial survival for patients transplanted using HLA-identical sibling donors is around 70% at 2 years.[10] The results using unrelated donors have been less good with 2 year survival between 20–40%.[11] In order to improve these results conditioning protocols have been modified further. Additionally long-term follow-up of patients who have survived following SCT show a much higher incidence of malignancies, particularly of the head and neck usually 8–10 years after the transplant. This in part relates to the inherent predisposition of FA patients to malignancy (which is now understandable from the role of the FA proteins in maintaining genomic stability) and in part to factors such as the use of radiotherapy in the conditioning. Results using Fludarabine based protocols which avoid[12,13] or use lower doses[14] of radiotherapy seem to be more encouraging for both sibling and unrelated stem cell transplants with 2 yr survival rates between 65–90%. This represents a major advance in outcome following SCT in FA patients.

The identification of the FA genes combined with the *in-vitro* gene transfer data which show that FA haemopoietic stem cells rescued by gene therapy should have a *selective growth advantage* within the hypoplastic BM environment, have resulted in gene therapy studies. One clinical study in FA recruited 4 FA-C patients. In this study although there were no serious side effects there was no significant therapeutic benefit,[15] possibly due to low transduction efficiency. Gene therapy

for FA is not yet a reality in the clinic and continues to be an area of active investigation.

Additionally, the case for future trials of gene therapy is strengthened by reports of FA *mosaic* patients. In such cases the DEB/MMC test may be negative or only demonstrate chromosomal hypersensitivity in a sub-group of cells. Somatic mosaicism is due to reversion of a pathogenic allele to ''wild'' type in a single haemopoietic (somatic) cell. The mechanisms involved in this reversion can vary[16] but in each case it generates one ''functionally normal'' FA allele and the resulting cell effectively becomes a ''heterozygous cell'' which would be expected to have a growth/survival advantage in the background of FA cells. These ''mosaic patients'' often have reasonable blood counts that keeps them free of haematological problems, suggesting that a single pluripotent stem cell may be sufficient to restore adequate haemopoiesis.

Dyskeratosis congenita (DC)

Clinical features

Classical DC is an inherited bone marrow failure syndrome characterized by the muco-cutaneous triad of abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia.[17] A variety of other (dental, gastrointestinal, genitourinary, neurological, ophthalmic, pulmonary and skeletal) abnormalities have also been reported. BM failure is the major cause of mortality with an additional predisposition to malignancy and fatal pulmonary complications. X-linked recessive, autosomal dominant and autosomal recessive subtypes of DC are recognised.

Clinical abnormalities in DC often appear during childhood. The skin pigmentation and nail changes typically appear first, usually by the age of 10 years. BM failure usually develops below the age of 20 yrs; 80–90% of patients will have developed BM abnormalities by the age of 30 yrs. Occasionally the BM abnormalities may appear before the muco-cutaneous manifestations. There is considerable variation between patients with respect to the age of onset and severity of the muco-cutaneous abnormalities. This can cause difficulties in making a clinical diagnosis. The main causes of death are BM failure/immunodeficiency (~60–70%), pulmonary complications (~10–15%) and malignancy (~10%).

Molecular and cell biology

Lymphocytes from DC patients typically show no significant difference in chromosomal breakage compared to those from normal controls with or without the use of bleomycin, DEB, MMC and γ-irradiation. This enables it to be distinguished from FA. Primary DC skin fibroblasts are abnormal both in morphology and in growth rate. They are also predisposed to developing unbalanced chromosomal rearrangements (dicentrics, tricentrics, and translocations) in the absence of any clastogenic agents. In addition, PB and BM metaphases from some patients show unbalanced chromosomal rearrangements in the absence of any clastogenic agents. These studies provide evidence for a defect, which predisposes DC cells to developing chromosomal rearrangements including end-to-end fusions. Like FA, DC may thus be regarded as a genomic instability disorder.

Haemopoietic progenitor studies have shown reduced numbers of all types of progenitors (consistent with a haemopoietic defect at the stem cell level) compared to controls. The progenitors can be reduced even when the PB count is normal. The demonstration of abnormalities of growth and chromosomal rearrangements in fibroblasts suggests that the BM failure is likely to be a consequence of abnormalities in both haemopoietic stem cells and stromal cells.

X-chromosome inactivation patterns (XCIPs) have been studied in PB cells of women from X-linked DC families by investigating a methylation sensitive restriction enzyme site in the polymorphic human androgen receptor locus at Xq11.2–Xq12 (HUMARA). All carriers of X-linked DC showed complete skewing in XCIP. The presence of the extremely skewed pattern of X-inactivation in PB cells suggests that cells expressing the defective gene have a growth/survival disadvantage over those expressing the normal allele.

Linkage analysis in one large family with only affected males made it possible to map the gene for the X-linked form of DC to Xq28.[18] The availability of additional X-linked families facilitated positional cloning of the gene (*DKC1*) that is mutated in X-linked DC.[19] The identification of the *DKC1* gene has made available a genetic test that can be used to confirm diagnosis in suspected cases and antenatal diagnosis in X-linked families. It has also led to the demonstration that the Hoyeraal-Hreidarsson (HH) syndrome is due to mutations in the *DKC1* gene.[20] HH is a severe multi-system disorder characterized by severe growth failure, abnormalities of brain development, bone marrow failure and immunodeficiency. The *DKC1* gene is expressed in all tissues indicating that it has a vital function in the human cell. This correlates well with the multi-system phenotype of DC. The *DKC1* gene and its encoded nucleolar protein, dyskerin, are highly

conserved throughout evolution. Dyskerin associates with the H/ACA class of small nucleolar RNAs (snoRNAs) and is involved in pseudouridylation (conversion of uracil to pseudouracil) of specific residues of ribosomal RNA (rRNA). This step is essential for ribosome biogenesis and therefore initially suggested that DC arises largely because of a problem with ribosome biogenesis.

Subsequent studies have shown that dyskerin also associates with the RNA component of telomerase (TERC, Fig. 2) that too contains an H/ACA consensus sequence.[21] Telomerase is an enzyme complex that is important in maintaining chromosomal telomere length after cell division. Two essential components of the telomerase complex are the RNA component (TERC) and the catalytic reverse transcriptase (TERT). In patients with X-linked DC it was demonstrated that the level of TERC was reduced and that telomere lengths were much shorter than in age matched normal controls. Subsequently it was found that telomeres are also shorter in cells from patients with autosomal forms of DC. This therefore suggested that DC might be a disease of telomere maintenance rather than ribosomal biogenesis. Further clarification came from the demonstration that one sub-type of autosomal dominant DC is due to mutations in the *TERC* gene.[22]

Since the *DKC1* encoded protein dyskerin and TERC are both key components of the telomerase complex it now appears that DC arises principally from an abnormality in telomerase activity. The brunt of the disease falls on tissues that need constant renewal, consistent with a basic deficiency in stem cell activity due to defective telomerase activity. The demonstration of *DKC1* and *TERC* mutations in DC families provides an accurate diagnostic test, including antenatal diagnosis, in a significant subset of cases (Table 3b). For DC patients this now also provides the basis for designing new treatments. Scientifically it provides the first genetic link between a human disease which is characterized by features of premature ageing (premature grey hair/hair loss, bone marrow failure, increased predisposition to malignancy) and short telomeres. Therefore unravelling the biology of DC has had important implications not only for patients with DC but also for the age-related disorders such as cancer and AA which too are associated with abnormal telomeres.

Recently heterozygous *TERC* mutations have been observed in a subset of patients with AA, myelodysplasia (MDS) and paroxysmal nocturnal haemoglobinuria (PNH) but who lacked classical features of DC.[23–25] Furthermore, AA patients

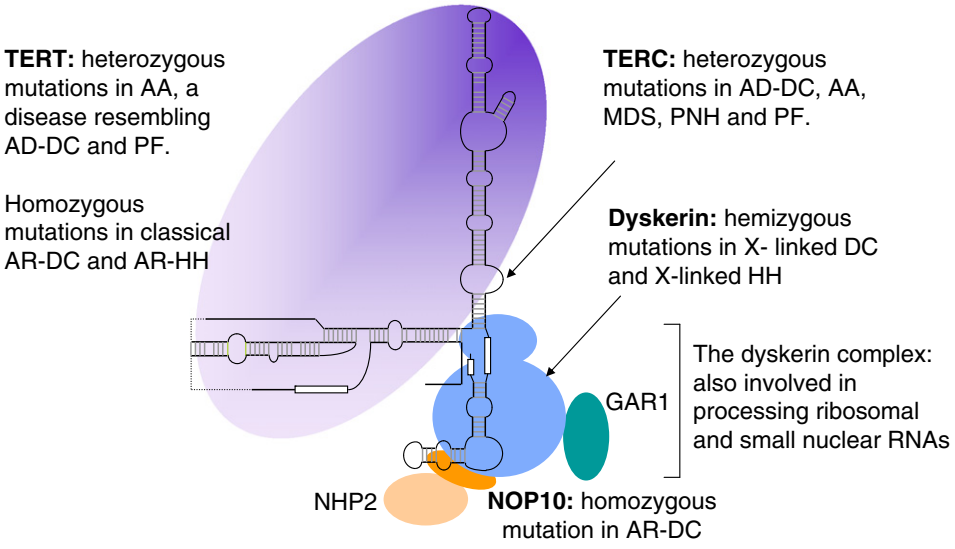

Figure 2 A schematic representation of the interaction between dyskerin and the other molecules (GAR1, NHP2, NOP10, TERC and TERT) of the telomerase complex (and their association with different disease categories). This is a RNA-protein complex since TERC is a 451b RNA molecule which is never translated. The other molecules (dyskerin, GAR1, NHP2, NOP10 and TERT) are proteins. Recent studies suggest that the minimal active telomerase enzyme is composed of two molecules each of TERT, TERC and dyskerin. Dyskerin, GAR1, NHP2 and NOP10 are believed to be important for the stability of the telomerase complex. AA = aplastic anaemia; AD—DC = autosomal dominant dyskeratosis congenita; AR—DC = autosomal recessive dyskeratosis congenita; AR—HH = autosomal recessive Hoyeraal Hreidarsson syndrome; MDS = myelodysplasia; PNH = paroxysmal nocturnal haemoglobinuria; PF = pulmonary fibrosis; X-linked DC = X-linked dyskeratosis congenita; X-linked HH = X-linked Hoyeraal Hreidarsson syndrome.

associated with *TERC* mutations had significantly shorter telomeres than age-matched controls. These data indicate that, in a subset of patients with AA, MDS and PNH the disorder is associated with a genetic defect in the telomere maintenance pathway. It also suggests treatments aimed at restoration of telomere length might be useful in this group of patients. Heterozygous mutations in *TERT*[26–28] have been recently found in some patients with bone marrow failure and disease resembling AD—DC. These findings further support the model that DC is principally a disorder of telomere maintenance. Additionally, telomerase dysfunction (heterozygous mutations in TERT or TERC) has also been identified as the likely cause of a subset of familial idiopathic pulmonary fibrosis, a disease in which fibrotic tissue forms in the lungs, eventually leading to respiratory failure.[29]

The genetic basis of many cases of AR—DC remains unknown. In one large AR—DC family the disease has been found to be due to homozygous mutations in the telomerase associated protein NOP10. Patients with homozygous NOP10 mutations, like patients with dyskerin and TERC mutations were also found to have short telomeres, further highlighting that DC is principally a disease of defective telomere maintenance.[30] In two additional AR families, one with AR—DC and the other with AR—HH, it has been found that the disease is due to homozygous mutations in TERT, suggesting that a pure but severe deficiency in telomerase can produce a phenotype of classical AR—DC and its severe variant, the HH syndrome. It is noteworthy, that one study relating to the X-linked form of DC suggests that dysfunctional dyskerin impairs translation from internal ribosome entry site (IRES)-containing genes. Since several tumour-suppressor and anti-apoptotic proteins are translated from IRES sequences it is conceivable that this may contribute to the susceptibility of X-linked DC patients to tumour development.[31] It has also been observed that hypomorpic *Dkc1* mice, displaying some clinical features that overlap with X-linked DC patients, have defective pseudouridylation.[32] However such defects in pseudouridylation have not been observed in X-linked DC patient cells[21] to date.

Treatment

As in FA, oxymetholone can produce an improvement in haemopoietic function (including trilineage responses) in approximately 50—70% patients for a variable time. Transient successful responses to granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF) and erythropoietin have also been reported.[33] The main treatment for severe BM failure however is allogeneic SCT and there is some experience using both sibling and alternative stem cell donors. Unfortunately because of early and late fatal pulmonary/vascular complications following SCT the results of allogeneic SCT have been less successful than in FA. The presence of pulmonary disease in a proportion of DC patients explains the high incidence of fatal pulmonary complications in the setting of SCT. It also highlights the need to avoid agents that are associated with pulmonary toxicity (e.g. radiotherapy and busulphan). Since BM failure is the main cause of premature death in DC patients and SCT is currently the only curative option for the BM failure SCT should continue to be performed on carefully selected patients. The best candidates for SCT are patients with no pre-existing pulmonary disease and who have sibling donors. SCT using Fludarabine based protocols appears to be giving encouraging preliminary results in some patients.[34]

In the future it will be important to determine whether treatments (for example by transfer of wild type *TERC* or *DKC1* into DC haemopoietic cells) aimed at restoration of telomere length will be possible.

Shwachman-Diamond syndrome (SDS)

Clinical features

Shwachman et al and Bodian et al first reported this disease independently in 1964.[35,36] It is now recognised as an autosomal recessive disorder characterized by exocrine pancreatic insufficiency (e.g. low serum trypsinogen and isoamylase), bone marrow failure and other somatic abnormalities (particularly metaphyseal chondrodysplasia).[37] Features of pancreatic insufficiency (malabsorption, failure to thrive) are apparent early in infancy (the pancreatic function can improve in up to 50% of SDS patients by 5 yrs of age). Other common clinical features include short stature, protuberant abdomen, and an icthyotic skin rash. Metaphyseal dysostosis is seen on radiographs in ~75% of patients. Hepatomegaly, rib/thoracic cage abnormalities, hypertelorism, syndactyly, cleft palate, dental dysplasia, ptosis and skin pigmentation may also be observed in some patients. Thus like FA and DC, SDS is a pleotropic multisystem disorder.

The spectrum of haematological abnormalities includes neutropenia, other cytopenias (approximately 20% have pancytopenia), myelodysplasia and leukaemic transformation (~25%). The age at

which leukaemia develops varies widely from 1 to 43 years. Acute myeloid leukaemia is the commonest category and there is a preponderance of cases of leukaemia in males (M: F, ~3:1). There is no explanation for this sex difference.

Molecular and cell biology

The SDS gene (*SBDS*) within 7q11 was identified in 2003.[38] The majority (>90%) of SDS patients have been found to have mutations in this gene (Table 3c). The lack of mutations in <10% of SDS patients suggests there may be at least one more gene responsible for SDS, the identity of which presently remains unknown. Based on the function of its homologues the *SBDS* gene is predicted to have a role in RNA metabolism and/or ribosome biogenesis.[39] Recent data from yeast studies provides compelling evidence that the SBDS protein has an important in role in the maturation of the 60S ribosomal subunit.[40] Several abnormalities in SDS cells have been observed including haemopoietic stem and stromal defects, increased rates of apoptosis and short telomeres. It will be important to determine how mutations in the *SBDS* gene lead to all these cell defects, the increased frequency of isochromosome 7q [i(7q)] and the clinical abnormalities characteristic of SDS. More immediately, this advance provides a genetic test facilitating accurate diagnosis and a link between BM failure/leukaemia and ribosomal dysfunction.

Treatment

The malabsorption in SDS patients responds to treatment with oral pancreatic enzymes. For those with neutropenia, G-CSF may produce an improvement in the neutrophil count. By analogy with FA and DC some patients with anaemia and/or thrombocytopenia may achieve haematological responses with oxymetholone. As in other types of BM failure, appropriate supportive treatment with red cell and platelet transfusions and antibiotics is very important. The main causes of death are infection or bleeding.

Recent analysis of SDS patients has showed that the incidence of myelodysplasia and transformation to acute myeloid leukaemia (~15–25%) is higher than previously reported.[37] The development of leukaemia, often with features of myelodysplasia, usually has a poor prognosis. SDS patients with leukaemia treated with conventional courses of chemotherapy usually fail to regenerate normal haemopoiesis. Since this is a constitutional disorder all somatic cells, including haemopoietic stem cells, are abnormal. In addition the haemopoietic stem cells may have accumulated secondary abnormalities as suggested by complex karyotypes (especially involving chromosome 7) often observed in the BM from such patients. Therefore for those who develop leukaemia usually the only successful treatment is allogeneic SCT.[41] In the future conditioning regimens that include Fludarabine might be associated with improved outcome as has been the case in patients with FA and DC. The similarities between SDS and the other inherited bone marrow failure syndromes emphasize that SDS should be regarded as a disorder with high propensity to develop both AA and leukaemic transformation. Since these complications may not develop until adult life, it is important for adult haematologists to be aware of SDS. Surprisingly non-haematological malignancies have not been observed in SDS patients.

Diamond-Blackfan anaemia (DBA)

Clinical features

Red cell aplasia was first reported in 1936 by Josephs. In 1938, Diamond reported on four children with hypoplastic anaemia and this has now come to be recognised as Diamond-Blackfan anaemia (DBA) or congenital pure red cell aplasia. DBA usually presents in early infancy with symptoms of anaemia such as pallor or failure to thrive. The hallmark of classical DBA is a selective decrease in erythroid precursors and normochromic macrocytic anaemia associated with a variable number of somatic abnormalities such as craniofacial, thumb, cardiac and urogenital malformations. Conventional haematological diagnostic criteria for DBA have included: (i) normochromic, usually macrocytic, but occasionally normocytic anemia developing in early childhood; (ii) reticulocytopenia; (iii) normocellular BM with selective deficiency of erythroid precursors (erythroblasts <5%); (iv) normal or slightly decreased leucocyte counts; and (v) normal or often increased platelet counts. More recently, an elevated erythrocyte deaminase activity, macrocytosis and elevated foetal haemoglobin have been added to the list of supportive features of DBA. It has also been recognised that in a subset of cases the presentation may be in adulthood.

There is marked heterogeneity in the associated somatic abnormalities and response to therapy. Informative analysis of 420 cases recruited to the DBA registry of North America (DBAR) was published recently.[42] The annual incidence of DBA is ~ 5 per million live births. The median age at presentation was 8 weeks, 93% of patients presented

in the first year of life. 79% were initially responsive to steroids, 17% were non-responsive and 4% were never treated with steroids. 31% of patients were receiving transfusions at analysis. The actuarial survival rates at older than 40 yrs was 100% for those in sustained remission, 87% for steroid—maintained patients and 57% for transfusion dependent patients. Of the 36 deaths reported to the registry 25 were treatment related: 5 from infections, 5 from complications of iron overload, 1 vascular access related death and 14 from transplant related complications.

In the DBAR 8.8% of families had more than one affected individual. Most of the familial cases displayed autosomal dominant pattern of inheritance. Somatic anomalies, excluding short stature, were found in 47% of patients. Of these, 50% were of the face and head (cleft lip, high arched palate, hypertelorism and flat nasal bridge), 38% were upper limb and hand (flat thenar eminence, triphalangeal thumb), 39% genitourinary, 30% cardiac. Height was below the third centile for age in approximately 30%.

MDS and AML have been reported in a few patients with DBA suggesting an increased predisposition to haematological malignancies. Non-haematological malignancies (e.g. osteosarcoma) have also been observed. There are also cases that have evolved into AA; neutropenia and thrombocytopenia are relatively common after the first decade. Giri et al[43] reported on moderate to severe BM hypocellularity in 21/28 (75%) with steroid refractory-DBA; marrow hypoplasia correlated with the development of neutropenia (9/21; 43%) and/or thrombocytopenia (6/21; 29%). Furthermore using *in-vitro* long-term-culture-initiating cell (LTC-IC) assay they provided evidence for a trilineage haemopoietic defect in patients with refractory DBA. Thus although classically DBA has been regarded as a pure red cell aplasia (and hence its ''inappropriate'' name) a more global haemopoietic defect is likely to be present and this may be seen more frequently in the future as patients survive longer due to improved medical care.

Molecular and cell biology

The typical haematological profile in DBA patients consists of normochromic macrocytic anemia, reticulocytopenia and a normocellular marrow with selective deficiency of red cell precursors. A number of different defects of *in-vitro* erythroid progenitor proliferation, differentiation, apoptosis and cytokine responsiveness have been reported but have not clarified the mechanism of *in-vivo* erythroid failure. For many years, based on the typical selective deficiency in red cell precursors researchers believed that DBA was due to an intrinsic problem confined to erythroid proliferation/differentiation. On the other hand the observation of a wide range of somatic abnormalities in a significant proportion of patients, reports of thrombocytopenia, neutropenia and AA together with the recent evidence for a trilineage haemopoietic defect suggests that the primary problem in DBA is not just confined to the erythroid lineage and that it is a multi-system disorder.

The establishment of DBA registries, recent advances in genetics and the identification of a female with a balanced X;19 translocation has facilitated a better understanding of DBA. Recent data from the UK show that $\sim$ 45% of DBA cases are familial.[44] The first DBA gene *DBA1* (*RPS19*) was identified in 1999.[45] The *RPS19* gene is located at 19q13.2 and encodes the ribosomal protein RPS19. Analysis of 172 DBA families (190 patients) by the DBA Working Group of the European Society of Paediatric Haematology and Immunology (ESPHI) has demonstrated heterozygosity for mutations affecting the *RPS19* gene in 42 of 172 index patients (24.4%).[46] Thus confirming the genetic heterogeneity of DBA. Interestingly, mutations in the *RPS19* gene were also found in some apparently unaffected individuals from DBA families, presenting only with an isolated elevation of erythrocyte adenosine deaminase activity (eADA). The lack of a genotype-phenotype correlation implies that other factors modulate the phenotypic expression of the primary genetic defect in families with *RPS19* mutations.

Further linkage analysis in DBA families has identified a second DBA locus on chromosome 8p23.—22 to which $\sim$ 40% of DBA families map. However a significant proportion of families map to neither the locus on chromosome 8 or 19 suggesting that there are likely to be at least 3 DBA genes. The *RPS19* gene shows a ubiquitous expression profile and encodes a 145 amino acid protein with a predicted molecular weight of 16kD. This protein has significant homologies with proteins from diverse species. Its precise function remains unknown although it is predicted to have a role in ribosome biogenesis.[47] The second *DBA* gene (*RPS24*) was identified recently.[48] This was only found to be mutated in a rare sub-group of DBA families ($\sim$2%). Interestingly this gene as well as *RPS17* (the third DBA gene)[49] are also believed to have a role in ribosomal function, thus substantiating further that DBA is likely to be disorder of ribosomal biogenesis.

The demonstration of *RPS19, RPS24* and *RPS17* mutations in $\sim$25—30% of DBA patients now makes it possible to confirm the diagnosis in a subset of DBA patients (Table 3d). This is useful in counselling

families. However the observed poor genotype-phenotype correlation means predictions regarding prognosis are not easy.

Treatment

The first line treatment for DBA remains corticosteroids. Once a maximal Hb response has been achieved the dose of prednisolone should then be tapered slowly until the patient is on the lowest dose possible on an alternate day regimen. The dose required to achieve this can vary considerably from patient to patient. For those patients who fail to respond or become refractory to steroids, blood transfusion is the mainstay of treatment. As in β-thalassaemia major, the main complication from transfusions is iron-overload and iron chelation with desferrioxamine should therefore be commenced as soon as patients have increased iron stores. The promising results with the new oral iron chelator, deferasirox, are likely to have a positive impact in the management of this group of DBA patients. Splenectomy may be indicated in the event of an increased transfusion requirement secondary to hypersplenism. For patients who are transfusion dependent and who have a compatible sibling donor haemopoietic SCT may be appropriate and is potentially curative.

Congenital amegakaryocytic thrombocytopenia (CAMT)

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare disorder, which usually presents in infancy and is characterized by isolated thrombocytopenia, reduction/absence of megakaryocytes in the BM with usually no somatic abnormalities. It is genetically heterogeneous with one autosomal recessive subtype characterized. Approximately 50% of patients will develop AA usually by the age of 5 yrs. They can also evolve into MDS or leukaemia. For patients with severe thrombocytopenia or AA the treatment of choice is SCT if a compatible donor is available.

In a subgroup of CAMT patients mutations in the gene encoding for the thrombopoietin receptor (C-MPL) have been identified.[50] As patients with *c-mpl* mutations can also have abnormalities in the leucocyte count, haemoglobin level and CNS abnormalities (e.g. cerebral and cerebellar hypoplasia) this study highlights the important role of the C-MPL receptor in haemopoiesis in general and in CNS development. It also substantiates the genetic heterogeneity of CAMT (Table 3e).

Conclusion

Over the last 15yrs there have been significant advances in our understanding of the molecular basis of several inherited bone marrow failure syndromes/inherited aplastic anaemias. These advances are facilitating diagnosis particularly when clinical features may be atypical. From a scientific perspective these disorders are providing important insights into several biological pathways of importance in normal human physiology. Clinical similarities between these syndromes have been observed for several years, it is no surprise that similar overlap is observed at the level of molecular pathology, for example SDS and DBA both appear to be disorders of ribosomal biogenesis and all patients with FA, DC, SDS and DBA are characterized by having short telomeres. The biological pathways disrupted in these disorders have severe consequences which include not only life threatening BM failure but also pleotropic effects on development culminating in complex pathologies such as premature ageing and increased predisposition to cancer which are at the heart of all these syndromes.

Practice points

- The inherited aplastic anaemias/bone marrow failure syndromes can have a very variable age of onset spanning from early childhood to adulthood.
- The presentation can be atypical in some cases and this includes presentation as aplastic anaemia or myelodysplasia.

Research agenda

- The genetic basis/molecular pathology of many uncharacterized cases of dyskeratosis congenita and Diamond Blackfan anaemia remains to be established. This is also the case for patients with BM failure associated with one or more physical abnormality but who do not have all the characteristic features of the recognised inherited bone marrow failure syndromes.
- What are the precise molecular mechanisms that lead to bone marrow failure in these syndromes?
- What are the ideal SCT conditioning regimens for these disorders?
- Can new treatments based on a better knowledge of the molecular pathology be developed for these syndromes?

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