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The landscape of pediatric Diamond-Blackfan anemia in Switzerland: genotype and phenotype characteristics

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Summary Statements

What is known?

- There is a large variation in the phenotype of Diamond-Blackfan Anemia (DBA) and diversity of genetic mutations.
- Malformation of the upper limbs, head and face, heart, and genitourinary system are frequently identified.

What is new?

- Patients with lower limb and anorectal malformations were repetitively found in our cohort enlarging the clinical spectrum of malformations.
- We show two patients of the same family with a DBA-like condition where the same *RPL17* variant was identified.

Key Words: Diamond-Blackfan anemia, genotype, phenotype, neonatal anemia, bone marrow failure disorders.

Acknowledgement

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Abstract

Background: Diamond-Blackfan anemia (DBA) is caused mainly by genetic mutations in large (*RPL*) or small ribosomal subunit genes (*RPS)* and presents with macrocytic anemia and congenital malformations. Clinical differences between genotypes are insufficiently understood. The aim of this study was to assess clinical features, treatment strategies, and genotypes in the Swiss pediatric DBA population.

Methods: We retrospectively reviewed medical charts of pediatric patients with DBA in Switzerland and stratified patients by *RPL* versus *RPS* mutations.

Results: We report 17 DBA patients in Switzerland who were all genetically investigated. In our cohort, patients showed a wide spectrum of clinical presentations and treatment needs. We found a high proportion of physical malformations (77%) including lower limb (17%) and anorectal (12%) malformations. The two patients with anorectal malformations presented both with antepositioning of the anus needing surgery within the first 15 months of life. One of these patients had sphincteric dysfunction, the other coccygeal agenesis. We found that included patients with an *RPL* mutation more frequently tended to have physical malformations and a milder anemia compared to patients with an *RPS* mutation (median hemoglobin at diagnosis 76 g/l versus 22 g/l).

Conclusion: We illustrate the wide clinical and genetic spectrum of DBA in Switzerland. Our findings highlight the need to take this diagnosis into consideration in patients with severe anemia but also in patients with mild anemia where malformations are present. Lower limb and anorectal malformation extend the spectrum of DBA-associated malformations.

Abbreviation Key

DBA	Diamond-Blackfan Anemia
eADA	Erythrocyte Adenosine Deaminase Activity
GATA-1	GATA binding protein 1 gene
HbF	Fetal Hemoglobin
Hgb	Hemoglobin
HSCT	Hematopoietic Stem Cell Transplantation
IBMFS	Inherited Bone Marrow Failure Syndrome
MCV	Mean corpuscular volume
n	number
RPS / RPL	Small / large Ribosomal Subunit Protein
SGA	Small for gestational age
SPOG	Swiss Paediatric Oncology Group

Introduction

Diamond-Blackfan Anemia (DBA) is a rare inherited bone marrow failure syndrome (IBMFS) characterized by normochromic macrocytic anemia. In patients where not all diagnostic criteria are met, the diagnosis can be supported by identifying a pathogenic gene mutation. Congenital physical malformations include craniofacial, upper limb and hand, cardiac, and urogenital malformations. (1)

Various countries have published their experience with diagnosis and treatment of patients with DBA. (2-4) The aims of this study were to describe the phenotype of DBA in Switzerland and to specify the clinical presentation including genetic characteristics in this population.

Methods

To identify pediatric DBA patients, we contacted all centers providing specialized hematology care to children in Switzerland (for additional information on methods, see **Supplementary File S1**). We included all patients up to the age of 18 years that were reported before July 2019 if (a) diagnostic criteria according to Diamond et al. were fulfilled without identified causal gene mutation; (5, 6) or (b) a pathogenic variant in a gene associated with DBA or (c) a pathogenic variant in a putative ribosomal protein in combination with a DBA-spectrum phenotype was identified. We stratified patients with a pathogenic ribosomal gene variant into two groups: (a) patients with large ribosomal subunit protein (*RPL*) gene mutations, and (b) with small ribosomal subunit protein (*RPS*) gene mutations, as previously described. (7)

Information was collected by reviewing patients' medical records.

We used descriptive statistics to compare phenotypes between the two groups. We obtained multicentric ethics approval for the conduct of the study and written

informed consent from patients and/or parents. This study was performed in accordance with the principles of the Declaration of Helsinki.

Results

Patient cohort and presentation

We received information whether pediatric DBA patients were identified from all nine centers with seven centers reporting 17 patients diagnosed with DBA undergoing regular follow-up care as of July 2019 (**Table 1**). Among them, no deaths and no lost to follow-up were reported. The majority of patients (n=12, 71%) presented with anemia during the first year of life. At first presentation, 4 patients (24%) had a hemoglobin below 30 g/l, and of those, two (50%) presented with low tissue perfusion and signs of hemodynamic shock.

Extra-hematopoietic abnormalities

We found congenital malformations in 13 patients (76%) and multiple malformations in 9 (53%). Craniofacial malformations (including cleft palate) (n=9, 53%), hand (including thumb) and cardiac anomalies (7 patients each, 41%) were the most prevalent **(Supplementary Table S1)**. Three patients (17%) presented with feet malformations. Two patients (12%) presented with anorectal malformations at birth. One of these patients had no sphincteric function and needed surgery at the age of 15 months. The other patient additionally had coccygeal agenesis and the malformation was surgically corrected at the age of 12 months.

Genetics

All patients were genetically tested and 14 (82%) were found to have a pathogenic mutation in known DBA genes **(Supplementary Table S2)**. Two related patients (12%) were identified with a variant in the large ribosomal protein subunit *RPL17* associated with a DBA phenotype. At the moment of presentation, patients with a

mutation in an *RPL* gene (n = 10, 59%) had a median hemoglobin level of 76 g/l (range 47 – 137 g/l). Patients with a mutation in an *RPS* gene (n = 6, 35%) had a median Hgb of 22 g/l (range 15 – 34 g/l).

All 10 patients with a mutation in an *RPL* gene (100%) had multiple organ systems affected by malformations compared to 33% of the patients (n=2) with *RPS* gene mutations (**Supplementary Figure S1**). Patients without any malformations had a mutation in an *RPS* gene (n=3) or no mutation identified (n=1). All patients with a cleft palate (n=4), a skeletal malformation such as hand (n=7), feet (n=3) or other skeletal malformations (n=4) had *RPL* gene mutations (*RPL5, RPL11, RPL17)*. No patients with *RPS* mutations showed any of the above-mentioned malformations. Patients with *RPS26* (n=1; no malformations) and *RPS17* mutations (n=2; 1 patient with an indistinct mild craniofacial dysmorphism, the other with no malformations) showed few malformations.

All patients with *RPL5* mutations in our cohort were responsive to steroid treatment and achieved stable hemoglobin levels **(Supplementary Figure S2)**. All 6 patients with *RPS* mutations (100%) needed treatment for anemia. In patients with *RPS* mutations, 1 patient *(*17%; *RPS17)* was steroid-responsive whereas 5 patients (83%) were dependent on regular transfusions.

Treatment

At the time of assessment, 11 patients (65%) were undergoing chronic treatment for their anemia. Initially, most patients were treated with red blood cell transfusions (n=11, 65%), the majority (n=10, 91%) had their first transfusion before the age of 1 year. All patients needing regular transfusions received iron chelation (n=6, 35%). Five chronically transfused patients (29%) were assessed for cardiac and liver iron overload by magnetic resonance imaging (T2*-weighted gradient-echo). None

showed any cardiac iron overload whereas all patients had a mild hepatic iron loading (n=5, 100%).

All patients receiving treatment for anemia (n=13, 77%) had at least one trial with corticosteroids. Six patients (46%) were not responsive to steroids, one patient lost response to steroids after 4 years and was treated with regular transfusions. The minimally effective dose for steroids ranged from 0.01mg/kg/d to 0.23mg/kg/d (median 0.17 mg/kg/d). Two patients underwent allogeneic HSCT due to steroid-dependency with poor tolerance in one and transfusion-dependency in the other patient. They were both treatment-free at time of study. No third-line treatment showed any efficacy in this patient cohort (**Supplementary Table S3**)

Discussion

We showed that patients with DBA in Switzerland present with varying degrees of anemia and a wide array of malformations including the lower limbs and anorectal region.

In our cohort, we observed a high proportion of physical malformations (13 patients, 76%). In the literature, 35-63% of patients were reported with physical malformations. (2, 8) We found 9 patients (53%) with malformations in multiple organ systems compared to approximately 25% in the Italian and North American cohort and 40% in the Dutch cohort. (1, 2, 7) This wide variation could partly be explained by differences in reporting and grouping of malformations. There is also a higher rate of malformations in cohorts performing organ screenings on all patients. In our cohort, all patients received an echocardiogram and all but one an abdominal ultrasound. Previous reports on skeletal malformations stated that mainly upper limbs are affected. (1) One report described malformation of the lower extremities (hip dysplasia, skeletal leg deformity or syndactyly of toes). (2) We found three patients

(18%) with malformations of their feet and two patients (12%) with hip dysplasia. This highlights that the spectrum of malformations in DBA patients is wide and malformations of the lower extremities can be associated. Additionally, two patients (12%) had anorectal malformations which have only been described in two patients with DBA to our knowledge so far. (2, 9)

In the past years, an increasing number of genotype-phenotype association analyses have been described in the literature. (10) We found a difference in the need for treatment for anemia between *RPL* (60%) and *RPS* (100%) gene mutations in our population. Other cohorts showed similar results (3), while some reported a good treatment response in *RPS* patients (11). We found that patients with an *RPL* mutation had a higher proportion of malformations (especially *RPL5* and *RPL11*). This is in line with previous publications. (7, 11)

Two patients of the same family (12%) were identified with a variant in *RPL17* associated with a DBA-like condition that segregated with affected family members and was predicted to be pathogenic using in vivo complementation studies in zebrafish. Its pathogenicity remains to be proven formally by further evidence and other affected families. (12) Facial dysmorphism, thumb malformations and a mild form of macrocytic anemia were seen in affected family members and none of the pediatric patients needed treatment for anemia.

A limitation of our study is the small sample size which is limited by the number of patients diagnosed with this rare disease in a small country like Switzerland. Furthermore, the diagnosis has been made by the local treating pediatric hematologists. We might have missed patients with unclassified anemia and no need for treatment that would fulfill criteria for an atypical form of DBA but have not been further evaluated due to their mild phenotype and were not included in our study.

This highlights the importance of considering DBA as a possible diagnosis in therapy-resistant anemia. A strength of our analysis is the high proportion of genotyped patients and the detailed information on clinical presentation and treatment. As all pediatric hematology centers in Switzerland participated in the study, we were able to show a nationwide overview of the currently treated patients with DBA at the time of data collection.

Conclusion

Our dataset contributes to the understanding of the large variability in the phenotype of DBA and the diversity of genetic variation and confirms many of the findings in other DBA cohorts. We found a high proportion of physical malformations including lower limb and anorectal malformations. Patients with an *RPS* gene mutation were treatment-dependent but showed less malformations than patients with an *RPL* gene mutation. The clinical spectrum of DBA is wide, including patients with malformations but only mild anemia. DBA should be considered in therapy-resistant chronic anemia not otherwise classified. Particularly patients with malformations should be tested for DBA even if mild anemia is present.

Declarations

Funding

Nicolas Waespe was supported by the CANSEARCH Foundation, Geneva, Switzerland.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability Statement

The datasets that where generated within this study will not be publicly available due to concerns for anonymity of patients with Diamond-Blackfan anemia being a rare disease with very few patients. Datasets that support the findings are available from the corresponding author upon reasonable request.

Author contribution statement

Nicole Vogel: Conceptualization, Methodology, Data preparation and curation, Writing - all stages, Visualization;

Markus Schmugge, Raffaele Renella: Writing - Reviewing and Editing;

Nicolas Waespe: Conceptualization, Methodology, Writing - all stages;

Heinz Hengartner: Supervision, Conceptualization, Methodology, Writing - all stages.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval number 2018-01793, leading committee: Eastern Switzerland. Date 09.10.2018. Written informed consent was obtained from patients and/or parents.

References

1. Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. Blood. 2010;116(19):3715-23.

2. van Dooijeweert B, van Ommen CH, Smiers FJ, Tamminga RYJ, Te Loo MW, Donker AE, et al. Pediatric Diamond-Blackfan anemia in the Netherlands: An overview of clinical characteristics and underlying molecular defects. Eur J Haematol. 2018;100(2):163-70.
3. Orfali KA, Ohene-Abuakwa Y, Ball SE. Diamond Blackfan anaemia in the UK: clinical and genetic heterogeneity. Br J Haematol. 2004;125(2):243-52.
4. Tamary H, Nishri D, Yacobovich J, Zilber R, Dgany O, Krasnov T, et al. Frequency and natural history of inherited bone marrow failure syndromes: the Israeli Inherited Bone Marrow Failure Registry. Haematologica. 2010;95(8):1300-7.
5. Diamond LK, Wang WC, Alter BP. Congenital hypoplastic anemia. Adv Pediatr. 1976;22:349-78.
6. Clinton C, Gazda HT, Adam MP, Ardinger HH, Pagon RA, Wallace SE, et al. Diamond Blackfan Anemia GeneReviews1993-2020 [Available from: https://www.ncbi.nlm.nih.gov/pubmed/20301769.
7. Quarello P, Garelli E, Carando A, Cillario R, Brusco A, Giorgio E, et al. A 20-year long term experience of the Italian Diamond-Blackfan Anaemia Registry: RPS and RPL genes, different faces of the same disease? Br J Haematol. 2020.
8. Bartels M, Bierings M. How I manage children with Diamond-Blackfan anaemia. Br J Haematol. 2019;184(2):123-33.
9. van den Hondel D, Wijers CH, van Bever Y, de Klein A, Marcelis CL, de Blaauw I, et al. Patients with anorectal malformation and upper limb anomalies: genetic evaluation is warranted. Eur J Pediatr. 2016;175(4):489-97.
10. Da Costa L, O'Donohue MF, van Dooijeweert B, Albrecht K, Unal S, Ramenghi U, et al. Molecular approaches to diagnose Diamond-Blackfan anemia: The EuroDBA experience. Eur J Med Genet. 2018;61(11):664-73.
11. Ulirsch JC, Verboon JM, Kazerounian S, Guo MH, Yuan D, Ludwig LS, et al. The Genetic Landscape of Diamond-Blackfan Anemia. Am J Hum Genet. 2018;103(6):930-47.
12. Davis EE, Reid DW, Liang J, Willer JR, Fievet L, Bhuiyan ZA, et al. Mutations in RPL17 expand the molecular basis of Diamond-Blackfan anemia and guide insights into unique biochemical signatures underscoring ribosomopathies. https://www.ashg.org/wp-content/uploads/2019/10/2014-platform-abstracts.pdf2014 [Available from: https://www.ashg.org/wp-content/uploads/2019/10/2014-platform-abstracts.pdf.

Legends

TABLE 1 Baseline characteristics of 17 children with Diamond Blackfan anemia being treated in Switzerland (as of July 2019). [a] including one patient without identified causal mutation; [b] 8 patients not included as 4 patients had no measurement of eADA activity and 4 patients only had measurements post transfusion of red blood cells; [c] patients with *RPL17* and *RPL11* mutations

	All patients (n=17)[a]	RPL mutation (n=10)	RPS mutation (n=6)
Demographics			
Male (n, %)	9 (53)	6 (60)	3 (50)
Female (n, %)	8 (47)	4 (40)	3 (50)
Age at presentation (months; median, range)	2 (0-64)	0 (0-64)	2.5 (0-9)
Age at diagnosis (months; median, range)	8 (0-78)	7.5 (0-78)	5.5 (0-27)
Time from presentation to diagnosis (months; median, range)	2 (0-42)	3.5 (0-42)	4 (0-22)
Ethnic background (n, %)			
Swiss	10 (59)	6 (60)	3 (50)
Other Caucasian	5 (29)	2 (20)	3 (50)
Other Mixed	2 (12)	2 (20)	0
Laboratory results at diagnosis			
Severe anemia (Hb < 70g/l) (n, %)	12 (71)	5 (50)	6 (100)
Hemoglobin (g/l; median, range)	60 (15-137)	76 (47-137)	22 (15-34)
MCV (fl; median, range)	98 (81-150)	94 (81-120)	102 (92-150)
Reticulocyte count (‰; median, range)	2 (0.7-55)	2.6 (0.98-55)	2 (1.5-12.3)
Thrombopenia (n, %)	2 (12)	0	2 (33)
Neutropenia (n, %)	1 (6)	1 (10)	0
eADA measured pre-transfusion (n, %) [b]	9 (53)	6 (60)	2 (33)
U/l (median, range)	475 (232-1565)	421 (232-1543)	948 (475-1420)
eADA elevated (n, %)	6 (67)	3 (50)	2 (100)
HbF measured pre-transfusion (n, %)	11 (65)	7 (70)	3 (50)
%; (median, range)	9 (0.7-67.1)	6 (0.7-67.1)	20.8 (19.9-43.5)
HbF elevated for age (n, %)	9 (82)	5 (71)	3 (100)
Bone marrow aspiration and trephine biopsy (n, %)	13 (76)	6 (60)	6 (100)
Decreased or absent red cell precursors (n, %)	9 (69)	6 (100)	2 (33)
Overall reduced cellularity of bone marrow (n, %)	1 (8)	0	1 (17)
Organ screening (n, %)			
Abdominal ultrasound	16 (94)	9 (90)	6 (100)
Echocardiogram	17 (100)	10 (100)	6 (100)
MRI, T2*-weighted gradient echo imaging	5 (29)	2 (20)	3 (50)
Congenital malformations (n, %)			
No malformations	4 (24)	0	3 (50)
One or more malformation	13 (76)	10 (100)	2 (33)
Small for gestational age	7 (37)	7 (70)	0
Growth retardation	5 (29)	4 (40)	1 (17)
Developmental delay	6 (35)	4 (40)	2 (33)
Treatment at time of data collection (n, %)			
Corticosteroids	5 (29)	3 (30)	1 (17)
Chronic red blood cell transfusions	5 (29)	1 (10)	4 (67)
Corticosteroids and red blood cell transfusions combined	1 (6)	1 (10)	0
HSCT	2 (12)	1 (10)	1 (17)
No treatment [c]	4 (24)	4 (40)	0

eADA, erythrocyte Adenosine Deaminase Activity; growth retardation, height below the 3rd percentile for age and sex; HbF, fetal hemoglobin; HSCT, Hematopoietic Stem Cell Transplantation; MCV, mean corpuscular erythrocyte volume; MRI, magnetic resonance imaging; n, number; multiple malformations, >1 organ system affected with malformations; *RPL*, large ribosomal protein subunit; *RPS*, small ribosomal protein subunit; severe anemia, hemoglobin < 70 g/l; SGA, small for gestational age, birth weight below 10th percentile for gestational age and sex.

Supporting Information

SUPPLEMENTARY File S1 Methods

SUPPLEMENTARY TABLE S1 Congenital malformations in 17 Swiss pediatric Diamond Blackfan anemia patients. ASD, atrial septal defect; AV block I, atrioventricular block I; IP, interphalangeal joint; M. thenar, Musculus thenar; MCP, metacarpophalangeal joint; PFO, patent foramen ovale; VSD, Ventricular septal defect.

SUPPLEMENTARY TABLE S2 Specific gene mutations in a Swiss cohort of 17 pediatric Diamond Blackfan anemia patients. *RPL/RPS*, large/small ribosomal subunit protein gene; wt, wildtype; [a] Genes analyzed: RPL11, RPL26, RPL35a, RPL5, RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, GATA1

SUPPLEMENTARY FIGURE S1 Malformations according to casual genetic mutations in 17 Swiss pediatric Diamond Blackfan anemia patients. *RPL/RPS,* large/small ribosomal subunit protein gene; Multiple malformations, >1 organ system affected with malformations; References for graphics: clipartqueen.com; thenounproject.com: man by Brad Avison, heart by Akhmad taufiq, mouth by Marco

Galtarossa, thyroid by Laymik, intestines by Maria Zamchy, joint by rivercon, eye by Jay Alfarez, nose by Lnhi, ear by Nicolas Morand, foot by Grégory Montigny, cleft palate by Olena Panasovska, thumb by Casey Hoerman.

SUPPLEMENTARY FIGURE S2 Patient-specific timeline showing course of treatment according to genetic mutation in 17 Swiss pediatric Diamond Blackfan anemia patients. *RPL/RPS,* large/small ribosomal subunit protein gene; P = Date of first presentation with anemia; x = Date of data collection; T = hematopoietic stem cell transplantation (HSCT); ▬ Time between presentation with anemia and date of data collection; ■ (Dark Grey) Corticosteroid therapy; ■ (Light Grey) Transfusion therapy

SUPPLEMENTARY TABLE S3 Third line treatment in a Swiss cohort of 17 pediatric Diamond Blackfan anemia patients