



## Introduction

In 1959, De Vaal and Seynhaeve<sup>1</sup> suggested the term “reticular dysgenesia” (RD) for a condition that they observed in newborn boy twins who had neither lymphocytes nor granulocytes in the peripheral blood and who both died within the first week of life from suspected bacterial infections. Lymph nodes, spleen, and thymus were devoid of lymphocytes, and the bone marrow showed a failure of myeloid maturation with a developmental arrest at the promyelocytic stage. With reticular cells being abundantly present in these tissues, the authors hypothesized that the disease originated from a failure of a “multipotent primitive reticular cell” to “develop into the mother cells of the myeloid series, or into lymphocytes and monocytes.” With a proportion of <2%, RD is a very rare SCID entity, and since its first description, ~20 patients have been reported in small series or in single case reports.<sup>2</sup> Besides the typical combination of T<sup>-</sup>B<sup>-</sup>NK<sup>-</sup> SCID and agranulocytosis, patients with RD were noted to suffer from a profound sensorineural hearing deficit.<sup>3</sup> Early hematopoietic stem cell transplantation (HSCT) was described as the only curative therapeutic option for this condition.<sup>3-6</sup> In 2009, mutations in the gene encoding adenylate kinase 2 (AK2) were identified as the molecular basis of the disease by 2 independent groups.<sup>7,8</sup> Adenylate kinases are phosphotransferases that are involved in intracellular energy transport by providing a shuttle system for high-energy phosphorylated adenine nucleotides. AK2 is a protein involved in the energy transfer in the mitochondrial intermembrane space that catalyzes the reaction ATP (adenosine triphosphate) + AMP (adenosine monophosphate) ⇒ 2ADP (adenosine diphosphate).

Patients with RD develop life-threatening infections very early in life, usually within the first days after birth. Immediate diagnosis and decisive therapeutic interventions are mandatory to offer a curative option in this otherwise fatal disease.

This survey was performed to comprehensively collect data on clinical presentation, transplantation, and outcome to create an objective basis for therapeutic decisions in this very rare disease.

## Patients and methods

Patient data were collected in an international survey between November 2010 and November 2015. Participating centers were recruited from repeat presentations of the project in annual meetings of the Inborn Errors Working Party of the European Group for Blood and Marrow Transplantation as well as the biannual meetings of the European Society for Immunodeficiency. In addition, major transplant centers in North America were contacted directly. Criteria to be included in the study were diagnostic findings consistent with severe combined immunodeficiency in combination with primary agranulocytosis. A questionnaire was completed by participating centers. Furthermore, if not already done, sequencing of AK2 was offered at the Institute for Clinical Transfusion Medicine and Immunogenetics Ulm (Ulm, Germany). Written informed consent was obtained from all families according to the regulations of local review boards.

Graft failure was defined as a situation in which neutrophil counts remained <500/μL after transplantation (primary) or fell below this threshold after initial recovery (secondary) irrespective of donor T-cell engraftment. Retransplantation included either an additional transplant procedure with conditioning or a change in the donor. A stem cell transfusion from the same donor and without chemotherapy was not defined as a repeat transplantation, but as a boost.

Chimerism analysis included phenotypic (HLA flow cytometry, red blood cell flow cytometry, and XY-FISH analysis) and genetic (STR-analysis of full blood or sorted subpopulations) methods.

Statistical analysis of overall survival was performed with a Kaplan-Meier survival analysis with 95% pointwise confidence intervals. Statistical significance

of differences in survival of defined groups was tested with a log-rank test. The significance level was set at 5%.

## Results

### Clinical presentation before HSCT

We received complete data sets of 32 patients originating from 29 independent families who were treated in 15 centers in 11 countries in Europe, Asia, and North America (Table 1). The ratio of boys to girls was 17:15. Premature birth was reported in more than one-third of patients with neonatal data available (11 of 29; 38%) and almost two-thirds of patients (18 of 29; 62%) were found to be small for gestational age.

Three patients with a positive family history of RD were identified with lymphopenia and agranulocytosis at birth. Two of these 3 patients developed bacterial sepsis despite the early diagnosis. Bacterial sepsis was the most frequent infectious complication and developed in 17 of the remaining 29 patients (59%), followed by omphalitis (5/29; 17%). The infections present at diagnosis in the remaining patients are given in Table 1. One patient (patient 15) developed the typical features of Omenn syndrome (ie, generalized erythrodermia, lymphadenopathy, and diarrhea) and lymphocytosis due to an oligoclonal expansion of T cells at the age of 8 weeks.<sup>9</sup>

Infectious agents were isolated from blood cultures in 9 of 19 patients presenting with clinical signs of sepsis and included *Staphylococcus aureus* in 3 patients and group B Streptococci, *Staphylococcus epidermidis*, *Escherichia coli*, *Streptococcus viridans*, *Pseudomonas aeruginosa* and *Candida albicans* in 1 patient, respectively. Noninvasive candidiasis was reported in 2 infants. The age at presentation was within the first month in 27 of 29 patients (93%), and in 20 of these 27 cases it was within the first week of life. The 2 late-presenting patients (patients 6 and 14) were diagnosed at the age of 2.5 months with whooping cough and positive polymerase chain reaction for *Bordetella pertussis* in the nasopharyngeal aspirate, the other at the age of 1.8 months with a history of recurrent otitis (*Klebsiella pneumoniae* and *S aureus*).

### Laboratory findings at presentation

All patients presented with lymphopenia and with persistent agranulocytosis (Table 2; supplemental Figures 1-8, available on the Blood Web site). T-cell numbers were below normal limits in all patients.<sup>10</sup> Maternal T cells were detected in 13 of 23 patients tested (57%), and an exanthema consistent with an alloreaction was present in 4 of those 13 patients (31%). Natural killer (NK)-cell and B-cell numbers were within the normal range in just 2 of 24 and 4 of 25 patients tested, respectively.<sup>11</sup>

In addition to lymphopenia and agranulocytosis, almost half of the patients exhibited other hematological abnormalities. Hemoglobin levels were below the normal range in 14 of 32 patients (44%).<sup>12</sup> In 1 patient (patient 22) an umbilical blood sample was taken for prenatal diagnosis, which revealed a hemoglobin level of 5.8 g/dL and a platelet count of 48 000/μL without clinical signs of anemia. Thrombocytopenia was observed in 14 of 31 (45%) patients.<sup>13</sup>

Bone marrow aspirates were reported in 26 of 32 patients and revealed hypoplasia in 9 and hyperplasia in 5 of those 26 patients. The most common finding reported in 22 of the 26 patients was an arrest of myeloid differentiation at the promyelocytic stage (Figure 1; supplemental Table 1). In 9 of 26 aspirates, dysmorphic lymphopoiesis was described, which was difficult to distinguish from malignant disease in some cases (Figure 1).

**Table 1. Clinical presentation of 32 patients with RD**

Patient No.	Year of birth	Sex	Gestational age, wk	Weight at birth, g	Percentile	Age at presentation, d	Infection/problem at presentation
1	2006	F	31	930	3-10	1	Neonatal sepsis ( <i>C albicans</i> ), died before HSCT
2	1988	F	33	1150	<3	1	Neonatal sepsis
3	2011	F	n.r.	n.r.	n.r.	4	Diarrhea
4	2008	M	27	1135	75-90	1	Neonatal sepsis ( <i>S epidermidis</i> )
5	2008	M	37	2270	<3	19	Neonatal sepsis ( <i>S aureus</i> )
6	2010	M	40	3000	10-25	53	Chronic otomastoiditis ( <i>K pneumoniae</i> , <i>S aureus</i> )
7	2011	M	39	2470	<3	3	Neonatal sepsis
8	2005	F	36	2515	25-50	16	Fever, weight loss
9	2005	F	37	2400	3-10	1	Neonatal sepsis ( <i>S agalactiae</i> )
10	2003	M	36	1910	<3	2	Mild respiratory distress, abdominal distension
11	2004	F	33	1100	<3	7	Neonatal sepsis
12	2006	M	39	2616	<3	7	Neonatal sepsis
13	2007	F	39	2900	10-25	5	Omphalitis, pneumatosis intestinalis
14	2009	F	37	2000	<3	75	Whooping cough ( <i>B pertussis</i> )
15	2011	M	39	2430	<3	1*	Neonatal sepsis, Omenn syndrome (8 wk)
16	1982	M	38	2400	<3	11	Neonatal sepsis
17	1987	M	42	2550	<3	1	Neonatal sepsis, diarrhea, failure to thrive
18	1987	F	40	3684	50-75	1	Neonatal sepsis ( <i>S viridans</i> ), UTI ( <i>C albicans</i> )
19	1993	M	31	1870	75-90	1	Ascites, hepato/splenomegaly, cholestatic liver disease
20	1998	F	36	2300	10-25	7	Neonatal sepsis ( <i>E coli</i> ), pneumonitis, omphalitis
21	1993	F	38	1310	<3	19	Neonatal sepsis, omphalitis
22	2009	F	36	2000	3-10	1*	Neonatal sepsis
23	1986	M	40	2850	3-10	1	Neonatal sepsis
24	1991	M	37	2100	<3	1*	No clinical signs or symptoms
25	1995	M	37	1920	<3	1	Prenatal anemia, neonatal sepsis ( <i>P aeruginosa</i> )
26	1997	F	n.r.	n.r.	n.r.	18	n.r.
27	1999	M	36	3000	50-75	7	Neonatal sepsis ( <i>S aureus</i> ), omphalitis
28	2000	M	31	1500	25-50	1	Ileus due to intestinal dilatation (terminal ileum) of unknown origin
29	2000	F	37	1750	<3	3	Neonatal sepsis ( <i>S aureus</i> )
30	2001	F	37	2770	25-50	14	Prenatal anemia
31	2002	M	n.r.	3350	n.r.	7	Petechiae/thrombocytopenia
32	2003	M	40	3165	10-25	8	Omphalitis

F, female; M, male; n.r., not reported; perc., percentile; UTI, urinary tract infection.

\*Positive family history with diagnosis immediately after birth.

## AK2 mutations

For 30 patients originating from 27 independent families, 22 different mutations in *AK2* were reported. In 2 patients, no material was available to perform retrospective genetic analysis. Mutations in 14 patients (Figure 2 and Table 2) have been previously described.<sup>7-9</sup> Deletions (1-5000 nucleotides), missense, nonsense and splice-site mutations were detected in *AK2* sequences, leading to in silico predictions of single amino acid missense mutations or premature stop codons with truncated proteins.

Of the 23 patients with homozygous mutations, 16 reported a consanguineous background whereas 7 did not. As expected, identical homozygous mutations were found in 3 pairs of siblings (patients 2 and 24, patients 1 and 22, and patients 9 and 15). Common mutations were detected in patients originating from the same (patients 25 and 31 from Cape Verde; patients 2, 24, and 7 from Turkey; and patients 6, 9, and 15 from the Arabic peninsula) as well as from different geographical areas (patients 10 and 17; and patients 20 and 32).

## Transplantation

With the exception of 1 patient (patient 1), who died before transplantation from *Candida* sepsis, the other 31 patients underwent HSCT and received a total of 47 transplantations. The mean age at first HSCT was 3.5 months (range: 0.5-11.1 months; median: 2.4 months).

Thirteen patients required a second HSCT either because of engraftment failure without any donor cells detectable (patients 2, 18, 20, 21, and 29) or because of persistence or recurrence of agranulocytosis (patients 6, 9, 11, 16, 19, 26, 30, and 31). Secondary transplantations were performed more frequently for patients who received nonmyeloablative conditioning regimens or for those who were transplanted with a T-cell-depleted graft (Figure 3A-B; supplemental Table 2).

Overall survival (OS) after HSCT was 68% (21 of 31 patients). Seven deaths occurred within the first 6 months after transplantation (patients 15, 16, 17, 20, 24, 25, and 29) and were either related to infections (eg, encephalitis, respiratory infection of unknown origin, pulmonary aspergillosis, and systemic adenovirus infection) or, in 1 patient, severe acute graft-versus-host disease (GvHD) in combination with veno-occlusive disease. Three late deaths at >6 months after transplantation occurred in patients (patients 19, 26, and 30) for whom permanent myeloid engraftment had failed despite the presence of donor lymphocytes (Figure 4; supplemental Table 2).

## Patients transplanted with T-cell-replete grafts

OS in patients transplanted with T-cell-replete grafts donated from HLA-identical and HLA-mismatched donors was 93% (13 of 14 patients) (Figures 3A and 4; supplemental Table 2).

Six patients had an HLA-identical family donor available, and all of these patients survived. One of these patients achieved long-term cure after 2 transplant attempts without conditioning. In the other 5 patients, conditioning was used. Busulfan-based conditioning was successful in 2 of 2 patients (patients 4 and 5). Alternative regimens used in 3 patients (patients 3, 6, and 7) were sufficient to allow long-term engraftment in 2 of these patients. Patient 7 developed mixed chimerism after conditioning with treosulfan only, but had normal neutrophil counts 2 years after transplantation. Patient 6, in contrast, experienced a gradual recurrence of neutropenia and required a second transplant, which was successful.

Transplants from unrelated donors were used in 8 patients. Overall survival in this group was 88% (7 of 8 patients). In 6 of these 8 patients, unrelated cord blood grafts with variable numbers of mismatches were used (supplemental Table 2). Busulfan-based conditioning was given to 4 of these patients with successful long-term engraftment in all of them (patients 10, 12, 13, and 14). Two patients received alternative conditioning (patients 9 and 11) without long-term myeloid engraftment. Both of these patients developed secondary neutropenia and were successfully retransplanted, receiving a busulfan-based regimen after 2.9 years and 7 months, respectively.

Two patients were transplanted with bone marrow grafts from unrelated adult donors (patients 8 and 15). Both received busulfan-based conditioning. One died of veno-occlusive disease and multiorgan failure,<sup>9</sup> whereas the other is alive and well.

#### Patients transplanted with T-cell–depleted grafts from HLA-haploidentical donors

Seventeen patients (55%) were transplanted with T-cell–depleted grafts from HLA-haplo-identical donors (patients 16–32). OS in this group was 8 out of 17 patients (47%). In 5 patients (patients 16–20), the initial HSCT was performed without conditioning and resulted in primary graft failure in all of them. One patient died after the first transplant, and 4 patients received repeat transplants after conditioning 1.2 to 8.4 months after the first HSCT. Among them, only 1 patient (patient 18) survived (Figure 3B; supplemental Table 2).

In 12 of 17 patients, conditioning was used prior to the initial haploidentical HSCT (patients 21–32). In 11 patients, conditioning was based on busulfan, either alone (patient 22) or in combination with cyclophosphamide (patients 23–31) or fludarabine (patient 32). These regimens were successful and led to engraftment and permanent cure in 6 of 11 patients. Two patients died following this procedure, 1 of GvHD (patient 24) and the other of interstitial pneumonia (patient 25). Three patients required a second transplant because of primary (patient 29) or secondary (patients 26 and 30) graft failure. All 3 patients died of adenovirus infection or myelodysplastic syndrome, as detailed elsewhere.<sup>14</sup>

Conditioning with cyclophosphamide alone in 1 patient (patient 21) was not successful, but engraftment was achieved with retransplantation following conditioning with busulfan and cyclophosphamide 6 weeks later.

#### GvHD

If GvHD prophylaxis was given, either cyclosporin A (CSA) or tacrolimus was included in all cases (supplemental Table 2). Steroids, methotrexate, or mycophenolate mofetil were given as additional components. Serotherapy prior to HSCT with either antithymocyte globulin or alemtuzumab was used in 16 of 31 first transplants. Following haploidentical T-cell–depleted HSCT, CSA was given to a

minority of 4 out of 17 patients (patients 19, 28, 29, and 32). The overall incidence of GvHD was 52% (16 of 31 patients), developed de novo after transplantation in 11 of 31 patients (35%), and was preexisting due to engraftment of maternal T cells in 4 of 31 patients (13%). Only 5 patients (16%) developed GvHD of grade III or IV. GvHD was the cause of death in only 1 patient (patient 24).

#### Lymphohematopoietic reconstitution and long-term follow-up

Twenty-one (66%) of the 31 patients are alive after HSCT (Table 3; supplemental Table 2) with a mean follow-up of 7.9 years (range: 6 months to 23.6 years). No serious or life-threatening infectious problems were reported after HSCT. Regarding growth and development, 10 of 17 patients with data available remained <10th percentile with their body weight, 7 of 16 patients remained <10th percentile with their height, and 4 of 16 patients were reported to have learning disabilities (supplemental Table 3). In a cohort with a high incidence of premature birth, infections, and repetitive exposure to chemotherapy and with the majority (12 of 16 patients) reported to have normal development, it is difficult to characterize developmental delay as a component of RD.

Mixed chimerism was found in 7 patients (patients 2, 5, 7, 14, 23, 27, and 28). Variable proportions of autologous cells were detected in the lymphoid, erythroid, and/or myeloid compartment. T cells were of complete donor origin in all but 1 patient (patient 14 with 4% of autologous T cells). For neutrophils, complete donor chimerism was reported in all but 2 patients (patients 5 and 14). Chimerism for B cells was selectively tested in 5 patients (patients 3, 5, 7, 14, and 27) and was found mixed in 3 patients with autologous proportions ranging from 5% to 67%. Chimerism of CD34<sup>+</sup> cells was investigated in 3 patients. Autologous CD34<sup>+</sup> cells were detected in 2 of 3 patients tested with an autologous proportion of 90% (patient 2) and 20% (patient 28) (Table 4). Red blood cell chimerism was found mixed in 3 patients with an autologous proportion of 50% to 94%.

Absolute neutrophil counts <1500/μL were found only in patients with mixed chimerism (patients 2, 7, and 27). T-cell counts were >1000/μL in all patients except 2 (patients 27 and 31), which is explained by a history of GvHD in 1 patient and a short follow-up of 0.5 years in the other. The percentage of naive T cells (here, defined as CD45RA<sup>+</sup>) was >30% in 12 of 13 analyzed cases, indicating good thymic function and capacity of de novo T-cell formation. Three patients remained on immunoglobulin substitution. All patients tested had positive antibody responses to vaccinations.

Nineteen out of the 21 long-term survivors were reported to suffer from persistent hearing deficiencies and were supported by either conventional hearing aids (n = 10) or cochlear implants (n = 9). For the remaining 2 patients, this information was not available.

## Discussion

In this retrospective international study on patients with RD, we have identified a number of particular features with regard to the clinical presentation as well as the therapeutic requirements of the disorder, which are distinct from other variants of SCID.

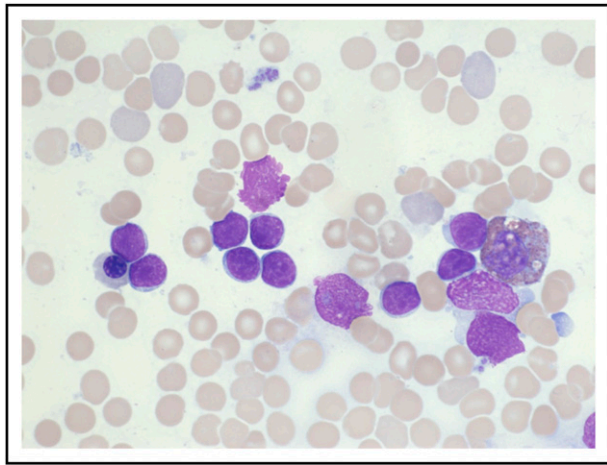
In contrast to other SCID patients who usually do not develop serious infections before 2 months of age,<sup>15</sup> patients with RD present much earlier with a predominance of invasive bacterial infections due to the associated agranulocytosis. Opportunistic infections, such as respiratory failure due to *Pneumocystis jirovecii* or systemic infections with cytomegalovirus, which are typical for other SCID entities, were not observed in this series of patients.



Table 2. (continued)

Patient No.	Age at blood analysis, d	Hemoglobin, g/dL	Platelet count ( $\times 10^9$ /mL)	Neutrophils/ $\mu$ L	Monocytes/ $\mu$ L	Lymphocytes/ $\mu$ L	MFT	Exanthema	T cells/ $\mu$ L	B cells/ $\mu$ L	NK cells/ $\mu$ L	Geographic origin	Parental consanguinity	Mutation AK2
20	199	9	23	150	400	700	-	-	100	500	50	Portuguese	-	c.[307C>T];[307C>T]; p.[Arg103Trp];[Arg103Trp]
21	19	8.6	330	30	10	40	n.r.	-	0	n.d.	n.d.	United States (white/ non-Hispanic)	n.r.	c.[614-615del];[614-615del]; p.[Gly205Aspfs*92]; [Gly205Aspfs*92]
22	1	5.8	41	0	0	100	+	-	100	0	0	Turkish	+	c.[498+1G>A];[498+1G>A]; p.[?];[?]
23	1	16.4	104	0	0	500	+	+	100	60	100	German	-	c.[94-2287_219+542del/2956]; [94-2287_219+542del/2956]; p.[Ala32_Leu73del]; [Ala32_Leu73del]
24	2	18.3	126	0	12	150	+	-	5	15	8	Turkish	+	c.[453delC];[453delC]; p.[Tyr152Thrfs*12]; [Tyr152Thrfs*12]
25	1	8	n.d.	0	200	300	n.r.	-	270	0	17	Cape Verde	+	c.[494A>G];[494A>G]; p.[Asp165Gly];[Asp165Gly]
26	17	15	442	150	550	900	-	-	90	0	90	French	-	c.[556C>T];[94_219del]; p.[Arg186Cys]; [Ala32_Leu73del]
27	15	14.9	296	76	45	207	-	-	4	14	33	Italian	+	c.[556C>T];[556C>T]; p.[Arg186Cys];[Arg186Cys]
28	1	15.2	460	85	12	940	+	+	30	22	5	German	+	c.[331-1G>A];[331-1G>A]; p.[?];[?]
29	3	7.5	16	0	400	0	+	-	0	0	0	Caucasian	-	c.[400-401del];[614-615del]; p.[Leu134Alafs*32]; [Gly205Aspfs*29]
30	14	10	100	0	150	260	-	n.r.	10	165	8	Turkish	+	c.[473del];[473del]; p.[Pro158Leufs*6]; [Pro158Leufs*6]
31	103	15	62	30	555	670	n.r.	n.r.	70	556	53	Cape Verde	+	c.[494A>G];[494A>G]; p.[Asp165Gly];[Asp165Gly]
32	8	14.3	293	100	200	300	n.r.	-	2	60	n.d.	Palestinian	+	c.[307C>T];[307C>T]; p.[Arg103Trp];[Arg103Trp]

Mutations are indicated according to GenBank reference sequences NM\_013411.4 for AK2 complementary DNA (isoform B) and NP\_037543 for AK2 protein. MFT, maternal-fetal transfusion; n.d., not determined; n.r., not reported.



**Figure 1.** May-Grunwald-Giemsa stain of a bone marrow aspirate from a patient with RD (original magnification  $\times 1000$ , immersion oil, Zeiss Axio Imager M1, AxioCam MRc [Carl Zeiss Microscopy, Jena, Germany]) showing immature lymphoid cells (hematogones) with slim cytoplasm as well as myeloblasts and a dysplastic promyelocyte with nuclear and cytoplasmic vacuoles. In the work-up of the bone marrow with immunostaining and flow cytometry, immature lymphocytes were identified as polyclonal B-cell precursors (flow cytometry of the bone marrow revealed a population of 46% with markers of pre-B cells CD10<sup>+</sup>/CD19<sup>+</sup>/cyIgM<sup>+</sup>/TdT<sup>+</sup>/CD34<sup>-</sup>; clonality was tested in a multiplex polymerase chain reaction for the rearranged B-cell receptor heavy chains [data not shown]).

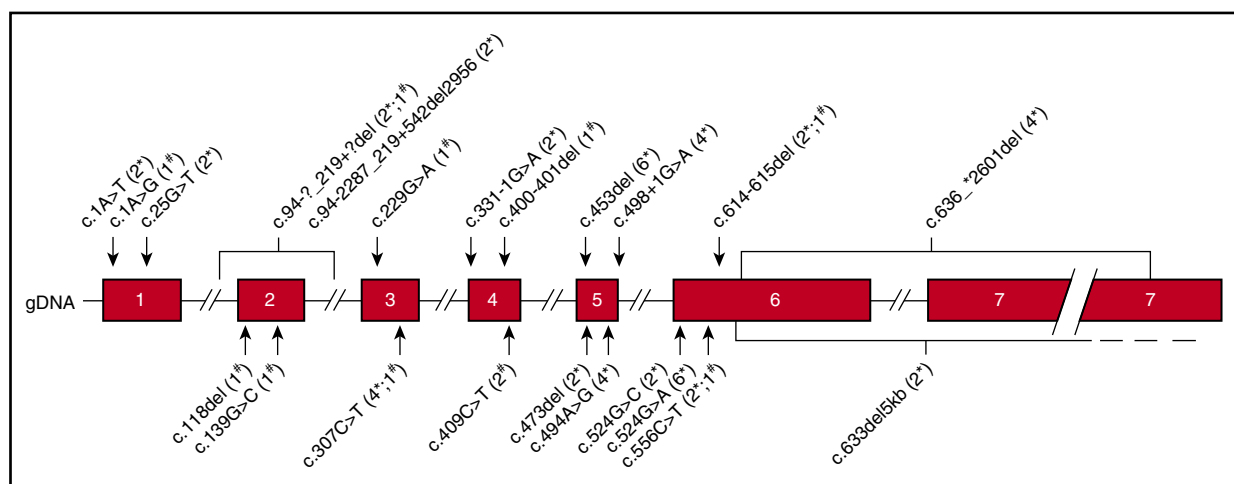
Another unique observation in this cohort is the high proportion of patients who were born prematurely and and/or small for gestational age. Because AK2 is a ubiquitously expressed protein involved in the basic mechanisms of cellular energy supply, this finding suggests that its deficiency may cause fetal stress, leading to premature birth.

Moreover, about half of the patients presented with additional hematological abnormalities affecting erythropoiesis and thrombopoiesis. Because these were also present in the absence of systemic infection or before birth, primary involvement can be suspected (supplemental Figures 1 and 2). These findings indicate that the enzyme deficiency may have a broader, although variable, effect on hematopoiesis not previously recognized.

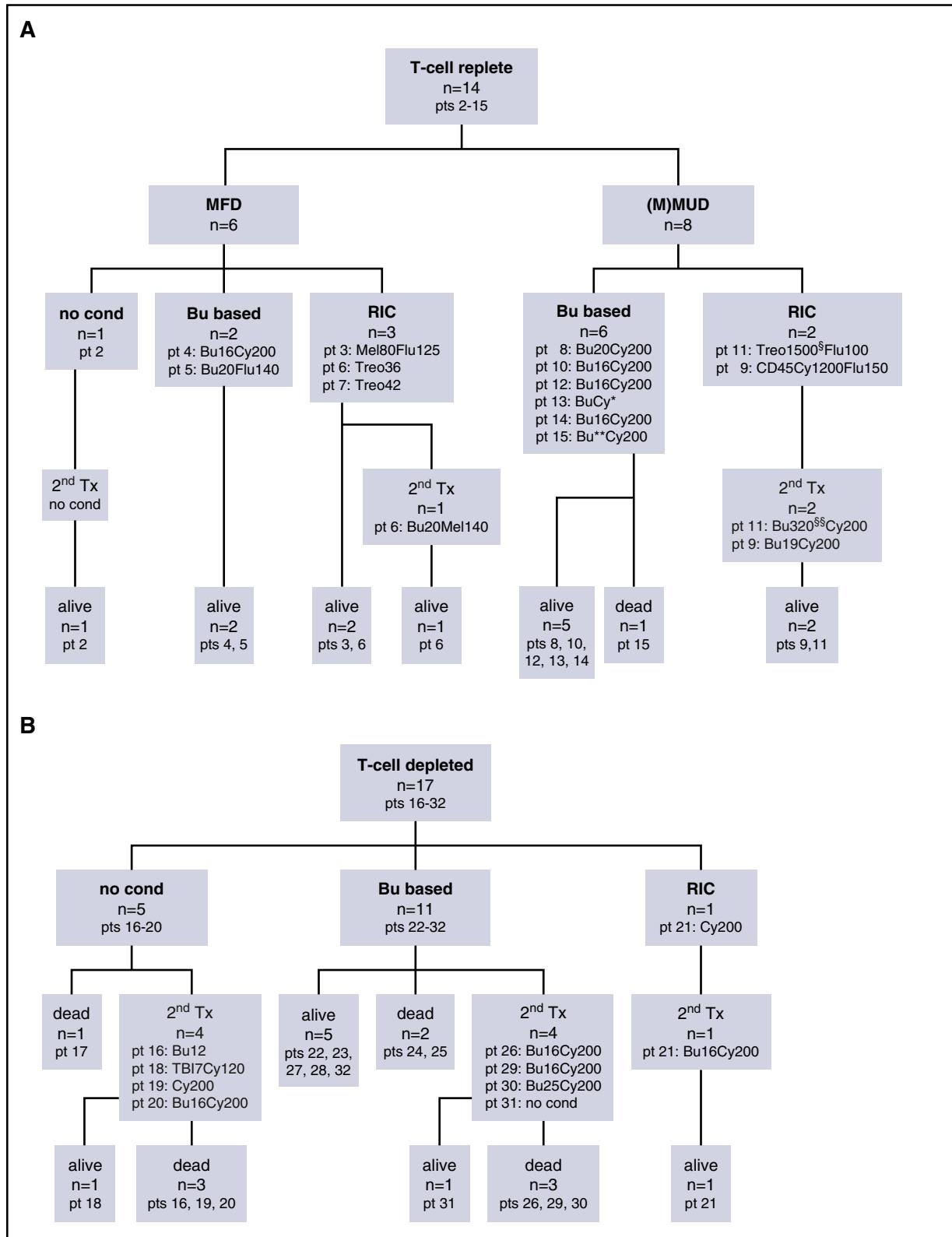
One could suspect that AK2 deficiency remains unrecognized in a proportion of patients dying from severe fulminant bacterial infections in early infancy. RD should be considered and ruled out in any neonate with unexplained leukopenia. Absent hearing or the typical abnormalities in a bone marrow aspirate, as described above, can provide helpful additional clues. However, both are difficult to perform and potentially misleading in a seriously sick newborn. Finally, sequencing of AK2 will confirm the diagnosis. Newborn screening for SCID, which at present is routinely performed in the majority of states in the United States,<sup>16</sup> will most likely identify these patients easily due to the absence of T cells and therefore low to absent T-cell receptor excision circle levels. Some children, however, might experience their first life-threatening infection before these results are available and the benefit for this group in comparison with other SCID entities might therefore be lower.

We were able to identify genetic defects in AK2 in all patients in whom this gene was sequenced (30 of 32 patients). The mutations included missense, nonsense, and splice-site mutations and large and small deletions and affected each of the 7 coding exons. The missense mutation c.524G>A was the most frequently detected genetic change and affected 3 independent families originating from the Arabic peninsula, which could indicate a higher frequency of this allele and possibly a common founder. In 2 of these patients, the mutation was associated with a leaky phenotype of SCID. One patient (patient 15) presented with an Omenn phenotype and a T-cell count of almost 2000/ $\mu$ L. The second patient (patient 6) with the same mutation presented with a high B-cell number (1850/ $\mu$ L) and abundant B-cell precursors in the marrow. This finding was initially misinterpreted as B-cell leukemia, but polyclonality was demonstrated (data not shown). The third patient (patient 9) with this same homozygous mutation had “classical” aleukocytosis (350/ $\mu$ L), which indicates that there is no reliable genotype-phenotype correlation.

In contrast to other SCID patients, permanent cure in patients with RD requires the correction of the myeloid lineage in addition to lymphoid reconstitution. According to the experience presented in this study, stable engraftment of hematopoietic stem cells is a crucial prerequisite to achieve normal myeloid function in patients with RD. Conditioning and alloreactive donor T cells are important factors that promote stem cell engraftment. Thus, in T-cell-depleted haploidentical

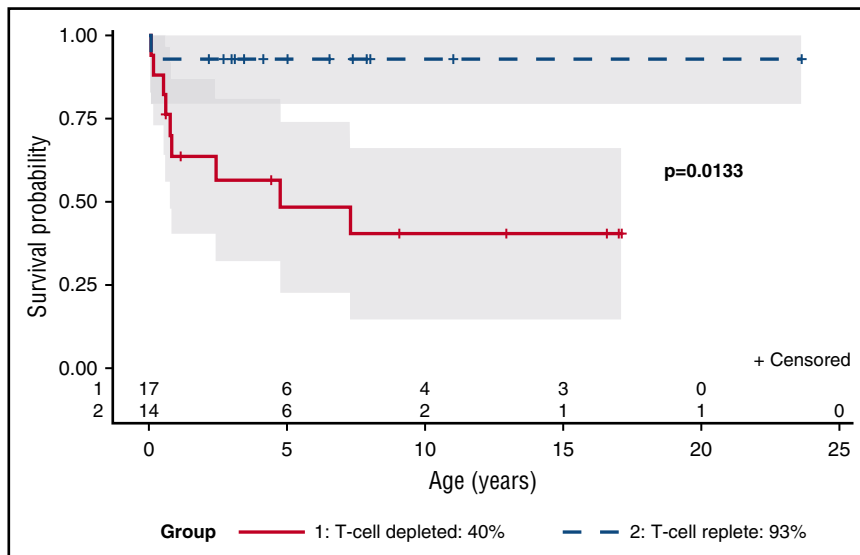


**Figure 2.** Genomic map of AK2 isoform B with mutations identified in 29 patients. GenBank reference sequence NM\_013411.4 for AK2 complementary DNA, isoform B. The numbers in brackets indicate the frequency of the mutations and whether they were identified as part of a homozygous mutation (\*) or a compound heterozygous mutation (#).



**Figure 3. Tree diagrams for patient transplantations.** (A) Tree diagram for 14 patients transplanted with T-cell–replete grafts. (B) Tree diagram for 17 patients transplanted with T-cell–depleted grafts. \*\* indicates dosage not reported; \*\*\* indicates targeted Bu 800-1200  $\mu\text{mol}/\text{min}$  per L; <sup>S</sup> indicates treosulfan dosage given in mg/kg; <sup>SS</sup> indicates busulfan dosage given in mg/m<sup>2</sup>. Bu, busulfan dosage given in mg/kg; Bu based, conditioning regimen contains busulfan; CD45, anti-CD45 antibody; Cy, cyclophosphamide dosage given in mg/kg; Flu, fludarabine dosage given in mg/m<sup>2</sup>; Mel, melphalan dose given in mg/m<sup>2</sup>; MFD, matched family donor; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; no cond, no conditioning; pt, patient; RIC, reduced intensity conditioning; TBI, total body irradiation dosage given in Gy; 2nd Tx, second transplant; Treo, treosulfan dosage given in g/m<sup>2</sup>.





**Figure 4.** Kaplan-Meier survival estimation with 95% pointwise confidence intervals. Comparison of the survival probability of patients after HSCT with T-cell replete and T-cell depleted grafts.

transplantation, donor cell engraftment is dependent on the conditioning regimen. Accordingly, reduced intensity conditioning regimens without a myeloablative agent ( $n = 1$ , patient 16) or HSCT without any conditioning ( $n = 5$ ; patients 17-21) were not successful in T-cell-depleted transplants. Even after conditioning with busulfan, only 6 of 11 patients (55%) engrafted, and 4 of the remaining patients experienced primary or secondary graft failure. In contrast, no graft failure was reported with T-cell-replete grafts after conditioning with busulfan. In this latter group, engraftment was not reliably achieved with less intense conditioning regimens: 3 out of 4 patients needed a second transplant because of either primary or secondary graft failure. Without any conditioning, T-cell-replete transplantation led to stable engraftment in a single patient after 2 attempts with a matched sibling graft. The assessment of the effectiveness of busulfan for myeloablation in this study is based on the combination with cyclophosphamide. Whether the combination with fludarabine, which was administered to 1 patient only, is equally effective in this disease remains unclear. The same applies for busulfan alone without additional components.

Without any previous experience, the necessity for conditioning was unknown in the early patients reported in this study, who were mostly transplanted from haploidentical donors. This fact at least partially contributes to the inferior results of T-cell-depleted transplantations, as depicted in Figure 3. Due to the limited number of patients per group, survival data should be interpreted very cautiously. In principle haploidentical T-cell-depleted transplantation makes sense for patients with RD if no well-matched donors are available, particularly utilizing newer haploidentical approaches. None of the patients in this cohort suffered from viral infections before transplant. Other SCID entities most frequently present with T-cell-dependent viral or opportunistic infections. For them, rapid T-cell reconstitution is clearly beneficial to clear these infections, and delayed T-cell reconstitution after haploidentical transplantation is a major disadvantage. RD patients, who most frequently present with bacterial infections, need rapid neutrophil engraftment to overcome their infections. Neutrophil reconstitution is not delayed after haploidentical transplantation, and haploidentical parental donors are readily available for the vast majority of patients.

Complete donor chimerism prevents secondary autologous reconstitution, which, in some patients, causes posttransplant neutropenia. Prolonged stimulation with granulocyte-colony stimulating factor may

lead to the development of myelodysplastic syndrome as reported for 2 patients.<sup>14</sup> In the case of a loss of myeloid engraftment and posttransplant neutropenia, retransplantation seems inevitable and should be considered early. Why neutropenia develops in some but not all patients with partial autologous reconstitution remains unclear.

In recent publications, new insight was gained into the molecular pathophysiology of AK2 deficiency.<sup>17</sup> In a zebrafish model, Rissone et al<sup>18</sup> were able to demonstrate that AK2 deficiency equally affects the myeloid lineage as well as hematopoietic stem and precursor cells. In an in vitro model for proliferation and differentiation of human bone marrow cells, Six et al<sup>19</sup> demonstrate that lineage-negative CD34<sup>+</sup> stem cells reside in hypoxic bone marrow niches and are not affected by AK2 deficiency because their energy needs are covered by anaerobic glycolysis.<sup>20</sup> With proliferation and differentiation, cells switch to aerobic metabolism and become dependent on mitochondrial function and AK2.

The clinical data from our cohort are mostly in accordance with data generated in the zebrafish model and the in vitro assays on human bone marrow. Long-term survivors with stable mixed chimerism in their CD34<sup>+</sup> compartment demonstrate that donor cells do not have any selective advantage in the competition with AK2-deficient lineage-negative cells. However, these autologous CD34<sup>+</sup> cells do not contribute to granulopoiesis, as shown for patients 2 and 28 in Table 4.

There is some conflicting data regarding hematopoiesis. Rissone et al<sup>18</sup> demonstrate in their zebrafish model that AK2 deficiency affects proliferation and maturation of red blood cell precursors. This is confirmed by the same group in a human in vitro differentiation model with induced pluripotent stem cells originating from a patient with AK2 deficiency, as the capacity of these cells to differentiate to erythroid cells is severely compromised.<sup>18</sup> Although almost half of the patients reported in our cohort (44%) suffered from anemia at presentation (suggesting that AK2 deficiency negatively affects red blood cell maturation), autologous CD34<sup>+</sup> cells seem to significantly contribute to the peripheral red blood cell pool in long-term survivors with stable mixed chimerism. These conflicting results from animal models, in vitro assays, and patient data cannot be explained. Functional assays on mutated AK2 proteins might be helpful to better explain the initial phenotype as well as posttransplant chimerism. Residual function of AK2 in autologous cells or even somatic mosaicism due to genetic reversions in hematopoietic lineages could contribute to “leaky” phenotypes. These factors are not considered in current models.

**Table 3. Chimerism and immunological reconstitution in 21 survivors after HSCT**

Patient No.	Follow-up, y	Chimerism			Immunological reconstitution						
		Full blood, % donor	T cells, % donor	Other leukocyte subpopulations, % donor	Neutrophils/ $\mu$ L	T cells/ $\mu$ L	B cells/ $\mu$ L	NK cells/ $\mu$ L	Naïve T cells, %	IVIg	Specific antibodies
2	23.6	mixed	100	Non-T cells: >90, PMN: >95; CD34 <sup>+</sup> : <10; red blood cells: 6	1148	1270	109	109	34	No	Tet/Diph/HiB/Pneu
3	7.9	n.d.	100	B cells: 100	4100	n.d.	n.d.	n.d.	n.d.	No	n.d.
4	3.0	100	n.d.	CD34 <sup>+</sup> : 100	1760	5350	1110	490	58	No	Tet/Diph/HiB/MMR
5	8.0	n.d.	100	B cells: 90, PMN: 90	2410	1553	263	174	66	No	Tet/Diph/HiB/Pneu/MMR
6	2.7	100	100	PMN: 100	1600	3620	860	240	54	No	Tet/Diph/HiB/Pneu
7	2.1	87	n.d.	n.d.	400	1138	121	67	n.d.	No	n.d.
8	11.0	100	100	B cells: 100, PMN: 100	1780	2487	324	367	68	No	Tet/Diph/HiB/Pneu/MMR
9	4.1	100	n.d.	n.d.	1530	1060	620	70	70	No	Tet/Diph/HiB/Pneu
10	7.4	100	100	PMN: 99	2260	3290	870	786	52	No	Tet/Diph/HiB/Pneu/MMR
11	6.5	100	n.d.	n.d.	2400	3000	1300	807	n.d.	No	Tet/Diph/HiB/Pneu/MMR
12	5.0	100	100	B cells: 100; NK cells: 100, PMN: 100	1776	1520	830	200	n.d.	No	MMR
13	3.1	100	n.d.	n.d.	4563	2084	322	275	66	Yes	unknown
14	3.4	n.d.	96	B cells: 33, PMN: 75	2170	1262	177	397	n.d.	Yes	n.d.
18	1.1	100	n.d.	n.d.	3000	1580	200	360	n.d.	Unknown	n.d.
21	16.6	100	n.d.	n.d.	3700	n.d.	n.d.	n.d.	n.d.	No	Tet/Diph
22	4.4	100	100	n.d.	2110	3410	810	270	51	No	Tet/Diph/HiB/Pneu
23	17.1	100	100	red blood cells: 14	2960	1930	210	115	39	No	Tet/Diph/HiB/Pneu
27	17.0	n.d.	100	B cells: 95, PMN: 100	900	461	77	13	65	No	Tet/Diph/HiB/Pneu/MMR
28	12.9	mixed	100	CD34 <sup>+</sup> : 80, non-T cells: >95, PMN: 100; red blood cells: 50	1672	1315	307	68	49	No	Tet/Diph/HiB/Pneu
31	0.6	100	n.d.	n.d.	3946	480	70	50	0	Yes	n.d.
32	9.1	100	n.d.	n.d.	3400	2870	350	140	n.d.	No	VZV

HiB, *Haemophilus influenzae* type B; Diph, diphtheria; IVIG: IV substitution of immunoglobulins; MMR: measles, mumps, and rubella; n.d., not determined; PMN, polymorphonuclear cell; Pneu, *S pneumoniae*; Tet, tetanus; VZV, varicella zoster virus.

From a clinical point of view, sensorineural hearing disability has a strong impact on the development of these children after successful HSCT. The molecular pathology leading to this nonhematological manifestation of RD is unclear, and animal models have been unable to clarify the mechanism. An AK2-deficient mouse model is not yet established, because a complete knockout of AK2 seems to be lethal in early embryonic development (K.S., unpublished data). Cochlear implants

are chosen by the majority of patients diagnosed and treated in recent years to correct the hearing disability. Older patients get along with conventional noninvasive hearing aids, which indicates some residual hearing capacity.

In summary, this international initiative has put together clinical and genetic data on the largest cohort of patients with RD so far. We have demonstrated the necessity for early diagnosis, rapid and decisive

**Table 4. Diagnostic and therapeutic recommendations for patients with RD**

Diagnosis	Therapy
Consider RD in any newborn with unexplained neutropenia	Myeloid engraftment is crucial for permanent cure
Confirmation by	Myeloid engraftment is supported by
Immunophenotyping	Conditioning with myeloablative agents
Hearing test	Transplantation with T-cell replete transplants
Bone marrow morphology	In case of primary or secondary graft failure, early retransplantation is recommended
Genetic analysis of AK2	

therapeutic measures, the superiority of T-cell-replete grafts, and the need for conditioning to reliably achieve myeloid engraftment (Table 4).

## Acknowledgments

The authors thank Beate Einsiedler and Rainer Muche from the Institute for Epidemiology and Medical Biometry (University of Ulm) for statistical support. The authors also thank Lueder Hinrich Meyer for immunophenotyping of bone marrow cells.

This work was supported by a grant 01GM1517B from the German Federal Ministry of Education and Research (M.H. and K.S.), grants U54 AI 082973 and R13 AI 094943 from the National Institute of Allergy and Infectious Diseases and Office of Rare Diseases, National Center for Advancing Translational Sciences, National Institutes of Health (M.J.C., L.D.N., and S-Y.P.), and by the Great Ormond Street Hospital Children's Charity and the Great Ormond Street Hospital/University College London National Institute for Health Research Biomedical Research Centre (H.B.G.).

## References

- de Vaal O, Seynhaeve V. Reticular dysgenesis. *Lancet*. 1959;274(7112):1123-1125.
- Friedrich W, Hoenig M, Schwarz K. Reticular Dysgenesis. In: Ochs HD, Smith EC, Puck JM, eds. *Primary Immunodeficiency Diseases: A Molecular and Cellular Approach*. Oxford, New York: Oxford University Press; 2014:269-273.
- Small TN, Wall DA, Kurtzberg J, Cowan MJ, O'Reilly RJ, Friedrich W. Association of reticular dysgenesis (thymic aplasia and congenital aleukocytosis) with bilateral sensorineural deafness. *J Pediatr*. 1999;135(3):387-389.
- De Santes KB, Lai SS, Cowan MJ. Haploidentical bone marrow transplants for two patients with reticular dysgenesis. *Bone Marrow Transplant*. 1996;17(6):1171-1173.
- Friedrich W, Goldmann SF, Ebell W, et al. Severe combined immunodeficiency: treatment by bone marrow transplantation in 15 infants using HLA-haploidentical donors. *Eur J Pediatr*. 1985;144(2):125-130.
- Levinsky RJ, Tiedeman K. Successful bone-marrow transplantation for reticular dysgenesis. *Lancet*. 1983;321(8326 pt 1):671-673.
- Lagresle-Peyrou C, Six EM, Picard C, et al. Human adenylate kinase 2 deficiency causes a profound hematopoietic defect associated with sensorineural deafness. *Nat Genet*. 2009;41(1):106-111.
- Pannicke U, Höning M, Hess I, et al. Reticular dysgenesis (aleukocytosis) is caused by mutations in the gene encoding mitochondrial adenylate kinase 2. *Nat Genet*. 2009;41(1):101-105.
- Henderson LA, Frugoni F, Hopkins G, et al. First reported case of Omenn syndrome in a patient with reticular dysgenesis. *J Allergy Clin Immunol*. 2013;131(4):1227-1230.e3.
- Weinberg AG, Rosenfeld CR, Manroe BL, Browne R. Neonatal blood cell count in health and disease. II. Values for lymphocytes, monocytes, and eosinophils. *J Pediatr*. 1985;106(3):462-466.
- Shearer WT, Rosenblatt HM, Gelman RS, et al; Pediatric AIDS Clinical Trials Group. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol*. 2003;112(5):973-980.
- Brugnara C. Reference Values in Infancy and Childhood. In: Nathan DG, Ginsburg D, Orkin SH, Look TA, eds. *Hematology of Infancy and Childhood*. Vol. 2. Philadelphia, PA: Saunders; 2003:1835-1964.
- Wiedmeier SE, Henry E, Sola-Visner MC, Christensen RD. Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system. *J Perinatol*. 2009;29(2):130-136.
- Lagresle-Peyrou C, Neven B, Six E, et al. Occurrence of myelodysplastic syndrome in 2 patients with reticular dysgenesis. *J Allergy Clin Immunol*. 2011;128(1):230-232.e2.
- Stephan JL, Vlekova V, Le Deist F, et al. Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. *J Pediatr*. 1993;123(4):564-572.
- Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. *Semin Perinatol*. 2015;39(3):194-205.
- Dzeja PP, Terzic A. Phosphotransfer networks and cellular energetics. *J Exp Biol*. 2003;206(12):2039-2047.
- Rissone A, Weinacht KG, la Marca G, et al. Reticular dysgenesis-associated AK2 protects hematopoietic stem and progenitor cell development from oxidative stress. *J Exp Med*. 2015;212(8):1185-1202.
- Six E, Lagresle-Peyrou C, Susini S, et al. AK2 deficiency compromises the mitochondrial energy metabolism required for differentiation of human neutrophil and lymphoid lineages. *Cell Death Dis*. 2015;6:e1856.
- Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell*. 2011;9(4):298-310.

## Authorship

Contribution: M.H., U.P., K.S., and W.F. designed and performed the study and prepared the initial draft of the manuscript in close cooperation with C.L.-P. and M.C.; M.H., C. L.-P., U.P., L.D.N., F.P., A.R.G., M.S., M.J.C., P.S., H.A.-M., D.A.-Z., S.-Y.P., W.A.H., H.B.G., P.V., K.O., K.I., H.Y., L.M.N., N.M.W., P.S.-P., K.-W.S., H.M., M.A.H., K.-M.D., C.S., D.M., E.-M.J., A.S.S., K.S., A.F., W.F., and M.C. were responsible for patient care and/or provided clinical, genetic, or laboratory data; and all authors contributed to the discussion and the preparation of the draft and have read and approved the final version of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of the members of the European Society for Blood and Marrow Transplantation Inborn Errors Working Party appears in the online appendix.

Correspondence: Manfred Hoenig, Department of Pediatrics, University Medical Center Ulm, Eythstrasse 24, 89075 Ulm, Germany; e-mail: manfred.hoenig@uniklinik-ulm.de.