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Regular Article

RED CELLS, IRON, AND ERYTHROPOIESIS

A novel syndrome of congenital sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD)

Daniel H. Wiseman,[1] Alison May,[2] Stephen Jolles,[3] Philip Connor,[4] Colin Powell,[5] Matthew M. Heeney,[6] Patricia J. Giardina,[7] Robert J. Klaassen,[8] Pranesh Chakraborty,[8] Michael T. Geraghty,[8] Nathalie Major-Cook,[8] Caroline Kannengiesser,[9,10] Isabelle Thuret,[11] Alexis A. Thompson,[12] Laura Marques,[13] Stephen Hughes,[14] Denise K. Bonney,[1] Sylvia S. Bottomley,[15] Mark D. Fleming,[16] and Robert F. Wynn[1]

[1]Department of Haematology, Royal Manchester Children's Hospital, Manchester, United Kingdom; [2]Department of Haematology, Cardiff University School of Medicine, Cardiff, United Kingdom; [3]Department of Immunology, University Hospital of Wales, Cardiff, United Kingdom; [4]Department of Paediatrics, Children's Hospital for Wales, Cardiff, United Kingdom; [5]Institute of Molecular and Experimental Medicine, School of Medicine, Cardiff University, Cardiff, United Kingdom; [6]Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA; [7]Division of Hematology-Oncology, Children's Cancer and Blood Foundation Laboratories, Weill Cornell Medical College, New York, NY; [8]Department of Pediatrics, Children's Hospital of Eastern Ontario, University of Ottawa, Ottawa, ON, Canada; [9]Assistance Publique des Hospitaux de Paris, Département de Biochimie Hormonale et Génétique, Hôpital Bichat-Claude-Bernard, Paris, France; [10]INSERM UMR773, Université Paris Diderot, Paris, France; [11]Department of Pediatric Hematology, Hôpital de la Timone, Marseilles, France; [12]Department of Pediatrics, Northwestern University Feinberg School of Medicine, Ann & Robert H. Lurie Children's Hospital, Chicago, IL; [13]Paediatric Department, Infectious Diseases and Immunodeficiencies Unit, Centro Hospitalar do Porto, Porto, Portugal; [14]Department of Immunology, Royal Manchester Children's Hospital, Manchester, United Kingdom; [15]Department of Medicine, Hematology-Oncology Section, University of Oklahoma College of Medicine, Oklahoma City, OK; and [16]Department of Pathology, Boston Children's Hospital, Boston, MA

Key Points

- A novel clinical syndrome of CSA, B-cell immunodeficiency, periodic fevers, and developmental delay is described.
- Bone marrow transplant resulted in complete and durable resolution of the hematologic and immunologic manifestations.

Congenital sideroblastic anemias (CSAs) are a heterogeneous group of inherited disorders identified by pathological erythroid precursors with perinuclear mitochondrial iron deposition in bone marrow. An international collaborative group of physicians and laboratory scientists collated clinical information on cases of CSA lacking known causative mutations, identifying a clinical subgroup of CSA associated with B immunodeficiency, periodic fevers, and development delay. Twelve cases from 10 families were identified. Median age at presentation was 2 months. Anemia at diagnosis was sideroblastic, typically severe (median hemoglobin, 7.1 g/dL) and markedly microcytic (median mean corpuscular volume, 62.0 fL). Clinical course involved recurrent febrile illness and gastrointestinal disturbance, lacking an infective cause. Investigation revealed B-cell lymphopenia ($CD19^+$ range, 0.016-0.22 × 10^9/L) and panhypogammaglobulinemia in most cases. Children displayed developmental delay alongside variable neurodegeneration, seizures, cerebellar abnormalities, sensorineural deafness, and other multisystem features. Most required regular blood transfusion, iron chelation, and intravenous immunoglobulin replacement. Median survival was 48 months, with 7 deaths caused by cardiac or multiorgan failure. One child underwent bone marrow transplantation aged 9 months, with apparent cure of the hematologic and immunologic manifestations. We describe and define a novel CSA and B-cell immunodeficiency syndrome with additional features resembling a mitochondrial cytopathy. The molecular etiology is under investigation. (*Blood*. 2013;122(1):112-123)

Introduction

Sideroblastic anemia (SA) describes a heterogeneous group of acquired and inherited disorders of erythropoiesis characterized by the presence in bone marrow (BM) of erythroid precursors with pathological, perinuclear mitochondrial iron deposition ("ringed sideroblasts"). During the past 2 decades the genetic bases of several distinct congenital SA (CSA) disorders have been defined. Some affect mainly or exclusively erythroid cells; other "syndromic" forms occur within multisystem disorders with extensive nonhematopoietic manifestations. The genes responsible for these phenotypes encode proteins involved in mitochondrial heme biosynthesis (eg, *ALAS2*; *SLC25A38*), iron-sulfur (Fe-S) cluster biogenesis/transport (*ABCB7*; *GLRX5*), and mitochondrial translation (*PUS1*; *YARS2*; mitochondrial deletions).[1-3] Anemia can be microcytic, normocytic, or macrocytic; can vary from mild to transfusion dependent; can present at any age up to the ninth decade; and may or may not be associated with spontaneous iron overloading. However, ⩾40% of CSA cases remain unexplained at the molecular level.[4]

As with other rare disorders, collection and collaborative review of patient data by expert centers allows for classification based on clinical characteristics that may aid discovery of novel, genetically-defined

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disorders.[4-6] Here we describe a novel syndrome identified among a group of mutation-negative CSA patients, associated with B-cell immunodeficiency, periodic fevers, developmental delay, and other recurring multisystem features. Although the molecular basis remains under investigation, detailed review of these cases through an international collaborative effort has revealed a consistent phenotype strongly suggesting a shared etiology.

Methods

Case histories and diagnostic samples from children with CSA were sent to reference laboratories in the United Kingdom and North America for molecular studies. Subsets of mutation-negative patients presenting with coexistent B-cell lymphopenia and panhypogammaglobulinemia were identified independently by each group. Informal communication between groups acknowledged a recurring phenotype, prompting further communication internationally and subsequent recognition of a larger cohort of similar cases at other referral centers. Exhaustive literature searches failed to identify any previous reports of this combination of features. Recognition of a potential novel syndrome prompted formation of an International Collaborative Group, comprising all physicians and scientists involved in treating or investigating these cases. Additional cases were sought by retrospective review of case histories within local registries, focusing on those lacking an identified genetic abnormality. Where potentially relevant cases were found, direct communication with referring teams sought additional clinical information to support or refute a common clinical phenotype.

The aims of the collaborative group were to: (1) collate clinical information to characterize the clinical phenotype and natural history of the putative syndrome; (2) propose provisional diagnostic criteria; and (3) collect patient samples for molecular investigation (coordinated at Boston Children's Hospital). A data collection tool was designed and completed for each case from retrospective review of medical records. Details were collated centrally and clinical summaries were produced. Diagnostic criteria emerged during the course of the study and were agreed upon by consensus opinion.

Genetic testing for CSA (*ALAS2, SLC25A38, GLRX5, ABCB7, PUS1, YARS2*, mtDNA deletion), periodic fever syndromes (*MEFV, TNFRSF1A, MVK, NLRP3*), or defects in B-cell maturation (*BTK*, *λ5/14.1*) had been variously performed in different diagnostic and research laboratories involving standard techniques (sequence analysis of coding regions, exon-intron boundaries, and important 5′ and 3′ regulatory regions of the genes; full mtDNA sequence analysis and Southern blot analysis for mitochondrial DNA [mtDNA] deletion detection). In some cases, muscle biopsy cytochrome oxidase staining and other investigations for altered respiratory chain activities were performed, using standard techniques.

Results

We identified 11 children (5 male; 6 female) from 10 families resident in Europe and North America (3 United Kingdom; 1 France; 1 Portugal; 4 United States; 2 Canada) with the clinically distinctive combination of CSA and B lymphopenia and/or panhypogammaglobulinemia. An additional case (deceased brother of child 3) was added on recognition of the index child's family history and review of historical case records. Multiple ethnicities were represented, including North European Caucasian, Pakistani, and Spanish/Hispanic. All were born to apparently healthy parents following uncomplicated pregnancies. Selected demographics and results are presented in Table 1. Summaries of individual clinical courses are provided online as supplemental data (available on the *Blood* website).

Family history

Consanguineous parentage was present in only 3 families and most children had several healthy/unaffected siblings. However, the cohort did include 2 sibling pairs. Children 1 (male) and 2 (female) were born to consanguineous parents and shared the described phenotype. Pedigree analysis was complicated by presence of other siblings unaffected by severe anemia or immunodeficiency, but who displayed a different cluster of syndromic features (consistent with the Cenani-Lenz syndrome[7]) not shared with the affected children, indicating another recessive condition in this family (Figure 1). Child 3 (female) conclusively displayed CSA with documented B immunodeficiency, having had an older brother (child 4) almost certainly affected by the same condition. This sibling had been diagnosed with a severe congenital dyserythropoietic anemia many years earlier, and had died before 5 years of age. Although SA was not specifically documented, retrospective review revealed a clinical course including hypogammaglobulinemia, periodic fevers, developmental delay, seizures, lymphadenopathy, brittle hair, and avascular necrosis. Autopsy was remarkable only for adrenal hemorrhage. Clinical and laboratory details are scant and no patient material is available; however, consensus opinion was that he almost certainly shared the same multisystem CSA disorder as his fully investigated younger sister. No other familial relationships exist between any of the other included children, and no family history of sideroblastic (or other) anemia was evident in any pedigrees analyzed. In aggregate, the pedigree information is most consistent with autosomal-recessive inheritance of this condition.

Clinical presentation

Two-thirds of cases (8 of 12) presented in the neonatal period or within first 3 months of life; all had done so by 7 months except child 1, who presented at 18 months. Diagnosis of SA preceded laboratory confirmation of immunodeficiency in all cases. The manner of initial presentation was similar for most cases. Typically, it involved a febrile illness characterized by elevated inflammatory markers, poor feeding, and gastrointestinal upset, for which no infective agent could be identified and during which anemia was detected. Two children (numbers 3 and 4) were diagnosed when severe pallor was noted at birth, before the first febrile illness. Another (child 9) suffered cyclical vomiting with metabolic acidosis but without prominent fevers.

Anemia

At presentation, anemia was generally severe (median hemoglobin [Hb], 7.1 g/dL; range 4.8-8.3) and markedly microcytic (median mean corpuscular volume [MCV], 62.0 fL; range 53.6-73.2). Peripheral blood smears typically revealed hypochromasia, microcytosis, variable schistocytosis, basophilic stippling, and frequent nucleated erythrocytes. All children underwent investigation for common causes of congenital anemia, yielding identification of sickle cell trait in one (child 8) and heterozygous α^+ thalassemia in another (child 1); however, none had significant hemoglobinopathies to explain the clinical presentation. Iron deficiency, red blood cell (RBC) membrane defects, enzymopathies, and porphyrias were consistently excluded.

BM examination revealed plentiful ringed sideroblasts in all cases tested, typically representing >45% to 50% of developing erythroblasts (particularly the latest forms; Figure 2). The exception was child 4, in whom a Perls stain was not

Table 1. Selected demographics and laboratory parameters for the 12 children identified with CSA, B-cell immunodeficiency, periodic fevers, and developmental delay

Case no.	Country of birth	Year of birth	Gender	Ethnicity	Consanguinuity?	Age at presentation	Blood count (at diagnosis)		RS, % of BM erythroblasts	Iron studies (pretransfusion unless indicated by *)		Serum Igs at diagnosis, mg/dL (pre-IVIg unless indicated by *)			B-cell numbers at diagnosis, $\times 10^9$/L (% total lymphs)	Notes
							Hb, g/dL	MCV, fL		Transferrin saturation, %	Serum ferritin, ng/mL	IgG	IgA	IgM		
1†	Wales	1996	F	Pakistani	Yes	18 mo	7.2	53.6	52-65	19	139	352	20	25	0.016 (1)	α+ thalassemia trait; low IgG1 and IgG3; progressive fall in Igs, T and NK cells; suboptimal response to immunizations
2†	Wales	2005	M	Pakistani	Yes	8 wk	8.2	56.0	>75	ND	95	266	27	25	0.17 (2.8)	Progressive fall in Igs, T and NK cells; suboptimal response to immunizations
3‡	USA	1991	F	Caucasian	No	Neonatal	5.2	73.2	>50	?	41 290*	Low	Low	Normal	Low	—
4‡	USA	1985	M	Caucasian	No	Neonatal	Low	Low	ND	?	?	Low	Low	Low	ND	—
5	USA	1998	M	Caucasian	No	3 wk	7.0	61.0	Frequent	33	ND	?	?	?	?	—
6	Portugal	2003	F	Caucasian	No	Neonatal	8.3	68.0	>40	93*	1998*	90	<6	15	0.035 (2)	—
7	France	2003	M	Caucasian (Spanish)	No	7 mo	6.0	62.0	>40	ND	729	<200	23	<30	0.05 (1.5)	B-cell numbers fluctuated (acute drop during febrile episodes)
8	USA	1993	F	Caucasian (Hispanic)	Yes	3 mo	7.1	54.0	40	90*	361*	606*	58*	46*	0.16 (9)	Sickle cell trait; B-cell numbers fluctuated (acute fall during febrile episodes)
9	Canada	2007	F	Caucasian	No	7 mo	6.6	63.4	5	48	290	368	26	99	Low	—
10	Canada	2007	F	Pakistani	Yes	7 mo	8.2	71.1	>15	44	5730	70	<7	7	0.07 (2)	Progressive fall in B and T cells
11	England	2009	M	Caucasian (UK)	No	7 wk	4.8	66.0	>50	37	926	45	<7	9	0.22 (5)	Increased naive IgD^+ $CD27^-$ B cells; 'leaky' maturation arrest in B precursors
12	USA	2009	M	Caucasian (Irish/ Polish/ Lithuanian)	No	Neonatal	7.3	57.0	40	30	302	731	35	62	0.655 (17)	—

Laboratory results are the earliest recorded results available, unless otherwise stated. Missing data (indicated by ?) resulted from inability to retrospectively retrieve laboratory records despite best efforts of treating physicians; where possible, a qualitative indication is instead provided, based on available documentation and correspondence and physician recall of the case/s. Cases were numbered in the order they were included for analysis.

IVIg, intravenous immunoglobulin replacement therapy; ND, not done; NK, natural killer; RS, ringed sideroblast.

*First iron studies performed (or retrievable) were measured after commencing regular transfusion program.

† and ‡ indicate sibling pairs.

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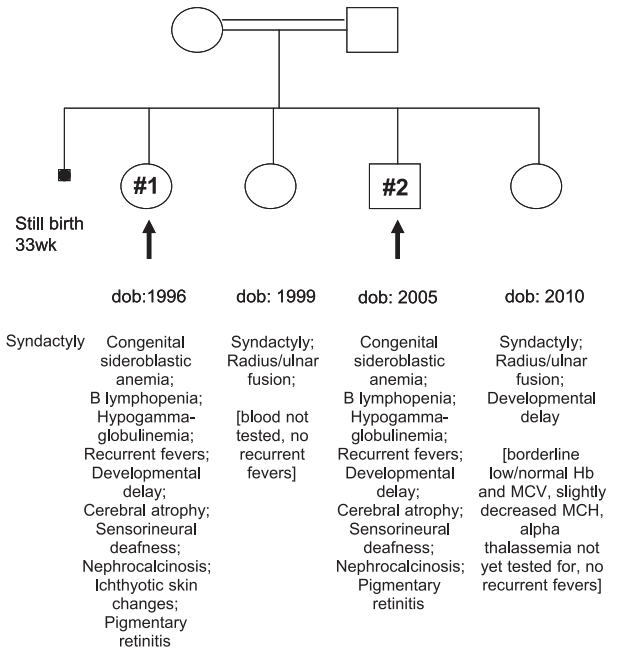


Figure 1. Pedigree for cases 1 and 2 with descriptions of phenotypes displayed. The other siblings unaffected by SA and immunodeficiency exhibited a different cluster of features consistent with the Cenani-Lenz syndrome.

performed/reported (as discussed in "Family history"). Erythroid hyperplasia and dyserythropoiesis were universal features, with deficient cytoplasmic hemoglobinization consistently reported. Other myeloid lineages appeared unaffected. BM cytogenetics showed a normal karyotype for every case.

Most children displayed hyperferritinemia that was often marked. This was in part ascribed to the inflammatory nature of presenting episodes, but also likely reflected secondary iron overload as documented on BM examination. Child 1 underwent more extensive ferrokinetic investigation that revealed rapid uptake of iron into BM but suboptimal incorporation into RBCs, together with increased serum transferrin receptor levels, indicative of dysfunctional iron metabolism and ineffective erythropoiesis. Elevated transferrin receptor was noted in at least 1 other case (child 12). Two children (numbers 6 and 8) had grossly elevated transferrin saturation on first biochemical iron studies. Both had only been transfused to a modest degree and iron loading was attributed at least in part to increased gut absorption, as observed in certain other CSAs.

Periodic fevers

A recurrent fever syndrome requiring multiple hospitalizations through infancy and early childhood was documented in at least 11 cases. Episodes were characterized by high fevers, systemic upset, and elevated inflammatory markers, with repeated investigation failing to attribute an infective cause; vomiting and diarrhea were prominent features. In some individuals the documented frequency and duration of episodes were remarkably consistent, suggesting a periodic fever syndrome. For example, child 11 was hospitalized for fever at predictable 3- to 4-week intervals, with resolution within 5 to 7 days apparently uninfluenced by antibiotics. Periodicity varied between individuals and in some cases declined or increased in frequency over time. Child 2, for instance, initially suffered febrile episodes every 6 days, each lasting 4 to 5 days, but over several years the interval between attacks extended to >2 months.

Immunodeficiency

Recurrent inflammatory episodes prompted screening for coexistent immunodeficiency, which was investigated in different ways and at different stages across the cohort. Some individuals also suffered episodes of sinopulmonary bacterial infection, clinically distinct from the periodic fever episodes. Eleven children examined displayed significant B-cell lymphopenia and hypogammaglobulinemia. Serum immunoglobulin (Ig) levels at diagnosis varied considerably, reflecting differences in both severity of clinical phenotype and age when first tested (3 months to 5 years), given the different age-specific reference ranges and potentially confounding influence of maternal IgG transfer. When first tested, IgG levels ranged from severely deficient to normal, with frank panhypogammaglobulinemia developing later in the more mildly affected children. Where IgG subclasses were quantified, IgG1 and IgG3 levels were relatively lower than IgG2 and IgG4. Review of serial Ig measurements is further confounded by the influence of regular intravenous Ig (IVIg) replacement therapy in most cases; even so, a progressive decline in serum Ig levels over time was typically observed.

The pattern of hypogammaglobulinemia was mirrored by peripheral blood B-cell ($CD19^+$) quantification. B lymphopenia was a unifying feature (range $0.016\text{-}0.22 \times 10^9$/L; normal ~0.6-3.0), in the context of an initially normal total lymphocyte count. In several cases, B-cell numbers fluctuated, dropping to nadir levels during inflammatory crises but partially recovering between attacks. While B-cell numbers were significantly reduced, other lymphocyte classes were initially preserved but progressively fell, resulting in profound B, T, and NK lymphopenia (Figure 3). The degree of hypogammaglobulinemia and B lymphopenia at presentation broadly correlated with anemia severity and prognosis, irrespective of when in the disease course these were first measured. Additionally, several children failed to mount durable serological response to both conjugated and unconjugated vaccinations.

The nature of child 11's immunodeficiency was investigated in particular detail. In addition to low circulating B cells and panhypogammaglobulinemia, skewed B-cell maturation was identified in peripheral blood: analysis of B-cell maturation showed the vast majority of circulating B cells (~90%) to be naive $CD27^-/IgD^+$, with class-switched $CD27^+/IgD^-$ B cells representing only 4%, suggesting a relatively early defect in B-cell maturation. Detailed maturation analysis by flow cytometry of BM samples revealed the presence of early B precursors but with a "leaky" maturation arrest before the cytoplasmic $Ig\mu^+$ pre-B-II stage (Figure 4).

The only child without documented hypogammaglobulinemia or B lymphopenia was child 12, in whom both parameters tested early in life were in the low-normal range. However, he has suffered unexplained and severe recurrent febrile episodes from infancy, in the context of a severe microcytic, mutation-negative CSA. His inclusion within the cohort was carefully considered but agreed upon by consensus on review of clinical records. Currently 3 years of age, he is being recalled for repeat immunology investigation.

Developmental delay

Developmental delay was observed in all children except child 11, who underwent BM transplantation (BMT) at 9 months having yet to demonstrate any significant delay; he continues to meet normal milestones posttransplant. Although variably manifested and documented, delay was typically significant and

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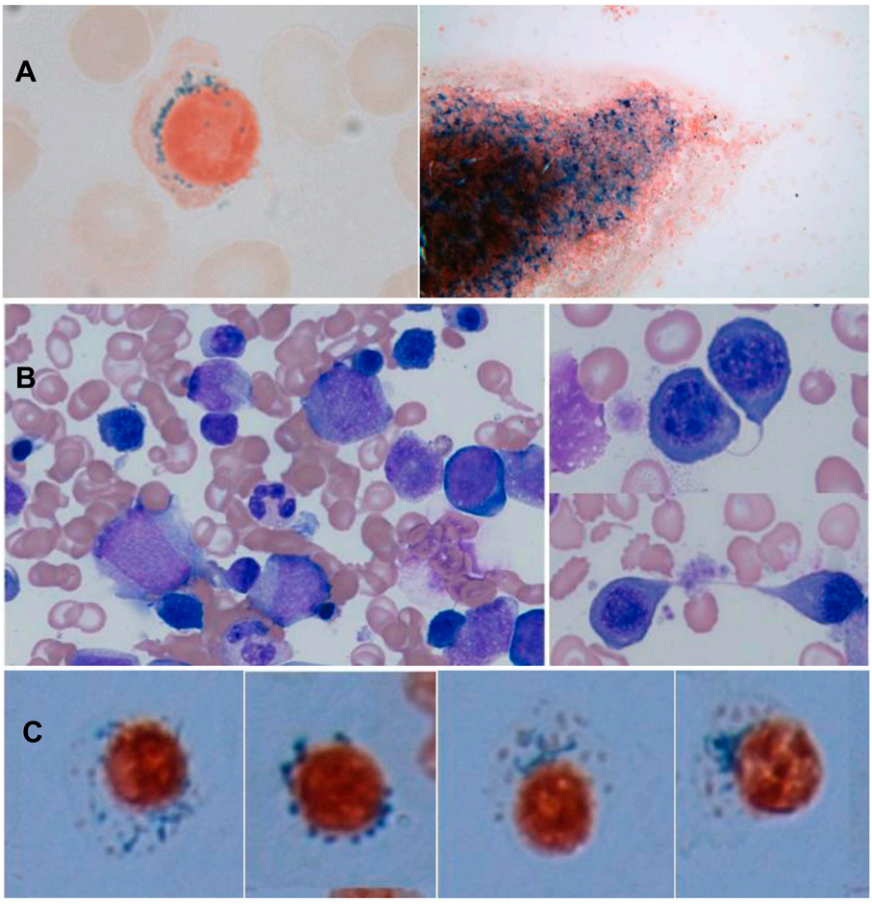


Figure 2. Photomicrographs of BM smears, demonstrating presence of ringed sideroblasts and erythroid hyperplasia with dyserythropoietic features. (A) Case 1 (Perl stain). (B) Case 11 (H&E). (C) Case 11 (Perl stain).

sufficiently alarming to prompt further neurological investigation. Generalized and truncal hypotonia were recurring features, often severe, progressive, and associated with gross motor developmental delay. Comprehension and communication were profoundly impaired in many children. In keeping with his milder immunodeficiency phenotype child 12's developmental delay was relatively modest, essentially confined to delayed speech development; at latest follow-up, he remains developmentally normal but under close observation.

Additional syndromic manifestations

The similarities in clinical presentation demonstrate a convincing link between the cases described. In addition, most members of

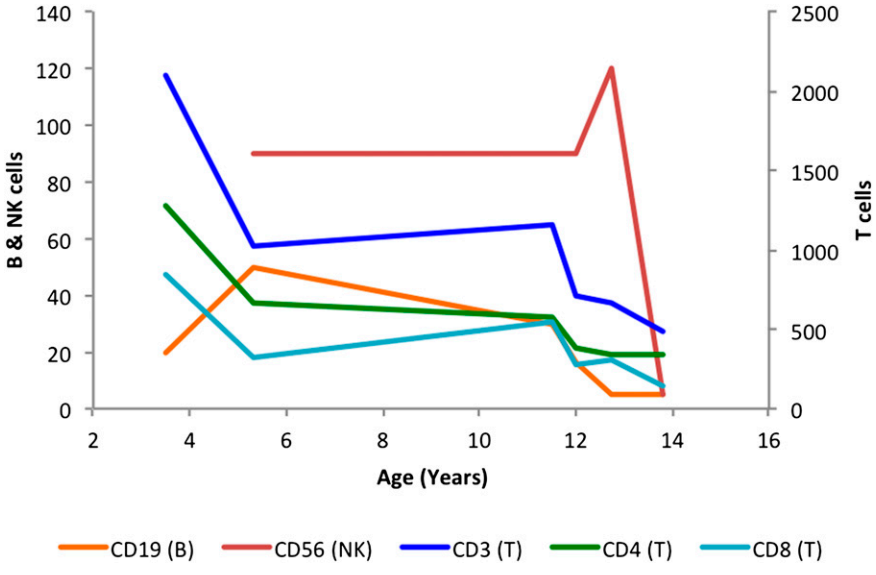
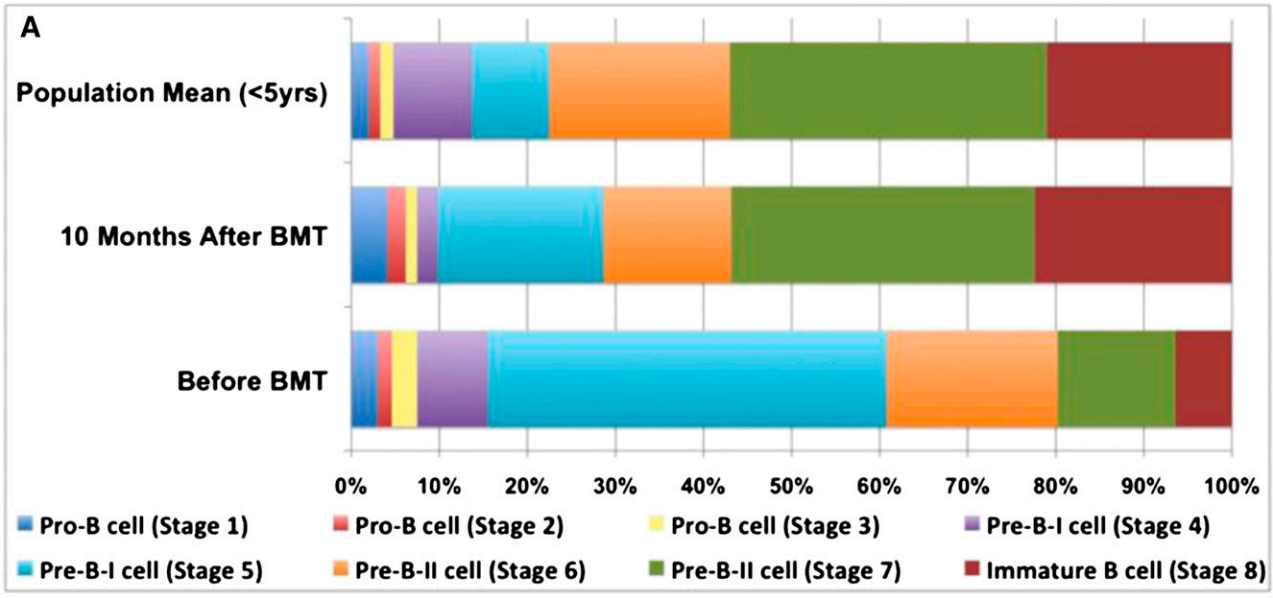


Figure 3. Graph showing decline in circulating B-, NK-, and T-cell numbers in case 1 with increasing age. This child died during the 15th year of life, at which point she displayed profound lymphocytopenia of all 3 lymphocyte classes (units are in cells per microliter).

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B	% Positivity Pre-Transplant	% Positivity Post-Transplant
Pro-B-cell (CD22+/CD79+/CD19–)		
- Stage 1	2.6%	3.6%
- Stage 2	1.4%	1.8%
- Stage 3	2.5%	1.1%
Pre-B-I-cell (CyIgμ–/CD19+)		
- Stage 4	6.9%	2.0%
- Stage 5	**39.0%**	**16.6%**
Pre-B-II-cell (CyIgμ+/CD19+)		
- Stage 6	16.7%	12.7%
- Stage 7	11.5%	30.2%
Immature B-cell (SmIgM+/SmIgD–)		
- Stage 8	5.5%	19.6%
Mature B-cell (SmIgM+/IgD+)		
- Stage 9	14.4%	16.8%

Figure 4. Detailed B-cell maturation analysis performed on BM from case 11. (A) Bar chart displays proportion of B cells at different stages of maturation within the BM compartment as determined by flow cytometric analysis. The bottom bar is from child 11 aged 8 months, demonstrating a leaky maturation block before the cytoplasmic Igμ-positive pre-B-II cell stage. The middle bar shows repeat analysis in the same patient 10 months after BMT, at which time the pattern had reverted to that observed in age-matched normal controls (top bar). (B) Relative proportions of B cells at different maturation stages in BM samples before and after BMT (analysis performed by M. van der Burg and J. J. M. van Dongen [Erasmus Medical Center, Rotterdam, The Netherlands]).

the cohort displayed other variable but recurring multisystem abnormalities (summarized in Table 2):

Sensorineural hearing impairment. At least 5 children developed severe bilateral sensorineural deafness. One received bilateral cochlear implants.

Recurrent seizures. Five children suffered recurrent seizures early in life. These ranged from partial complex to generalized tonic-clonic, displaying a variety of related electroencephalography (EEG) abnormalities.

Other neurological/neuromuscular abnormalities. In addition to those features already mentioned, gross ataxia and other cerebellar signs occurred in several cases. Neuroimaging variously revealed cerebral atrophy, delayed cortical white matter myelination, abnormal enhancement of external capsule and thalamus, communicating hydrocephalus, and cerebellar abnormalities including decreased perfusion.

Nephrocalcinosis/renal tubular dysfunction. Three children developed renal calculi at an early age: one (child 9) underwent thorough metabolic investigation and was found to have significant hypercalciuria (3.0 mol/mol Cr; normal range 0.08-0.6). A fourth (child 3) was diagnosed with renal tubular Fanconi syndrome, a renal tubular acidosis frequently associated with nephrocalcinosis; this resulted in chronic hypokalemia and hypophosphatemia although no actual calculi were documented during life. Most children, however, were not specifically evaluated for these entities.

Aminoaciduria and other metabolic abnormalities. Six children displayed excessive generalized aminoaciduria, with increased urinary metabolites of the tricarboxylic acid pathway additionally detected in child 2. One (child 8) had grossly elevated plasma alanine levels, which have decreased over time. In no cases were abnormalities diagnostic of any specific inborn error of metabolism.

Table 2. Summary of clinical phenotype for each of the cases in the cohort

	Case no.											
Clinical feature	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Microcytic anemia	+	+	+	+	+	+	+	+	+	+	+	+
SA	+	+	+	ND	+	+	+	+	+	+	+	+
B lymphopenia and/or hypogamma globulinemia	+	+	+	+		+	+	+/−	+/−	+	+	−*
Recurrent inflammatory epsidodes	+	+	+	+	+	+	+	+	+†	+	+	+
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	−*
Sensorineural deafness	+	+	−		−	+	−	+	+	−	−	−
Ataxia/cerebellar signs	−	−				+	+	+	+		−	−
Seizures	−	−	+	+	+	−	+	+	−	−	−	−
Neuroimaging abnormalities	Cerebral atrophy	Cerebral atrophy	Cerebral atrophy; decreased cerebellar perfusion		Communicating hydrocephalus; macrocephaly	−	−	Cerebral atrophy; abnormal enhancement of external capsule and thalamus	Delayed white matter myelination	−	−	−
Nephrocalcinosis	+	+	−					−	+		−	−
Aminoaciduria ± hyperalaninemia	+	+	+	−			+	+	+		+	−
RP	+	+				+	−	−			+	−
Cardiomyopathy (diagnosed during life)	−	−	−			+	−	−		+	−	−
Splenomegaly	−	−	+/−		+		+/−	+/−	−	−	−	−
Lactic acidosis	+	+		−							+	
Brittle hair			+	+								+
Other features	Ichthyotic skin changes; abnormal high amplitude slow activity on EEG	Increased urinary TCA intermediates	Renal tubular Fanconi syndrome	Malabsorption; small bowel villous atrophy; avascular necrosis (hip)	Pancreatic insufficiency			Hypercalciuria	Torticollis	Hypertension	Nonspecific metabolic myopathy on muscle biopsy	

Detailed clinical vignettes describing each child's clinical course are provided online as supplemental data.

+, feature confirmed during life; −, feature confirmed to be absent during life or at latest follow up; +/−, borderline/mild presence of abnormality or clinical feature; blank cells, insufficient information, usually as feature was not specifically investigated or considered during life; ND, BM ringed sideroblastosis not confirmed but highly likely given similarities to older sibling with confirmed CSA (discussed in "Family history").

*Low normal results when tested early in life; see "Immunodeficiency" section for explanation of rationale for child's inclusion within cohort.

†Clinical course characterized by recurrent inflammatory episodes with gastrointestinal upset and raised inflammatory markers, although high fevers were not recalled as a prominent feature.

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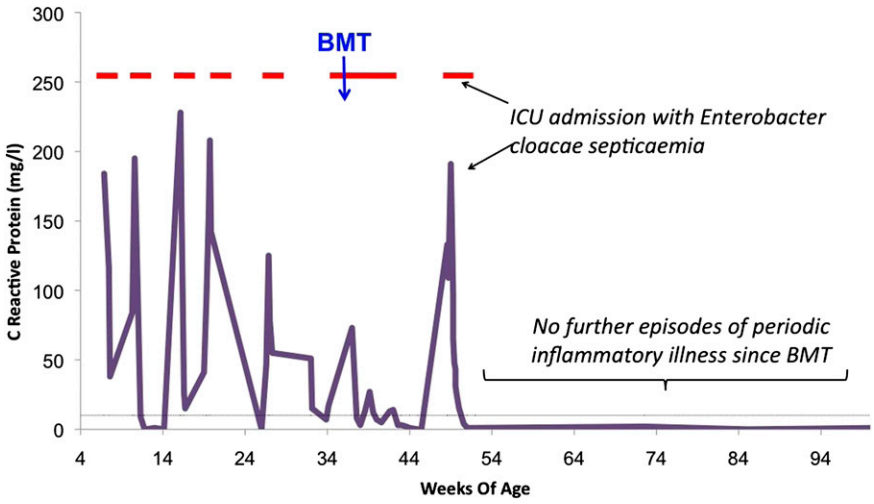


Figure 5. Graphical representation of selected clinical events in child 11's early disease course. Line graph traces serial C-reactive protein levels, illustrating recurrent inflammatory episodes occurring with periodicity of 4- to 6-week intervals; red lines represent periods of hospitalization. Other than a single early episode of *Enterobacter* septicemia, BMT appears to have effectively cured the immunologic and fever components of the disease as well as the SA.

Most displayed further nonspecific metabolic and biochemical abnormalities but with no clear pattern, including hyperglycemia and chronic hypokalemia, hypophosphatemia and hypocalcemia. Metabolic acidosis was a common feature, particularly during acute febrile crises.

Cardiomyopathy. Two children were diagnosed with moderate or severe symptomatic dilated cardiomyopathy during second year of life; notably, both had expressed a particularly severe hematologic/immunologic phenotype. Moreover, cardiac failure was the primary or a contributing cause of death in at least 5 of the 7 deceased children. Monitoring revealed only modest cardiac and systemic iron overload, indicating that cardiac disease (typically diagnosed within the first 2 years, or causing death within 5 years of life) was not directly attributable to pathological effects of iron overload.

Pigmentary retinitis. At least 4 children were diagnosed with a retinitis resembling retinitis pigmentosa (RP); in one, this manifested as the atypical RP variant retinitis punctata albescens. However, again most were not screened for this.

Other features. Several other abnormalities were noted in isolated cases and their relevance to the syndrome remains unclear. Hepatosplenomegaly was documented in 4 children but when present was typically mild. Brittle hair was noted in 3 cases. Another (child 1) displayed chronic ichthyotic skin changes, punctuated with eruptions of erythema and/or hypopigmentation. Skin biopsy revealed a perivascular lymphohistiocytic infiltrate within papillary dermis but lacked specific diagnostic features; electron microscopy showed small foci of fibrillar material resembling amyloid. One child (number 11) had detailed investigation of mitochondrial respiration in muscle tissue, revealing moderately reduced activity of respiratory chain complex I with borderline low complex II and IV activity. Muscle histology revealed accentuated staining for lipid and the oxidative enzymes cytochrome oxidase, nicotinamide adenine dinucleotide hydrogen dehydrogenase and succinate dehydrogenase, suggesting a nonspecific metabolic myopathy.

Clinical interventions

Ten children required regular or intermittent RBC transfusional support from infancy or early childhood. This resulted in secondary transfusional iron overload necessitating iron chelation therapy in surviving cases. The other 2 displayed a milder phenotype in which anemia remained stable without clinical indication for regular transfusion. Pyridoxine was administered to most children on recognition of CSA, with no discernible impact on Hb, symptoms, or transfusion requirement. Other dietary supplements (eg, thiamine, riboflavin) were attempted in certain cases, without clinical benefit. Ten children received regular or intermittent IVIg replacement therapy. This was observed in some cases to reduce bacterial sinopulmonary infections but had minimal influence on the "sterile" periodic fever episodes. One (child 1) received anakinra, which was successful in alleviating the febrile episodes; however, development of allergy necessitated treatment discontinuation.

One child (number 11) underwent myeloablative allogeneic BMT from an unrelated donor at 9 months of age. He was heavily transfusion dependent since presentation at 7 weeks and subsequently suffered debilitating periodic fevers requiring frequent hospitalization. Recognition of other cases forming this cohort suggested an unacceptably poor life expectancy. Moreover, at 9 months of age, organ function remained good, permitting myeloablative transplantation with busulfan, cyclophosphamide, and alemtuzumab conditioning. Full donor chimerism was confirmed by day +28 and is maintained beyond 3 years posttransplant. Around day +100, he became unwell with an episode initially mimicking his pretransplant "sterile" inflammatory crises; on this occasion, *Enterobacter cloacae* was isolated from blood cultures and he responded to appropriate antibiotics. He remains clinically well with normal blood count and serum Ig levels, normal growth and development, and no admissions for unexplained inflammatory illness since transplant (Figure 5). However, 32 months posttransplant, a pigmentary retinitis was detected on routine surveillance. He has yet to declare any other syndromic manifestations.

Outcome

The pace of progression and prognosis reflected the considerable heterogeneity of the disorder, with distinctly more and less severe phenotypes observed. At time of publication, 7 of the children have died (58%), at median 4 years of age (range 16 months to 14 years). Those more severely affected failed to survive beyond the third year of life, having suffered severe transfusion-dependent anemia and very low B-cell numbers and Ig levels from diagnosis, with cardiomyopathy seemingly associated with poorer prognosis and early death. Cases 3 and 1 survived until age 7 and 14 years,

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Table 3. Summary of selected interventions attempted and outcome for each of the children within the cohort

	Interventions			Outcome		
Case	Regular transfusions	IVIg replacement	BMT	Alive/Dead	Age at death	Terminal event/Cause of death
1	Yes	Yes	No	Dead	14 y	Sepsis with multiorgan failure and toxic epidermal necrolysis (attributed to a cephalosporin)
2	No	Yes	No (awaited)	Alive (7 y)	n/a	n/a
3	Yes	Yes	No	Dead	61 mo	Multiorgan failure
4	Yes	Yes	No	Dead	4 y	Admission with seizures, hypokalemia and fevers; rapid onset multiorgan failure; adrenal hemorrhage on autopsy
5	Yes	?	No	Dead	56 mo	Arrived in shock with severe hypoglycemia; died within hours in multiorgan failure
6	Yes	Yes	No	Dead	25 mo	Cardiac failure secondary to cardiomyopathy (not related to iron overload)
7	Yes	Yes	No	Dead	16 mo	Multiorgan failure (pneumonitis; cardiac failure)
8	Yes	Yes	No	Alive (19 y)	n/a	n/a
9	No	No	No	Alive (5 y)	n/a	n/a
10	Yes	Yes	No	Dead	28 mo	Cardiac failure secondary to cardiomyopathy (not related to iron overload)
11	Yes*	Yes*	Yes (aged 9 mo)	Alive (4 y)	n/a	n/a
12	Yes	No	No	Alive (45 mo)	n/a	n/a

n/a, not applicable.
*Child 11's transfusion dependence and immunodeficiency fully resolved after BMT.

respectively, despite both suffering significant neurodegenerative complications from early childhood. All deaths were from cardiac or multiorgan failure, most in the context of sepsis unresponsive to broad-spectrum antibiotics. Of the 5 surviving children, 3 have displayed a noticeably milder phenotype: 2 (numbers 2 and 9) without regular transfusion requirement who remain alive in the eighth and sixth years of life, respectively, and another (child 12) with a noticeably milder immunodeficiency. The longest surviving child remains alive aged 19 years, but with significant neurological impairment and transfusional iron overload. Child 11 remains alive into the fifth year of life, 41 months post-BMT; the hematological and immunological manifestations have apparently been completely corrected. Interventions and outcomes are summarized in Table 3.

Discussion

We describe a novel syndrome of CSA, B-cell lymphopenia, panhypogammaglobulinemia, periodic fevers, and developmental delay, with additional recurring syndromic features. The similarities in phenotype suggest a probable shared etiology. Following recognition of these features, the search for additional cases has been remarkably successful, suggesting the likelihood that further cases exist.

The defining hematologic feature is that of severe microcytic CSA, usually presenting in infancy and conferring transfusion dependence. SAs are a heterogeneous group of inherited or acquired anemias with the shared hallmark of the ringed sideroblast in BM. Ringed sideroblasts are pathological erythroblasts with a ring of perinuclear, iron-positive granules that represent iron-laden mitochondria, reflecting disrupted iron metabolism.[8] Genetically defined CSAs are uncommon or extremely rare, have a variety of inheritance patterns and may result from an erythroid-specific defect or as part of a multisystem syndrome.[1-3] None of the previously defined causes of CSA is associated with immunodeficiency.

The archetypal "pure" SAs result from defects in heme biosynthesis, causing mitochondrial siderosis, microcytic anemia, and secondarily progressive systemic iron overload.[2,9] X-linked SA (XLSA) is the most common and results from mutations in *ALAS2,* with new mutations continuing to be described.[10] Nevertheless, XLSA accounts for <40% of cases of CSA[4] and was an unlikely explanation in our cohort given the purely erythroid-specific expression of ALAS2. Nonetheless *ALAS2* mutations were excluded in all cases. A more attractive candidate was SLC25A38, which encodes a putative inner mitochondrial membrane glycine importer. Recessive SLC25A38 mutations cause a phenotypically similar SA,[5,6] with failure to deliver glycine having a similar impact on heme synthesis as *ALAS2* deficiency. Intriguingly, the second highest tissue expression (after the erythron) is in $CD19^+$ B cells.[5] However, SLC25A38 sequencing was normal in our cohort.

Mitochondria also serve as a major site of Fe-S biogenesis.[11,12] Functional defects in this system cause severe mitochondrial iron overload, defective activity of Fe-S–dependent enzymes and oxidative damage, explaining several syndromic forms of SA. Autosomal-recessive *GLRX5* defects can cause a microcytic SA, although the single described case had far milder anemia and fewer ringed sideroblasts than our cohort, presenting in the fifth decade of life.[13,14] Missense mutations of ABCB7 result in the XLSA and ataxia syndrome (XLSA/A),[15-17] of potential interest given the cerebellar abnormalities observed in several of our cases. However, neither gene has any reported impact on lymphopoiesis and mutations were excluded by sequence analysis.

Another defining feature of our syndrome was clinically significant immunodeficiency with panhypogammaglobulinemia and/or B lymphopenia, often profound and present from an early age. Because this is not a feature of any described CSA syndrome, coincidental occurrence of a separate cause for immunodeficiency was considered. However, the reduction in mature $CD19^+$ B cells in peripheral blood and early onset argue against coincidental common variable immunodeficiency, the most common cause of

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selective B immunodeficiency, which generally presents later in life with normal circulating mature B-cell numbers.[18] The leaky maturation block within the B-precursor compartment at the pre-B-II stage (Figure 4) most closely resembles that observed in X-linked agammaglobulinemia, which results from mutations in Bruton tyrosine kinase (Btk) and accounts for 85% of patients with defects in early B-cell development.[19] However, direct sequencing of BTK gene coding exons, splice sites, and BTK transcript products failed to identify any mutations; likewise for the λ5/14.1 gene, which encodes a component of the pre-B-receptor complex transiently expressed by B precursors, deficiency of which causes a similar phenotype.[20,21] Pertinently, SA has not been described with any congenital immunodeficiency syndrome. Moreover, the progressive decline in T and NK cells seen in several cases suggests a more general impact on lymphopoiesis than a purely selective B-cell maturation defect.

Another recurring feature was periodic episodes of fever and inflammation, clinically resembling acute sepsis but typically lacking a causative organism and associated with gastrointestinal upset. Several cases documented remarkably consistent periodicity, cycling every 2 to 4 weeks and resolving within 5 to 7 days. This pattern resembles that observed in the periodic fever syndromes: autoinflammatory disorders arising from mutations (usually autosomal recessive) in genes regulating interleukin-1β secretion and other elements of innate immunity.[22-24] However, these have no documented (or intuitive) impact on B-cell maturation and direct sequencing of relevant genes excluded familial Mediterranean fever (MEFV), TNF-receptor associated period fever (TNFRSF1A), hyperimmunoglobulin D syndrome (MVK), and the cryopyrinopathies (NLRP3) in several cases.

The patients displayed additional nonhematologic/immunologic manifestations. Most common was progressive neurodegeneration and developmental delay, often with other neurologic, cardiologic, metabolic, and other multisystem abnormalities. These disparate features recall syndrome complexes associated with the mitochondrial cytopathies (MCs), a diverse group of disorders characterized by impaired mitochondrial respiratory chain function and energy production. Mitochondrial proteins are the products of both nuclear DNA (nDNA) and separately inherited mitochondrial DNA (mtDNA), with the latter displaying up to 100-fold higher mutation rate.[25] mtDNA contains 37 genes encoding 13 mitochondrial proteins (subunits of respiratory chain enzyme complexes), 22 transfer RNA (tRNA) species, and 2 ribosomal RNAs.[26,27] Mutations of mtDNA and related nDNA genes can cause major disruption of mitochondrial biogenesis and function, resulting in distinct multisystem disorders with protean (but overlapping) clinical features.[28-30] Neurological involvement is frequent, including neurodegeneration, ataxia, sensorineural deafness, and seizures.[26] Lactic acidosis, hyperalaninemia, renal tubular dysfunction, nephrocalcinosis, RP, and brittle hair are also common features, resembling the spectrum of abnormalities seen in our cohort.

Hematologic manifestations are less prominent but do feature in some MCs,[31] including rare syndromic CSAs. Large mtDNA deletions are responsible for Pearson marrow-pancreas syndrome, characterized by exocrine pancreatic dysfunction with SA and BM failure.[2,32] Nearly half display heteroplasmy for a canonical 4977-bp mtDNA deletion, although others have different, occasionally nonoverlapping deletions removing tRNA species required for mitochondrial translation.[33] However, the anemia is typically macrocytic, with other cytopenias and characteristic vacuolization of early erythroid/myeloid precursors. Moreover, pancreatic abnormalities were not prominent in our cohort. Large mtDNA deletions can alternatively (or eventually) cause the distinct Kearns-Sayre syndrome, characterized by progressive ophthalmoplegia, RP, cardiac conduction defects, cerebellar ataxia, deafness, myopathy and endocrinopathies. SA can feature, but despite phenotypic overlap with our cohort the anemia is typically normo-/macrocytic and less severe. Significant mtDNA deletions, mutations and rearrangements were excluded by direct sequencing of the entire mitochondrial genome in most of our cases. Furthermore, immunodeficiency is not a typical feature of most MC syndrome complexes.

However, precedent for MCs involving perturbed immune function does exist, with a neonatal-onset mitochondrial respiratory chain disease associated with mtDNA depletion and progressive T-cell immunodeficiency described. Additional features included cytopenias, psychomotor retardation, axial hypotonia, hypoplasia of corpus callosum, and impaired myelination.[34] Moreover, the lack of overt mtDNA depletion in our cases does not exclude a MC. Most genes involved in mitochondrial protein synthesis, expression, and function are nuclear in origin, with >100 nDNA genes implicated in various MCs.[29] Indeed, nDNA defects account for >80% of pediatric MC cases (>50% in adults).[35] A pertinent association between defective mitochondrial protein expression and CSA is provided by the mitochondrial myopathy with lactic acidosis and SA (MLASA) syndrome. This was first identified in patients of Persian-Jewish descent with recessive mutations of *PUS1* (on chromosome 12q24.33), resulting in decreased pseudouridylation of mitochondrial tRNAs, reduced tRNA stability/function, and impaired protein translation.[36,37] Clinical expression is variable but typically manifests with myopathy, lactic acidosis, SA, and occasionally with mental retardation and dysmorphia. Biochemical investigation may reveal decreased activity of respiratory chain complexes I to IV.[2] A similar syndrome is described in patients with mutations of *YARS2* (chromosome 12p11.21), which encodes an enzyme (tyrosyl-tRNA synthetase) that catalyses binding of tyrosine to its cognate tRNA. Reduced enzymatic activity results in decreased mitochondrial protein synthesis and mitochondrial respiratory chain dysfunction involving complexes I, III, and IV.[38] Some patients developed cardiomyopathies. Neither *PUS1* nor *YARS2* mutations were found in our cases, and important phenotypic differences remain: notably, immunodeficiency is not described in MLASA. However, alternative nuclear genes important in the synthesis, function, and maintenance of tRNA and mitochondrial protein translation seem logical candidates and are currently being investigated. The primary defect in our syndrome must embrace both B-cell development and mitochondrial iron metabolism, in an as yet-undefined manner.

This study is limited by its retrospective nature. The patients had been diagnosed and investigated sporadically, in different centers and countries, most long before initiation of this project. Consequently, a standardized, consistent approach to investigating all aspects of this multisystem disorder was impossible. Moreover, given that some historical cases were managed at multiple centers, comprehensive and specific clinical details were sometimes difficult to obtain. This resulted in considerable variation in amount and quality of information gathered. Although these cases cannot yet be formally unified by a shared genetic insult, all have undergone detailed central review with consensus agreement of sufficient similarity to warrant collation and reporting as a single clinical entity at this time. Laboratory investigation on patient material is ongoing.

Until the molecular basis of this syndrome is formally established we propose naming the syndrome *"Sideroblastic anemia*

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with immunodeficiency, fevers and developmental delay" (SIFD), with the following diagnostic criteria:

Required

(1) SA: which is severe, microcytic, and of early onset
(2) B-cell immunodeficiency: panhypogammaglobulinemia and/or absolute reduction in mature $CD19^+$ B cells in peripheral blood
(3) Fevers: which are recurrent and associated with systemic inflammation in the absence of an identifiable infective cause
(4) Developmental delay

Additional features that may be present

(1) Growth retardation
(2) Sensorineural deafness
(3) Seizures
(4) Cerebellar signs
(5) Cerberal hemisphere and/or cerebellar neuroimaging abnormalities
(6) Dilated cardiomyopathy
(7) Nephrocalcinosis
(8) Aminoaciduria
(9) RP
(10) Brittle hair
(11) Hepatosplenomegaly

Conclusion

We describe a novel syndromic form of early onset, severe CSA associated with B immunodeficiency, periodic fevers, and developmental delay. Additional features reveal a multisystem disorder reminiscent of a MC but without evidence of mtDNA depletion and with some atypical features. Regular blood transfusion, IVIg replacement, and iron chelation are likely required treatments but have had little impact on quality-of-life determinants. Mortality within the first decade of life was high, typically in the context of acute sepsis without positive microbiology and unresponsive to broad-spectrum antibiotics. This might implicate the cytokine storm as contributing to these terminal events, suggesting a potential role for alternative interventions (eg, interleukin-1 blockade, employed with some success in 1 case). However, there is evidence of variable penetrance. Two affected siblings have demonstrated somewhat different clinical outcomes, and some cases have shown a distinctly milder phenotype with survival into teenage years and/or less intensive transfusion requirement. The only child to receive allogeneic BMT did so after careful consideration of his severe disease expression, and engraftment of donor hematopoiesis has successfully reversed the hematologic and immunologic manifestations. He has since developed retinitis but remains otherwise well more than 3 years posttransplant. This provides rationale for considering early BMT in other cases and emphasizes the importance of early recognition/diagnosis. To what extent multisystem involvement is preventable by BMT remains unknown.

Although a rare syndrome, the relative ease with which 12 cases have been identified in a short period of time suggests numerous other children may be affected. Diagnosis of this multisystem disorder requires specialist hematology and immunology investigations (eg, Perls examination of BM; B-cell enumeration) and given the diverse features, with variable expression, it might present to a range of specialists. Awareness of SA as a potential explanation for the anemia is particularly important because it may not be routinely considered alongside more familiar causes of microcytosis. Moreover, certain features (eg, retinitis; aminoaciduria) may not be clinically apparent and without specific investigations would remain undetected. Our study represents a first step in raising this awareness. We have established a database and welcome correspondence from colleagues recognizing similar cases. Work is ongoing to establish the molecular basis of the disorder using primary patient material and cell lines derived from these children.

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Authorship

Contribution: R.F.W. and D.H.W. conceived the project and identified cases referred from European centers, in collaboration with A.M.; concurrently, M.D.F. and M.M.H. identified a similar pattern of cases referred from several North American centers and compiled a similar database, in collaboration with the referring physicians; all authors were involved in the initial establishment of the International Collaborative Group; D.H.W. created the data collection proforma, provided clinical information for case 11, collated and analyzed returned information for all cases, and wrote the final manuscript and clinical vignette for case 11; S.J., P. Connor, C.P., P.J.G, R.J.K., P. Chakraborty, M.T.G., N.M.-C., C.K., I.T., A.A.T., L.M., S.H., and S.S.B. contributed cases with which they had direct clinical involvement, providing information through completion of a data collection proforma, drafting of clinical vignettes, and direct communication with D.H.W.; M.D.F. and A.M. were primarily responsible for molecular analysis for known CSA mutations on samples from the North American and European children, respectively; M.D.F. is coordinating the central database of putative SIFD cases and the work investigating its molecular basis using patient-derived cell lines and other primary material from these cases; and all authors reviewed and provided revisions of the full manuscript in the drafting process.

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Correspondence: Daniel H. Wiseman, Department of Haematology, Royal Manchester Children's Hospital, Oxford Rd, Manchester M13 9WL, United Kingdom; e-mail: dwiseman@picr.man.ac.uk.

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