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CONGENITAL CYTOPENIAS

The molecular basis of congenital thrombocytopenias: Insights into megakaryopoiesis

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Thrombocytopenia, defined as a platelet count less than 150,000 μl^{-1}, is a common problem in clinical hematology. In most patients, the cause is secondary to an acquired autoimmune disorder, a systemic illness or infection, or an adverse drug effect. Inherited thrombocytopenias are relatively rare, but the identification of genetic mutations in patients with these disorders has contributed significantly to our understanding of the molecular basis of megakaryopoiesis and platelet production. Although laboratory models based on engineered cell lines or transgenic mice are useful in the evaluation of genetic changes that affect megakaryopoiesis, they are not always faithful representations of the human conditions. Therefore, studies from affected patients continue to be vital for our understanding of these diseases.

The list of recognized syndromes of inherited thrombocytopenia is growing, reflecting our increased understanding of the variety of genes that regulate megakaryocyte development and the formation of platelets. Individual syndromes can be classified conceptually by grouping them into disorders with amegakaryocytosis, impaired megakaryocyte maturation, or more distal defects in platelet formation. Low platelets are usually the dominant feature, but abnormalities in other blood cell lineages may be present. In addition, features outside of the hematopoietic system may be associated, such as skeletal defects, hearing abnormalities, or renal dysfunction. Importantly, several of the inherited thrombocytopenias carry a risk of progression to aplastic anemia or leukemia and may occasionally present with these features if the preceding thrombocytopenic phase has not been detected.

Disorders associated with amegakaryocytosis include congenital amegakaryocytic thrombocytopenia (CAMT), thrombocytopenia with absent radii (TAR), and amegakaryocytic thrombocytopenia with radio-ulnar synostosis (ATRUS). These children typically present at birth with severe thrombocytopenia, platelets of normal size, and a paucity of bone marrow megakaryocytes. If plasma levels of thrombopoietin (TPO), the primary cytokine regulating platelet production, are obtained they are elevated due to decreased uptake by the decreased megakaryocyte mass [1]. The prototypical disorder in this category is CAMT, in which patients have severely reduced megakaryocytes and platelets due to mutations in the TPO receptor c-Mpl [2]. Because of the complete lack of receptor-mediated uptake, elevated TPO levels are particularly prominent in CAMT. Although infants present with low platelets, they usually progress to complete bone marrow aplasia within a few years, providing evidence for a critical role for TPO in the maintenance of the hematopoietic stem cell [3]. Mice engineered to have homozygous deletion of c-Mpl are thrombocytopenic and have a reduced number of stem cells [4,5], but the mice do not develop pancytopenia even with prolonged monitoring (unpublished observation). The basis for this difference between humans and mice deficient in c-Mpl is not understood. Identified mutations of c-Mpl are scattered throughout the gene and are inherited in an autosomal recessive pattern, as heterozygous expression of a wild type receptor is adequate for normal thrombopoiesis. Mutations can be classified as type I, in which there is complete loss of the receptor due to a truncation or nonsense mutation, or type II, in which an amino acid substitution results in a receptor with some partial function [2]. This distinction is clinically significant as it appears that patient with type II mutations tend to have a higher initial platelet count and a slower course of progression to aplasia. Because of the expected development of marrow failure, it is recommended that patients with CAMT be trans-

planted if a matched related stem cell donor is available.

Infants with TAR syndrome may resemble CAMT in that they present with severe thrombocytopenia from birth; however, they are distinguished by characteristic forearm abnormalities in which the radii are absent [6]. This abnormality is not typically seen in Fanconi anemia (FA), in which the thumbs may be missing but the radii are present. TAR usually exhibits autosomal recessive inheritance, though families with apparently autosomal dominant transmission have been reported [7]. Bone marrow megakaryocytes in TAR are reduced and may appear immature [8]. Curiously, the thrombocytopenia in TAR syndrome spontaneously improves after the first year of life, and unlike CAMT there is not a progression to marrow aplasia; however sporadic cases of late transformation to leukemia have been reported [9–11]. The genetic basis of TAR is not understood; investigations into members of the homeobox family genes known to be involved in limb development have not revealed any mutations [12]. Although platelets from individuals with TAR have defective signaling and do not show the expected phosphorylation of intracellular proteins in response to TPO stimulation, the lack of signaling is not due to a mutation in c-Mpl or its associated Jak2 kinase [8,13,14]. Elucidation of the genetic defect in TAR may therefore provide new insights into the mechanisms of TPO signaling.

Like TAR, ATRUS is characterized by amegakaryocytic thrombocytopenia from birth and the presence of a forearm defect, with proximal radioulnar fusion. This may be difficult to detect on exam and forearm radiographs are helpful. Other skeletal abnormalities including clinodactyly and shallow acetabulae may be present. In two pedigrees with ATRUS, autosomal dominant inheritance of mutations in the homeobox family gene HoxA11 have been described [15,16]. Unlike TAR, the thrombocytopenia in ATRUS does not improve over time, and case reports have indicated that these children may be at risk of progression to aplastic anemia. The molecular mechanism by which HoxA11 leads to thrombocytopenia is not clear because although both HoxA10 and HoxA11 are expressed in hematopoietic stem cells, only HoxA10 has been found in megakaryocytes and the HoxA11 knockout mouse does not have thrombocytopenia [17,18]. Target genes of HoxA11 that may be candidates for its role in megakaryopoiesis have not been identified. Mutations involving TPO in clinical syndromes of thrombocytopenia are notable for their absence; despite the fact that TPO deletion in knock-out mice cause a phenotype nearly identical to that of c-Mpl mutations [5], defects in this growth factor have not been reported in patients with low platelets or with aplastic anemia.

Disorders in which megakaryocyte maturation is impaired frequently involve mutations affecting specific transcription factors, such as RUNX1 (AML1), GATA1, and FLI1. These disorders are usually associated with a moderate thrombocytopenia and normal or large platelet size. Evaluation of the bone marrow reveals megakaryocytes that are small and may be hyperproliferative. If ploidy analyses are performed, megakaryocytes in the higher ploidy classes are reduced. Mutations in RUNX1, a transcription factor with a critical role in definitive hematopoiesis [19,20] and leukemogenesis [21], have been described in familial platelet disorder with predisposition to acute myelogenous leukemia (FPD/AML) [22,23]. Such families are characterized by autosomal dominant inheritance of mutations in RUNX1 either leading to haploinsufficient expression of the transcription factor or its inability to bind DNA. Because individuals with FPD/AML may be candidates for bone marrow transplantation (BMT) for the treatment of leukemia, it is important to screen potential sibling donors for the presence of this disorder. The molecular description of FPD/AML has provided new insight into the significant role of RUNX1 in normal megakaryopoiesis. The critical targets of RUNX1 in megakaryopoiesis are not known, but RUNX1 interacts with the megakaryocytic transcription factor GATA1 [24] and this interaction is likely important for its role in platelet production. GATA1 and its co-factor Friend of GATA1 (FOG1) are essential in both thrombopoiesis and erythropoiesis [25–27]. Mutations in the X-linked GATA1 gene have been found in 5 families with thrombocytopenia, often with large hypogranular platelets and accompanied by dyserythropoiesis or thalassemia [28–30]. All mutations to date have been described in the N-terminal zinc finger of GATA1 and either disrupt its interaction with FOG1 or with DNA [25]. Although mutations in FOG1 have not been described, they would be predicted to have a similar phenotype but autosomal inheritance. Another critical transcription factor that may interact with RUNX1 and GATA1 in megakaryopoiesis is the Ets family member FLI1 [31,32]. In murine models, deletion of FLI1 leads to defects in vascular development megakaryocyte maturation [33], and consensus sites for FLI1 are found in proximity to GATA1 sites in the promoters of many megakaryocyte-specific genes [32,34]. Although no specific mutations in FLI1 have been described in humans, children with the Paris-Trousseau syndrome have a constitutive deletion of 11q24 that encompasses FLI1 [33,35]. FLI1 is therefore a candidate as the critical gene in this disorder. Paris Trousseau is a variant of the Jacobsen syndrome, in which congenital heart defects, facial dysmorphism, and mental retardation are accompanied by thrombocytopenia with small marrow megakaryocytes [35–37]. Platelets in this syndrome demonstrate giant, fused alpha granules and may be large. In addition to these disorders, GATA1 may

regulate the expression of the heterodimeric transcription factor NF-E2 [38,39]. In mouse models, NF-E2 has been demonstrated to regulate a number of megakaryocyte-specific genes and to be required for thrombopoiesis [40–43]. Despite evidence of its importance as a transcription factor directing megakaryocytic differentiation, mutations in NF-E2 have not been described as a cause of inherited thrombocytopenia in humans. Not all mutations leading to impaired megakaryocyte maturation have been found in transcription factors; a large kindred with autosomal dominant thrombocytopenia linked to chromosome 10 led to the identification of a novel kinase, MASTL (previously FLJ14813), that appears to be important for megakaryocytic maturation and polyploidization [44]. The function of this kinase is sill unknown. Although the risk for progression to leukemia associated with mutations in RUNX1 is clear, it is not known if mutations involving GATA1, FLI1, or MASTL confer a similar predisposition to leukemic transformation. However, mutations in GATA1 are uniformly found in the megakaryoblasts of patients with Down syndrome [45], and mouse models of GATA1 knockdown are associated with the development of leukemias [46] whereas mouse models with lineage-specific GATA1 deletion are associated with the development of myelodysplasia [47]. In the clinical setting, patients with constitutive defects in megakaryocyte maturation should be monitored closely.

Thrombocytopenia syndromes in which platelet production is primarily affected are often marked by abnormal platelet size. The mutations described generally involve regulators of the cytoskeleton. Small platelets in males with moderate to severe thrombocytopenia are suggestive of Wiskott Aldrich Syndrome (WAS). WAS is an X-linked syndrome characterized by microthrombocytopenia, T-cell deficiency, and immune dysregulation resulting in infections, eczema and a predisposition to lymphoid malignancy [48]. Platelet function in WAS is also impaired, leading to bleeding out of proportion to the degree of thrombocytopenia. The mutated gene, encoding WAS protein (WASP), encodes a hematopoietic specific actin regulating protein that activates Arp2/3 to nucleate actin polymerization [49,50]. Binding domains within WASP mediate interactions with WASP-interacting protein (WIP), phospholipids, SH3-containing signaling proteins, and the active GTP-bound form of cdc42 [49,51]. Nonsense mutations or truncations that result in the absence of WASP lead to a more severe phenotype, whereas substitution mutations in which the protein retains some partial function may only result in an X-linked microthrombocytopenia without the features of immune dysfunction [52–55]. The diagnosis of WAS can be confirmed by flow cytometry of lymphocytes to evaluate levels of WASP protein and by sequencing for WASP mutations.

Disruption of specific protein–protein interactions may account for some variability in the phenotype. Young boys with the full WAS syndrome are generally transplanted if they have a suitable stem cell donor, due to the risk of life threatening infection and development of malignancy. It is thought that peripheral destruction contributes to the thrombocytopenia in WAS and XLT [56], and in many cases platelet counts may be significantly improved by splenectomy [48,57]. In contrast to WAS, Bernard Soulier syndrome (BSS) is characterized by macrothrombocytopenia, often with giant platelets. In addition to their large size, platelets in BSS are severely impaired in their function. This disorder can be attributed to mutations in GPIb or GPIX [58], which can be detected by flow cytometry as reduced expression of the GPIb/IX/V complex, or by platelet function testing as absent ristocetin-induced platelet aggregation (RIPA). Originally thought to be a recessive disorder, it is now appreciated that patients heterozygous for BSS mutations often have a partial phenotype with mild macrothrombocytopenia [59]. In addition, patients with DiGeorge syndrome may also have a mild macrothrombocytopenia due to the location of the GPIb beta gene within the deleted region of chromosome 22q11 [60,61]. The reason for the large platelet size in BSS is not clear but may be due to loss of the interaction between GPIb and filamin which serves to anchor the platelet membrane to the underlying actin cytoskeleton [62,63]. Management of bleeding in patients with homozygous BSS syndrome is problematic as platelet transfusion can result in allosensitization and the development of platelet refractoriness. DDAVP may have some benefit in improving bleeding times in affected patients, probably through a von Willebrand factor-independent mechanism [64,65]; alternatively recombinant factor VIIa has been used [66]. In addition to BSS, macrothrombocytopenia is also seen in the autosomal dominant May Hegglin anomaly and Sebastian's, Fechtner's, Epstein's, and Alport's-like syndromes, now collectively recognized as MYH9-related disorders [67,68]. MYH9 encodes the non-muscle myosin heavy chain IIA, which is expressed in platelets, neutrophils, the kidney and cochlear cells. Precipitates of myosin can be detected as neutrophilic inclusions, appearing like Dohle-bodies on a peripheral smear, which may facilitate diagnosis in this disorder. The macrothrombocytopenia in the MYH9-related disorders is associated with the variable expression of glomerulonephritis, hearing loss, and cataracts, and patients should be monitored for these complications. Recently a mouse model of MYH9-deficiency was reported; although the heterozygous mice show variable hearing deficits they do not manifest macrothrombocytopenia, neutrophil inclusions, or renal disease [69]. The reason for this finding is not understood but it may relate to the production of a
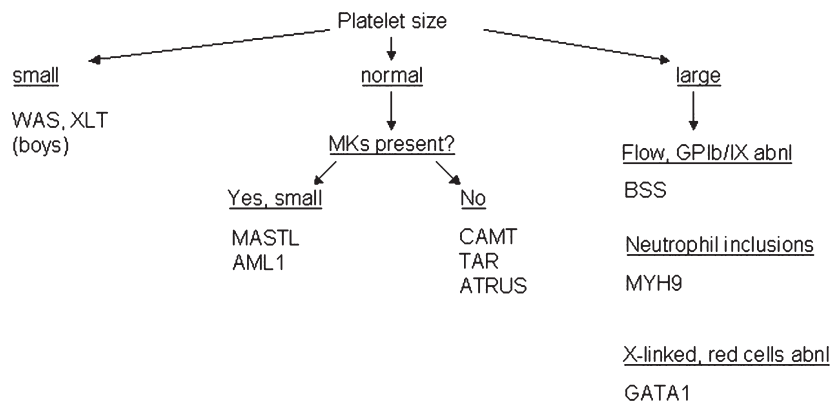
Figure 1. Algorithm for evaluation of candidate disorders of inherited thrombocytopenia.

mutant myosin in most cases of the human disorder, whereas in the mouse model there is only a reduced amount of the normal protein.

In addition to platelet number, platelet structure can be affected by inherited mutations. Abnormal platelet granules may have been noted as a feature of TAR, GATA1 mutations, Paris-Trousseau syndrome, and WAS. The gray platelet syndrome is marked by autosomal dominant inheritance of thrombocytopenia in which the platelets are deficient in α-granules, which is visualized on a Wright-Giemsa stain as gray appearing platelets and can be confirmed by electron microscopy. The genetic cause of gray platelet syndrome is not known, although it is thought to involve a transport or storage process rather than defective synthesis of a specific alpha-granule component [70]. Hermansky-Pudlak, Griscelli and Chediak-Higashi syndromes exhibit deficiencies of dense granules and platelet dysfunction [71]. Thrombocytopenia is variable in these disorders. Because the pathways regulating the formation and trafficking of dense granules are also involved in the trafficking of lysosomes and melanosomes, pigmentation abnormalities frequently accompany the platelet defects. Platelet dysfunction associated with abnormal granule release and defective platelet aggregation can also cause significant bleeding in the absence of thrombocytopenia [72]. In addition, inherited defects extrinsic to hematopoietic cells may lead to increased platelet consumption and thrombocytopenia; these include congenital TTP, in which a deficiency of ADAMTS 13 leads to ultralarge von Willebrand multimers and episodic thrombocytopenia and anemia, type IIb von Willebrand disease, in which a mutation in von Willebrand factor causes increased affinity for GPIb and platelet destruction, and platelet type von Willebrand disease, in which a mutation in GPIb causes increased affinity for von Willebrand factor and platelet destruction. Familial ITP has been reported but the genetic basis of this is not clear and it may represent a manifestation of an inherited immunodeficiency. Because these syndromes are not primarily associated with impaired platelet formation, for brevity these disorders will not

be addressed here and the reader is directed to other recent reviews [73–78].

Once artifact and acquired causes have been excluded, identifying the genetic basis for chronically low platelets in an individual or family remains difficult. Proper evaluation often requires a combination of clinical and research investigations, due to the rarity of these disorders and a lack of standardized testing. An algorithm has been developed by the Italian Gruppo di Studio delle Piastrine to facilitate the diagnosis of the major identified syndromes of inherited thrombocytopenia [79–81]. This algorithm utilizes generally available parameters such as platelet size, inheritance, and associated features to direct the evaluation for known platelet disorders. Specific testing is then applied to confirm the diagnosis (Figure 1). Using this algorithm and extensive laboratory evaluation, approximately half of the patients in that registry with could be diagnosed with a known platelet disorder.

In clinical practice, the diagnostic workup for inherited thrombocytopenia should first exclude the more common phenomena of immune and non-immune mediated platelet destruction, drug effects, infections, and malignancy. In addition, spurious thrombocytopenia, or pseudothrombocytopenia, due to clumping of platelets collected in EDTA based anticoagulants can be ruled out by examining the smear and repeating the platelet count on a specimen collected in citrated buffer. The patient's history should be reviewed for duration and degree of thrombocytopenia, symptoms of bleeding and the presence of associated abnormalities involving the skin, kidneys, or hearing. A careful family history will help to establish the mode of inheritance and the presence of associated features such as nephropathy, hearing loss, or a predisposition to leukemia. The patient should be examined for growth deficiency, the presence of skeletal or other congenital abnormalities, and skin or pigmentation defects. A complete blood count, leukocyte differential, and peripheral smear should be carefully reviewed for additional cytopenias, red cell indices, red cell morphology, neutrophilic

inclusions, and platelet size and granularity. It is important to remember that automated cell counters may not give an accurate platelet count if the platelets are very small. Patients should have a bone marrow aspiration and biopsy to assess the frequency and appearance of the megakaryocytes in the bone marrow and to exclude alternative diagnoses; in addition, cytogenetics should be sent on the bone marrow specimen. Forearm radiographs will help to exclude subtle defects such as radioulnar fusion. If suspected, FA should be ruled out by chromosomal breakage analysis. Von Willebrand disease should be excluded by standardized testing and multimer analysis if the history suggests it; a low dose RIPA using patient plasma will show increased platelet aggregation if the patient has type IIb von Willebrand disease, whereas a low dose RIPA using patient platelets is used to detect platelet-type von Willebrand disease. Although theoretically useful, platelet aggregation studies are often difficult to standardize in patients with platelet counts of less than 100,000 μl^{-1}. In syndromes of large platelets, flow cytometry to evaluate surface expression of GPIb/IX/V is clinically available and can be used to diagnose BSS, although reduced expression of GPIb/IX/V has been reported in MYH9-related disease and GATA1 deficiency and therefore this may test may not be specific [82]. Similarly, flow cytometry tests have been developed to assay for WASP in lymphocytes. A few clinical laboratories will provide diagnostic sequencing for candidate genes including c-Mpl and WAS. However, many potentially useful diagnostic tests are available only in the research setting and have not been developed for clinical use, including reticulated platelets, TPO levels, hematopoietic progenitor assays, slide-based immunofluorescence for myosin inclusions, and sequencing for the majority of candidate genes known to be associated with inherited thrombocytopenia. Once the diagnostic workup has been completed, it is important to follow the clinical course of patients with constitutive thrombocytopenia, especially those with amegakaryocytosis, impaired megakaryocytic maturation or without a definitive diagnosis, given the unclear risk for progression to aplasia or leukemia. Where possible, these patients should be entered into one of the several registries for inherited thrombocytopenia that exist in Europe, Canada, and in the US so that information regarding these rare but informative individuals can be captured.

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CONGENITAL CYTOPENIAS

Congenital neutropenias

CORNELIA ZEIDLER

Introduction

The term congenital neutropenia (CN) has been used for a group of hematological disorders characterized by severe neutropenia with absolute neutrophil counts (ANC) below $0.5 \times 10^9 \, L^{-1}$ associated with increased susceptibility to bacterial infections. This group of diseases includes primary bone marrow failure syndromes with isolated neutropenias like Kostmann syndrome and cyclic neutropenia, and neutropenias associated with metabolic or immunological disorders, like glycogen storage disease type 1b and Hyper IgM-syndrome, and neutropenias being one feature of a complex syndrome, like Shwachman-Diamond syndrome or Barth syndrome. To avoid confusion, we prefer using the term CN only for the most severe disorder among this group. Severe neutropenia characterized by an early stage maturation arrest of myelopoiesis leading to bacterial infections from early infancy. This disease has originally been described as Kostmann syndrome [14,15] with an autosomal recessive inheritance. Recent pathogenetic investigations have demonstrated that this clinical phenotype includes different disorders, with a heterogenous pattern of inheritance including autosomal recessive, autosomal dominant and sporadic cases. Different point mutations in the neutrophil elastase gene have been detected in a subgroup of patients. Data on over 400 patients with CN collected by the Severe Chronic Neutropenia International Registry demonstrate that independent from the CN-subtype more than 90% of these patients respond to recombinant human granulocyte-colony stimulating factor (rHuG-CSF) with ANCs that can be maintained around $1.0 \times 10^9 \, L^{-1}$. Adverse events include mild splenomegaly, moderate thrombocytopenia, osteoporosis and malignant transformation into MDS/leukemia. Development of additional genetic aberrations, e.g., G-CSF-receptor gene mutations, monosomy 7 or ras mutations during the course of the disease indicate an underlying genetic instability leading to an increased risk of malignant transformation. If and how rHuG-CSF treatment impacts on these adverse events remains unclear since there are no historical controls for comparison. Hematopoietic stem cell transplantation is still the only available treatment for patients refractory to rHuG-CSF treatment.

Severe congenital neutropenia

Pathophysiology

The underlying genetic defect of this group of disorders, including Kostmann syndrome is still only partially identified. The original hypothesis for Kostmann syndrome included a genetic predisposition resulting in defective production of G-CSF or defective response of the neutrophilic precursors to G-CSF or other hematopoietic growth factors. However, serum from CN patients contains normal or increased levels of G-CSF [18] and in vitro assays demonstrate a normal biological activity of the endogenous G-CSF.

Myeloid cells from CN patients express slightly increased numbers of G-CSF receptors [16] with a normal binding constant for G-CSF to its receptor.

With the recent detection of various mutations within the neutrophil elastase gene as the cause of cyclic neutropenia [13], genetic screening for mutations of the neutrophil elastase was also started in patients diagnosed with congenital neutropenia [7,11]. Inspite of phenotypical uniformity, characterized by severe chronic neutropenia and a maturation arrest of myelopoiesis in the bone marrow at the promyelocyte/myelocyte stage, in congenital neutropenia patients neutrophil elastase mutations were present only in a major subgroup. All mutations identified so far are present in one allele only. Analysis

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of CN families also discovered that only one parent carried the mutated elastase gene indicating an autosomal dominant inheritance.

In CN patients who developed leukemia, acquired G-CSF receptor mutations affecting the cytoplasmic domain were present in most patients tested so far [1,9,23,24], suggestive of an important role of these mutations in the leukemogenesis. None of the G-CSF receptor mutations was detectable from birth, indicating that these mutations are not causative for the neutropenia. A small subgroup of patients develops these mutations during the course of life, most likely caused by genetic instability. G-CSF receptor analyses cannot be used to discriminate between the different diseases causing severe neutropenia, but might be helpful in screening for risk of leukemia. The time between acquisition of a G-CSF receptor mutation and development of leukemia varies considerably [24]. In few patients a G-CSF receptor is only present in the leukemic cells whereas other patients show single or multiple mutations of the G-CSF receptor gene several years prior to leukemic transformation already [24]. Like the elastase mutations, also the G-CSF receptor mutations affect one allele only in the majority of patients. There is no evidence that a G-CSF receptor mutation leads to a change in the clinical response to G-CSF treatment irrespective of any increase or decrease in G-CSF dose.

Clinical features

All patients suffer from severe chronic neutropenia with absolute neutrophil counts continuously below $200 \; \mu^{-1}L^{-1}$, and in many patients peripheral blood neutrophils are completely absent. The estimated frequency of CN is approximately 1 to 2 cases per million with equal distribution for gender. If the disease is diagnosed during the first months of life, anti-neutrophil antibodies should be excluded.

In patients with CN severe bacterial infections frequently occur during the first year of life. Postnatal an omphalitis may be the first symptom, but later also otitis media, pneumonitis and infections of the upper respiratory tract, abscesses of skin or liver are common infections, which often lead to the diagnosis of CN. Blood cultures are mainly positive for Staphylococci or Streptococci, but also other bacteria, e.g., Pseudomonas, Peptostreptococcus, and fungi were reported. In addition, rare infections like a clostridial gas gangrene infection may occur in these patients. The outcome of these fulminant infections is often lethal due to lack of neutrophil defense. Most patients suffer from frequent aphthous stomatitis and gingivahyperplasia leading to an early loss of permanent teeth.

Blood values

To confirm the diagnosis repeated differential blood counts are required indicating persistent absolute neutrophil counts (ANC) within a range of $0-0.2 \times 10^9 \; L^{-1}$. Blood counts often indicate additionally mild anemia and thrombocytosis. There may also be increases in blood monocytes and eosinophils. Immunoglobulin levels for IgG are elevated in the majority of patients independent of their infectious status (unpublished data). The specific immunologic competence after vaccination is normal. Blood chemistry is within the normal, age-dependent range for electrolytes, kidney and liver function.

Bone marrow

The bone marrow usually shows a maturation arrest of neutrophil precursors at an early stage (promyelocyte/myelocyte level) with few cells of the neutrophilic series beyond the promyelocyte stage. Promyelocytes often reveal morphological atypical nuclei and vacuolization of the cytoplasma. The number of promyelocytes is slightly increased [27] with a median percentage of promyelocytes of 8% prior to G-CSF treatment. While on G-CSF treatment, the percentage of promyelocytes decreases to 3%, whereas myelocyte and neutrophil counts increase. Marrow eosinophilia and monocytosis is common, and monocyte counts may change during treatment. Cellularity is usually normal or slightly decreased. Megakaryocytes are normal in number and morphology. The invitro growth of granulocyte colonies in CFU-GM assays is often defective with a maturation arrest that mimics the disease.

Cytogenetic evaluation and molecular testing

Normal bone marrow cytogenetics at diagnosis may change during the course of the disease with monosomy 7 being the most frequent aberration in about 50% of abnormal cytogenetic results. Abnormal cytogenetics are often associated with morphological changes of the bone marrow indicating the onset of myelodysplasia or leukemia (see below).

Studies of the G-CSF receptor gene have shown that mutations occur mainly within a critical region (nucleotide position 2300 to 2500) of the intracellular part of the receptor. These mutations are acquired mutation by a subgroup of patients. Individual patients may develop single or multiple mutations within this critical region. Analysis of the G-CSF receptor gene in order to detect acquired mutations can be performed from blood and bone marrow.

Table I. Differential Diagnosis of Congenital Neutropenia

1. Other congenital neutropenias:
   Cyclic neutropenia
   Myelokathexis
   Chédiak-Higashi syndrome

   Inborn errors of metabolism:
   Shwachman-Diamond-Syndrome (SDS)
   Pearson-Syndrome
   Glycogen storage disease type Ib (GSD Ib)
   Methylmalonic aciduria (MMA)
   Barth syndrome

2. Immunodeficiencies
   Hyper IgM syndrome
   Agammaglobulinemia
   Large granular lymphocyte syndrome (LGL)
   Severe combined immunodeficiency (SCID)

3. Immune neutropenia
   Autoimmune neutropenia
   Alloimmune neutropenia

4. Idiopathic neutropenia

Differential diagnosis

The differential diagnosis of CN [29,30] includes a number of other congenital or inherited disorders as well as acquired diseases listed in Table I.

The most common of these rare diseases are cyclic neutropenia, Shwachman-Diamond syndrome [4], glycogen storage disease type 1b and autoimmune neutropenia in infancy. A very important differential diagnostic evaluation is testing for neutrophilic antibodies. In children aged 1–3 years suffering from autoimmune neutropenia the presence of neutrophil-specific auto-antibodies can result in increased peripheral destruction of neutrophils. Although peripheral blood neutrophil counts may be as low as in CN patients, these patients usually do not suffer from severe bacterial infections. In the serum of these patients granulocyte-specific antibodies are detectable by various immunologic tests [5].

Treatment

Since 1987 rHuG-CSF [19,22] is available for the treatment of CN. Phase I/II/III studies demonstrated the efficacy of rHuG-CSF on increasing the number of neutrophils and reducing infections [2,3,6]. In contrast, Granulocyte-macrophage colony stimulating factor (GM-CSF) treatment does not lead to an increase in blood neutrophils, but only blood eosinophils [26].

In 1994 the Severe Chronic Neutropenia International Registry (SCNIR) was established to collect data on clinical course and outcome of these rare disorders. As of December, 2004, 422 patients with CN were enrolled in the SCNIR. Of these 422 patients more than 95% responded to individual doses of rHuG-CSF treatment with an increase in

their absolute neutrophil counts to $1.0 \times 10^9 \, \text{L}^{-1}$ and above. Most CN patients respond to a dose between 3 and 10 mcg kg^{-1} per day with a few individuals that require doses higher than 80 mcg kg^{-1} per day to respond. Non-responders to rHuG-CSF are defined as patients failing to respond to rHuG-CSF levels exceeding 120 mcg kg^{-1} per day.

For patients, who do not respond to rHuG-CSF treatment alone or in combination with SCF, currently a transplantation of hematopoietic stem cells (HSCT) is the only treatment available [20,28]. After a successful HSCT, the patients have a normal hematopoiesis and do not require cytokine treatment anymore. It appears difficult to recommend transplantation, if CN patients benefit from rHuG-CSF and do not show any evidence of an impending malignant transformation. The risks associated with a transplant from an HLA-identical sibling may outweigh the risk of leukemic transformation when rHuG-CSF is continued in responding patients. If the risks of HSCT could be further decreased by employing new regimens, then HSCT from a matched sibling donor may be employed as an early curative option in the future.

Long term safety

Leukemia

Prior to the availability of cytokine therapy, it was already recognized that leukemic transformation occurred in patients with congenital neutropenia [12,21]. However, in the pre-cytokine era, in 42% of all published cases the patients died in the first two years of life usually from sepsis or pneumonia. Thus, the true risk of congenital neutropenia patients developing MDS/AML could not be defined. Since rHuG-CSF therapy is available, most of the patients survive well beyond two years of age. Therefore, it is unknown, whether prolonged survival unmasks the natural course of the disease with an increased risk of leukemic transformation independent of any treatment [10]. However, there is evidence that patients who require high rHuG-CSF doses for neutrophil response have a higher risk of leukemic transformation (paper submitted).

From the initiation of clinical trials with rHuG-CSF in 1987 through December 2000, a total of fourty-five patients with severe chronic neutropenia who developed MDS/AML were reported to the SCNIR, all of whom have a diagnosis of congenital neutropenia. The overall incidence or crude rate of MDS/AML conversion is 11.7% for CN patients (45 cases among 383 exposed cases), with an average follow-up of approximately five to six years. Two of the total fourty-five congenital patients with secondary MDS/leukemia were diagnosed as Shwachman-Diamond syndrome. No cases of MDS/AML occurred in the

subgroup of patients suffering from cyclic, or idiopathic neutropenia.

Conversion to MDS/AML in SCN patients was associated with one or more cellular genetic abnormalities, e.g., monosomy 7, ras mutation, or G-CSF receptor mutation, which may be useful to identify subgroups of patients at high risk [9,23].

Interestingly, marrow cells from eleven SCN patients who transformed to MDS/AML showed point mutations in the gene for G-CSF receptor resulting in a truncated C-terminal cytoplasmic region of the receptor that is crucial for maturation signaling [9,23,24].

As illustrated by the cases described herein, the development of MDS/AML is a multi-step process characterized by a series of cellular genetic changes indicating a genetic predisposition to malignant transformation.

Osteoporosis, splenomegaly, vasculitis

Osteopenia is reported in 54% of CN patients analyzed for bone mineral with varying degrees of abnormal results [25]. However, most patients did not suffer from clinical symptoms of osteopenia or osteoporosis, such as bone pain or fractures.

Splenomegaly

The incidence of a palpable splenomegaly (2 cm below the costal margin) was 21% in CN patients prior to treatment with rHuG-CSF. During initiation of rHuG-CSF therapy spleen size may further increase, but remains at the level of occurrence (33.8–47.6%). In some individuals, splenomegaly can be associated with infections or with transformation to MDS/AML.

Vasculitis

Vasculitis is a rare finding (3.3% of CN patients). Symptoms of vasculitis generally occur during the first ANC increase after G-CSF initiation and abate when ANC decreases. In patients with recurrent vasculitis an underlying autoimmune disorders or an underlying malignancy needs to be ruled out.

Monitoring

All patients should be seen by a physician at least twice a year. Blood counts (WBC, hemoglobin, platelets and differential blood counts) and a physical examination should be obtained at least every three months, including an assessment of weight and height and a documentation of intercurrent infections.

The SCNIR recommends annual bone marrow examination (morphology plus cytogenetics) to search for acquired cytogenetic abnormalities, such as

monosomy 7 or trisomy 21, and G-CSF receptor mutations [29,30].

Cyclic neutropenia

Pathophysiology

Cyclic neutropenia is another rare disorder characterized by repetitive infectious episodes, fever and mouth ulcers during regularly recurring phases of severe neutropenia. This disorder was first described by Leale in 1910 [17] as recurrent furunculosis in an infant showing an unusual blood picture. Many years later the autosomal dominant inheritance was first recognized by a collection of affected families.

An oscillatory production of precursor cells in the bone marrow causes fluctuations of almost all types of blood cells. In most patients the disease is autosomal dominantly inherited, but sporadic cases were also identified. Recent molecular genetic studies demonstrated that different mutations in the gene for neutrophil elastase (ELA2) located on chromosome 19 p13.3 are responsible for this disease in both autosomal dominant and sporadic cases [13,7].

Clinical features

The diagnosis of cyclic neutropenia should be considered, if a child presents with regularly recurring fever, mouth ulcers, pharyngitis, and lymphadenopathy, or recurrent skin infections. Usually these symptoms are already present in children less than one year of age. Symptoms may last for more than one week. Patients suffering from painful deep mouth ulcers are often unable to eat and may present with loss of body weight. Almost all patients with periods of severe neutropenia (ANC less than 200 cells $\mu^{-1}L^{-1}$) every 3 weeks show at least some symptoms with almost every cycle. The frequency of bacterial infections depends also on the length of the neutropenic phase, therefore patients with longer neutropenic periods are more susceptible to infections. However, severe bacterial infections like pneumonia and septicemia usually are rare. Inbetween the neutropenic phases, patients are without symptoms and have normal physical examinations [8].

Blood values

Blood cells show a cyclic pattern with a typical cycle length of 21 days. In clinically obvious cyclic neutropenia neutrophil counts fall to less than 200 $\mu^{-1}L^{-1}$. After 3 to 5 days neutrophils increase and reach counts within the lower normal level.

If cyclic neutropenia is suspected, serial blood counts need to be performed at least 3 times per week over six weeks to document the typical cyclic pattern of blood neutrophils. In most patients

periodic oscillations of reticulocytes and platelets are detectable as well, and sometimes even eosinophils and lymphocytes cycle.

Bone marrow

In cyclic neutropenia bone marrow morphology changes during a cycle. Serial bone marrow aspirates have shown an early maturation arrest of myelopoiesis, comparable to congenital neutropenia, at the onset of the neutropenic phase. Usually, within 3 to 5 days myeloid maturation recovers and myelopoiesis up to band neutrophils is present in bone marrow aspirates. Erythroid precursors also show oscillations in the bone marrow. Colony assays from patients with cyclic neutropenia have shown that various types of cells fluctuate at the same periodicity [17].

Treatment

For patients with cyclic neutropenia the availability of G-CSF changed the clinical course significantly, since there was no effective treatment before. In clinical trials it has been shown that daily application of G-CSF (2 to 5 $\mu g\,kg^{-1}$) increased the amplitude of neutrophil oscillations and shortened the duration of the neutropenic phase. Under G-CSF the cycle length changes from 21 to about 14 days [3,6]. The significant decrease of infectious episodes is mainly due to a shortening of the severe neutropenic period.

Long term safety

In cyclic neutropenia patients G-CSF treatment significantly reduced mouth ulcers, febrile and infectious episodes. A modest increase in spleen size is probably common, but significant splenomegaly was not documented.

During the past ten years leukemic development was not reported in cyclic neutropenia patients with or without G-CSF treatment. In contrast to congenital neutropenia, cyclic neutropenia seems not to be a pre-leukemic disorder.

Conclusion

In light of the reported studies and longitudinal data from the SCNIR, we suggest that the use of rHuG-CSF remains the first-line treatment for the majority of CN patients and clinically symptomatic patients with cyclic neutropenia.

Hematopoietic stem cell transplantations (HSCT) from HLA-identical sibling are beneficial for CN patients refractory to rHuG-CSF. For those patients in whom a G-CSF receptor mutation is identified, HSCT from an HLA-identical sibling is an option. Patients who develop monosomy 7, other significant chromosomal abnormalities or MDS/leukemia should proceed urgently to HSCT. Data on alternative sources of donor stem cells are insufficient to assess outcome in patients with CN. With the exception of those patients, who fail to respond to rHuG-CSF, the cytokine should be employed to maintain an ANC ranging from $1.0-5.0 \times 10^9\,L^{-1}$ with amelioration of symptomatology.

All CN patients, regardless of any treatment or their response to treatment, are at risk of developing MDS or leukemia at an incidence of about 11%. Careful monitoring for cytogenetic abnormalities and G-CSF receptor mutation is necessary to initiate HSCT as soon as any of these are detected. Despite the significant risk of leukemic development, it must be taken into consideration that HSCT-related morbidity is also significant. Therefore, HSCT should be restricted to G-CSF non-responders, if there are no signs of leukemia or a pre-leukemic state.

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CONGENITAL CYTOPENIAS

Congenital bone marrow failure involving the red blood cells

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Aplastic anaemia and red cell aplasias may be congenital or acquired. It has been usual to consider the acquired and inherited types as quite distinct but it is becoming evident as the genetic bases of the inherited diseases become better understood that some cases labelled acquired may be late onset genetic disorders. Furthermore, knowledge of the pathophysiology of the inherited disorders may help understand the evolution of acquired forms, particularly the development of malignant disease. The other disorders which display phenotypic overlap are Fanconi Anaemia (FA), dyskeratosis congenital (DC) and Diamond Blackfan Anaemia (DBA)

Fanconi Anaemia

Fanconi anaemia (FA) is an autosomal recessive disease in which the development of aplastic anaemia is coupled to developmental abnormalities (Table I). There is wide genetic and phenotypic heterogeneity [1]. Families have been described in all populations.

Genetics

The characteristic diagnostic test for FA is the increase of multiple chromosome abnormalities in peripheral blood lymphocytes in metaphase, further increased when exposed to DNA clastogenic agents which cause DNA cross-linking, such as diepoxybutane (DEB) or mitomycin C. The test is usually carried out on lymphocytes though cultured skin fibroblasts also show the chromosome sensitivity. The lymphocytes are stimulated with phytohaemagglutinin with and without the addition of the clastogen. Metaphases are examined for chromosomal breaks, rearrangements, gaps, exchanges and endoreduplications. A significant increase above the normal, control value is diagnostic of Fanconi Anaemia but false negatives occasionally occur as a result of *in vivo* "correction" of the genetic defect in compound heterozygotes.

The genetic basis of FA is complex. Somatic cell fusion studies showed that there are at least 9 distinct genes identifiable, mutations of which cause FA, *FANC(A-G and FANCL)*, including *FANCD1* and *FANCD2*. Seven of these genes have been cloned, *FANCA(16q24.3)*, *FANCC(9q22.3)*, *FANCD2(3p25.3)*, *FANCE (6p21.3)*, *FANCF (11p15)*, *FANCG(9p13)*. *FANCB (Xp22.31)* codes for FA-associated polypeptide 95Kda. *FANCG* is identical to a gene *XRCC9* which is thought to be involved in cell cycle regulation or post replication repair and *FANCD2* co-localizes with *BRCA2*, a DNA damage response agent and the major breast cancer susceptibility protein, and the genes may be identical. The Fanconi proteins. FA A, C, G and F, form a nuclear complex essential for protection against chromosome breakage.

For each of these genes involved in FA, multiple mutations have been described. Mutations identified in different FA cases involving the *FANCC* gene are shown in Table II. Well over 100 different mutations have been described for the *FANCA* gene.

Haematology

Patients usually have a normal or nearly normal blood count at birth. The age at which pancytopenia develop depends in part on the underlying genetic defect, most commonly appearing between 5 and 10 years, but in some families later. Attempts to identify clinical syndromes with different complementation groups suggests that there are different clinical presentations within these groups [1]. Nearly all patients with Fanconi anaemia develop bone marrow failure (90%) and an aplastic marrow, and there is a markedly increased risk of developing acute leukaemia (33%), mainly myeloid.

Table I. Common dysmorphisms in Fanconi Anaemia

System	Features	% affected
Skeleton	Short stature	57
	Upper limb abnormalities (Radial ray)	48
	Microcephaly	27
	Microphthalmia	26
Skin	Café-au-lait patches	60
	Hyperpigmentation	
	Hypopigmented spots	
Renal	Horseshoe kidney	23
	Pelvic kidney	
	Double ureter	
Low birth weight	Overall	12
	Diagnosed in first year of life	50

The IVS4+4A→T mutation results in total loss of protein and is common in the Ashkenazi Jewish population. The phenotype is particularly severe with early bone marrow failure, high incidence of leukaemia and multiple somatic abnormalities. The same mutation in Japanese apparently has a milder phenotype. Mutations in the *FANCD1 (BRCA2)* gene are associated with early onset acute leukaemia and high incidence of breast cancer in kindreds.

Clinical features

The main features of Fanconi anaemia are shown in Table I. Symptoms are dependent upon the degree of pancytopenia with anaemia, haemorrhage and infections being the main problems. Patients with Fanconi anaemia also have a high relative risk of cancer, particularly involving squamous epithelium of the tongue, pharynx, vulva or vagina.

Management

The outlook in Fanconi anaemia is poor with almost inevitable progress to bone marrow failure. Most patients will respond to treatment with anabolic steroids though such treatment only delays and does not prevent the development of bone marrow failure or acute leukaemia. Treatment with orally active, 17α

Table II. FANCC mutations (9q22.3)

Exon	Nucleotide changes	Amino acid changes	Mutation type
1	292C→T	Q13X	Stop codon
1	320G→A	W22X	Stop codon
1	322delG		Frameshift
4	IVS4+4A→T		RNA splicing
5	775C→T	R174X	AA substitution
6	808C→T	R185X	Stop codon
13	1742T→G	L496R	AA substitution
14	1806insA		Insertion
14	1897C→T	R548X	Stop codon
14	1916T→C	L554P	AA substitution

From Buchwald M and Carreau M. *Genetic Basis of Fanconi's Anemia* (2000)

alkylated androgens such as oxymetholone, may cause peliosis hepatis and hepatocellular carcinoma as well as severe virilising side effects.

Haematopoietic stem cells transplantation produces the only hope of avoiding marrow failure and acute leukaemia, though of course it will not prevent other consequences of FA including the development of solid tumours. The genetic defect in FA which produces chromosome instability, renders cells sensitive to clastogenic agents so that conditioning for allogeneic transplant needs to be considerably modified and reduced doses of alkylating agents and/or irradiation used. Modern conditioning regimes aim for non-ablative immunosuppression. The results with HLA-matched sibling donors are good with 80–90% survival, whereas results with unrelated volunteer donors have been somewhat disappointing. Historical records from the EBMT (23% survival) and the IBMTR (>10 years old, 15%, <10 years 20%) emphasize the poor outcome. Recent results avoiding radiation and using fludarabine and non-cytotoxic regimens (ALG or Campath 1H) are more encouraging.

Gene therapy

FA should provide an ideal disorder for gene therapy. Many cases are monogenetic or compound heterozygotes. Transfected cells should have a growth advantage over the mutated stem cells. Attempts have been to correct FANCC defective stem cells by gene transduction using retroviral [2] and adenovirus associated [3] vectors. Whilst *in vitro* results have been promising, clinical application only produced transient improvement in marrow cellularity.

Dyskeratosis congenital

Dyskeratosis congenital (DC) is a rare inherited disorder involving the mucocutaneous system. Bone marrow failure develops in about 50% of cases, usually in the second to the fourth decade

Genetics and pathophysiology

Inheritance may be autosomal dominant, autosomal recessive or X-linked, indicating that several different genetic disorders may lead to a common or similar phenotype. In the genetic disorders so far characterized, the common finding is excessive shortening of telomere repeats caused by relative failure of telomerase reverse transcriptase (TERT). The genetic sub types are shown in Table III.

Dyskerin is a 57kDa protein which is closely associated with the telomerase complex. Mutations in the *DKC1* gene are responsible for the X-linked DC. Telomerase has two components, a protein constituent which constitutes the catalytic site

Table III. Inheritance of Dyskeratosis congenital

DC subtype	Approx. percentage of DC patients	Chromosome location	Gene product	Mutations identified (n)
X-linked recessive	40	Xq28	Dyskerin	30
Autosomal dominant	5	3q21–3q28	hTR (TERC)	6
Autosomal recessive	50	?	?	?

After Dokal (2005), data based on Dyskeratosis congenital registry (Knight et al 1998).

(TERT) and a RNA component, human telomerase RNA (hTR), also known as telomerase RNA component (TERC). The autosomal recessive DC is caused by mutations in the hTR gene [4]. Mutations in either gene that lead to deficient activity of their product produce loss of telomerase activity and diminished proliferative capacity and replicative persistence in affected cells. Replication is associated with increasing chromosomal abnormalities, even in the absence of clastogens and hence an increased risk of malignant transformation.

Cinical features

DC is characterised by abnormalities of the skin, nails, oral mucosa and dentition. Other abnormalities are also found (Table IV). In both the X-linked and autosomal forms there is phenotypic heterogeneity. In most cases skin abnormities, reticular hyperpigmentaion commonly on neck, upper trunk and limbs, and nail dystrophy appear in the first 10 years. The nails develop longitudinal ridging and atrophy, sometimes going on to complete loss of nails from fingers and toes. Leukoplakia and excessive tears come next and bone marrow failure becomes apparent in the teens going on to become complete usually before the age of 30.

Table IV. Somatic anomalies in Dyskeratosis congenital

Abnormality	Percentage of patients
Abnormal skin pigmentation	89
Nail dystrophy	88
Bone marrow failure	86
Leukoplakia	78
Epiphora	31
Learning difficulties/developmental delay	25
Pulmonary disease	20
Short stature	20
Extensive dental caries	17
Oesophageal stricture	17
Premature hair loss/ greying	16
Hyperhiderosis	15
Malignancy	10
Intrauterine growth retardation	8
Liver disease/peptic ulcer/enteropathy	7
Ataxia/cerebellar hypoplasia	7
Hypogonadism / undescended testicle	6
Microcephaly	6
Urethral stricture/phimosis	5
Osteoporosis/aseptic necrosis/scoliosis	5
Deafness	1

Data from the DC Registry (Knight et al. 1998)

Pulmonary complications are less common but assume major importance if stem cell transplant is attempted. There is a strong vasculitic component, which is grossly exaggerated, often with fatal consequences, by allogeneic transplantation. In some patients bone abnormalities, particularly osteoporosis, are major symptomatic problems.

Death is mainly caused by the consequences of marrow failure or malignant disease. Most cancers are squamous or adenocarcinomas. The oropharynx and gastrointestinal tracts are most commonly involved. The incidence of leukaemia is not increased in contrast to FA.

Management

Treatment is difficult. Supportive care and genetic counselling are of considerable importance as is the management of the pulmonary and malignant complications. Anabolic steroids may improve the blood count temporally but at the cost of side effects. Conventional HSCT may correct the bone marrow failure but a sadly high proportion of successful grafts are followed by pulmonary failure, particularly in patients who have impaired lung function pre-transplant. Conditioning should avoid drugs toxic to the lung.

DC would be an ideal target for gene therapy since, as with FA, the transformed stem cells should have a growth advantage over the defective cells.

Diamond Blackfan Anaemia

Daimond Blackfan anaemia (DBA) is a congenital failure of erythropoiesis, characterized by a selective defect in red cell production, normochromic, macrocytic anaemia, reticulocytopenia and normal white cell and platelet counts. Somatic anomalies are found in about 50% of patients (Table V)

Genetics and pathophysiology

Some 20% of DBA patients have a family history indicating autosomal dominant inheritance. Consanguinity may suggest autosomal recessive inheritance for some cases and the remainder are sporadic. More recent family studies have shown that first degree relatives of apparently sporadic cases have raised red cell adenosine deaminase levels suggesting a complex dominant inheritance. Family studies using

Table V. Pevalence and pattern of physical anomalies in children with Diamond Blackfan Anaemia in the UK.

Physical anomalies	Percentage affected
Any	58
Craniofacial anomalies	52
Eyes	34
Palate/jaw	29
Thumbs	18
Growth retardation	27
With associated anomalies	17
Isolated retardation	10

the European DBA registry mapped a gene to chromosome 19q13, coding for ribosomal protein S19. *RPS19* mutations are found in about 25% sporadic cases, including family members with isolated raised red cell ADA. Other families have a gene mutation mapping to chromosome 8, as yet unidentified.

The common presentation is of anaemia and reticulocytopenia presenting at or shortly after birth. Recent studies have shown that that the defect in erythropoiesis lies in the erythropoietin dependent stage of expansion and maturation [5]. This occurs in both *RPS19* cases and the others suggesting pathways which may be disrupted by a number of gene defects. Studies on the proliferative capacity of other cell lineages in the DBA marrow reveal a more general marrow defect in some cases.

Clinical course and management

Anaemia develops shortly after birth in most cases though later and milder onset may occur in earlier generations of dominantly inherited disease. The haemoglobin often reaches very low levels before the diagnosis is made and transfusion is required. Prednisone, 2 mg Kg^{-1} per day, leads to remission of anaemia in about 70% of cases. About half of these remain steroid dependent though the dose required may be very low. Of the others about half maintain remission off steroids, the remainder become refractory and need to return to a transfusion regimen. Occasionally spontaneous remission may occur in transfusion dependent patients. Patients who are transfusion dependent need iron chelation therapy. With good compliance for chelation the life expectancy is nearly normal for patients in whom total marrow failure does not develop. MDS and AML are also more likely in patients with DBA [6]. Allogeneic HSCT has been curative for some patients but care should be taken to ensure the donor does not have a *forme fruste* of the disease such as raised ADA.

DBA must be distinguished at presentation from transient erythroblastopenia of childhood (TEC). This is an acquired, self-limited red cell aplasia which develops in a previously fit child. There is often a antecedent history of a virus infection thus the aplasia is more likely to be the result of the immune response rather than the virus itself. Reticulocytosis, indicating early remission may be found at diagnosis and recovery is usually complete by 2 months. TEC usually presents about 2 years of age, a little later than DBA.

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