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

SUPPLEMENTARY FILE

Authors: Nicole Vogel [1], Markus Schmugge [2], Raffaele Renella [3], Nicolas Waespe [4,5,6*], Heinz Hengartner [7*] on behalf of the Swiss Paediatric Oncology Group (SPOG) Hematology Working Group

Affiliations: [1] Department of Pediatrics, Cantonal Hospital Graubuenden, Chur, Switzerland; [2] University Children's Hospital Zurich, Switzerland; [3] Department of Pediatrics, Lausanne University Hospital CHUV, Lausanne, Switzerland; [4] Childhood Cancer Research Group, Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland; [5] Research Platform for Pediatric Oncology and Hematology (CANSEARCH Research Platform), Faculty of Medicine, University of Geneva, Geneva, Switzerland; [6] Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland; [7] Pediatric hospital of Eastern Switzerland, St. Gallen, Switzerland

*) equally contributing senior authors

Corresponding Author: Heinz Hengartner, heinz.hengartner@kispisg.ch

SUPPLEMENTARY File 1:

Methods

In Switzerland, pediatric patients with a treatment-dependent hematologic disease are treated and followed-up in one of the nine pediatric hematology centers. We contacted all nine centers to receive information on patients with a diagnosis of Diamond-Blackfan anemia (DBA) that were seen in regular follow-up care, were deceased, or no longer seen in clinical care.

We defined DBA using the diagnostic criteria according to Diamond et al. which include chronic anemia of no other identifiable cause, diagnosis during the first year of life, reticulocytopenia, and no other major cytopenia. (1)

The first author visited each center, which reported DBA patients and extracted the following data from patients' medical charts:

- demographic and clinical information (sex, gestational age at birth, birth weight/length, recent measurement of weight/height);
- family history (including country of origin, anemia, macrocytosis, genetic mutations of parents and siblings, physical malformations)
- diagnostic findings (age at presentation and reported diagnoses, symptoms at presentation, physical malformations, malignancies);
- information on treatment (red cell transfusions, chelation and steroid treatment with initial dose, treatment schedule, minimal effective dose and side effects, HSCT treatment with age at HSCT, donor information, indication and adverse events);
- laboratory data such as full blood counts (hemoglobin (Hgb), mean corpuscular volume (MCV), reticulocyte count, white blood counts, and

platelets), erythrocyte adenosine deaminase activity (eADA), fetal hemoglobin (HbF), ferritin at diagnosis, highest ferritin;

- bone marrow aspiration and trephine biopsy findings;
- genetic test results including interpretation.

Missing information was supplemented by interviewing the treating physicians. All data was encoded at time of evaluation.

Laboratory data was extracted from original laboratory reports at first presentation, wherever available. Results were interpreted according to age with laboratory-specific reference values. Severe anemia was defined as hemoglobin (Hgb) <70g/l. (2)

Genetic testing for DBA-associated gene mutations was conducted at different clinical molecular laboratories at the discretion of the treating physicians. We used the ACMG guidelines to classify variants. (3)

Post-transfusion eADA values were excluded from the analysis to avoid falsely normal eADA levels. (4) All eADA values were obtained from the same laboratory at the University Children's Hospital Zurich, Switzerland which is the only laboratory in the country to perform these analyses. Values between 100 – 400 U/l were considered within the normal range. Iron overload was defined as a serum ferritin level >1000 mcg/l without signs of inflammation as suggested previously. (5) Wherever available MRI T2* findings were considered.

We collected physical malformations according to organ involvement as previously described. (4, 6)

Small for gestational age (SGA) was defined as a birth weight below the 10th percentile for gestational age and sex. We did not summarize short stature (height

below the 3rd percentile for age and sex) in physical malformations due to its likely multifactorial etiology. (5, 7)

We defined treatment response to steroids as adequate when achieving transfusion independency. The treatment response in our cohort was evaluated by the treating hematologist.

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SUPPLEMENTARY TABLE S1 Congenital malformations in 17 Swiss pediatric Diamond Blackfan anemia patients

Organ system	Patients per organ system	Features	Patients per feature
Craniofacial	9	Hypertelorism	3
		Macrocephaly	1
		Retrognathia	1
		High-arched palate	1
		Low set ears	1
		Frontal bossing	1
		Microstomia	1
		Microphthalmia	2
		Mild facial dysmorphism	5
		Cleft palate	4
Cardiac	7	VSD	4
		PFO	2
		ASD	1
		AV block I	1
		Aortic valve insufficiency	1
		Aortic valve hypoplasia	1
Hand (Thumb)	7	Thumb hypoplasia	1
		Triphalangeal thumb	1
		Hypoplasia M. thenar	1
		Mirror hand	1
		Ankylosis MCP and IP	1
Hand (Finger)	3	Brachydactyly	2
		Hypoplasia Dig V	1
Feet	3	Clinodactyly Dig V bilaterally (phalanges)	1
		Metatarsus adductus	1
		Bilateral in-toeing and torsion of Dig III unilaterally	1
Other skeletal malformations	4	Pectus carinatum	1
		Agenesis os coccygis	1
		Hip dysplasia	2
Urogenital	4	Undescended testicles	3
		Duplex kidney	1
Anorectal malformations	2	Anteposition of anus without sphincteric function	1
		Antepositon of anus and coccygeal agenesis	1
Others	3	Hypothyroidism	2
		Thyroid hypervascularity	1

Legend: 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.

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SUPPLEMENTARY TABLE S2 Specific gene mutations in a Swiss cohort of 17 pediatric Diamond Blackfan anemia patients

Patient	Gene	Pathogenic variant	Predicted protein change	Inheritance		Previously reported
1	*RPL5*	*RPL5:c.132C>G*	p.Tyr44Ter	de novo	both parents tested	Muramatsu H, Okuno Y, Yoshida K, et al (2017) Genet Med 19:796–802. https://doi.org/10.1038/gim.2016.197
2	*RPL5*	*RPL5:c.516_522dup*	p.His175Tyrfs*12	de novo	both parents tested	No
3	*RPL5*	*RPL5:c.324+1G>C*	p.?	de novo	both parents tested	No
4	*RPL11*	*RPL11:c.408del*	p.Arg136Serfs*58	de novo	parents not tested, asymptomatic	No
5	*RPL11*	*RPL11:c.6+2T>C*	p.?	de novo	parents not tested, asymptomatic	Ulirsch JC, Verboon JM, Kazerounian S, et al (2018) American Journal of Human Genetics 103:930–947. https://doi.org/10.1016/j.ajhg.2018.10.027
6	*RPL11*	*RPL11:c.416dup*	p.Ser140Glnfs*32	inherited	sibling with identical mutation (patient 7), parents not tested	No
7	*RPL11*	*RPL11:c.416dup*	p.Ser140Glnfs*32	inherited	sibling with identical mutation (patient 6), parents not tested	No
8	*RPL11*	*RPL11:c.433_444dup*	p.Gly149Glnfs*46	inherited	mother with identical mutation, father not tested	No
9	*RPL17*	*RPL17:c.217-3C>G*	p.?	inherited	mother with identical mutation, father not tested; patient 9+10 related	Davis EE, Reid DW, Liang J, et al. ASHG 2014 Abstract #230
10	*RPL17*	*RPL17:c.217-3C>G*	p.?	inherited	mother with identical mutation, father no mutation; patient 9+10 related	Davis EE, Reid DW, Liang J, et al. ASHG 2014 Abstract #230
11	*RPS17*	*RPS17:c.(?_2)_(*104_?)del*	p.?	de novo	parents not tested, asymptomatic	Ulirsch JC, Verboon JM, Kazerounian S, et al (2018) American Journal of Human Genetics 103:930–947. https://doi.org/10.1016/j.ajhg.2018.10.027 (similar deletions reported)
12	*RPS17*	*RPS17:c.201_202del*	p.Gly68Tyrfs*19	de novo	both parents tested	Gazda HT, Sheen MR, Vlachos A, et al (2008) Am J Hum Genet 83:769–780. https://doi.org/10.1016/j.ajhg.2008.11.004
13	*RPS19*	*RPS19:c.1-6_1-2del*	p.?	de novo	parents not tested, asymptomatic	No
14	*RPS19*	RPS19:c.412-2A>G	p.?	de novo	parents not tested, asymptomatic	No
15	*RPS19*	*RPS19:c.172+1G>T*	p.?	de novo	both parents tested	Boria I, Garelli E, Gazda HT, et al (2010) Hum Mutat 31:1269–1279. https://doi.org/10.1002/humu.21383
16	*RPS26*	*RPS26:c.97G>A*	p.Asp33Asn	de novo	both parents tested	Doherty L, Sheen MR, Vlachos A, et al (2010) Am J Hum Genet 86:222–228. https://doi.org/10.1016/j.ajhg.2009.12.015
17	no mutation found [a]	NA			parents not tested, asymptomatic	

Legend: *RPL/RPS*, large/small ribosomal subunit protein gene; wt, wildtype; [a] Genes analysed: RPL11, RPL26, RPL35a, RPL5, RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, GATA1.

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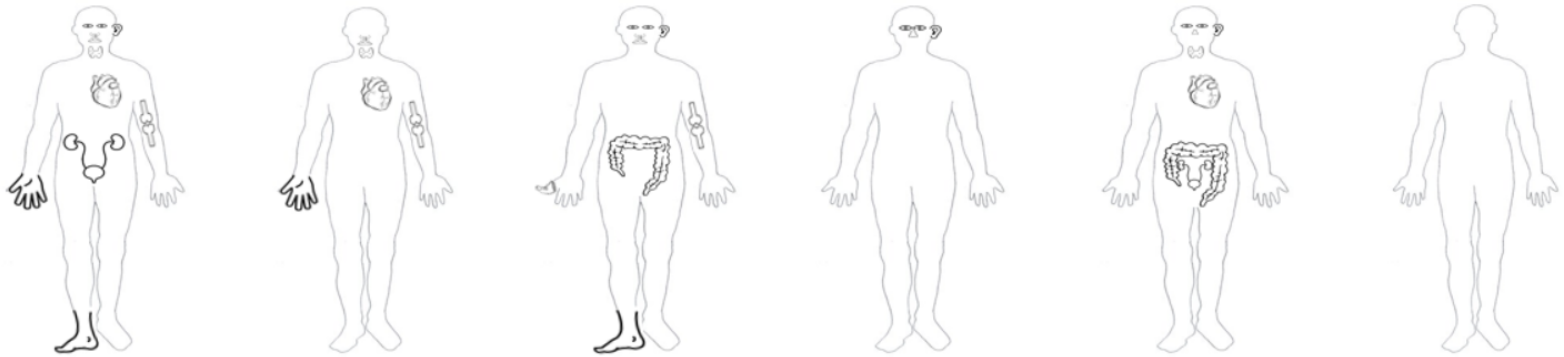
SUPPLEMENTARY FIGURE S1 Malformations according to casual genetic mutations in 17 Swiss pediatric Diamond Blackfan anemia patients.



		RPL 5	*RPL 11*	*RPL 17*	*RPS 17*	*RPS 19*	*RPS 26*
Total Patients		3	5	2	2	3	1
Patients with Multiple Malformations		2	3	2	0	2	0
Malformations							
Face	Craniofacial	3		2	1	1	
	Cleft palate	1	2	1			
Thyroid		1	1			1	
Cardiac		1	4			2	
Hand	Thumb		2	2			
	Finger	2	1				
Feet		2		1			
Other Skeletal Malformations		1	2	1			
Urogenital		1				2	
Anorectal				1		1	

Legend: *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.

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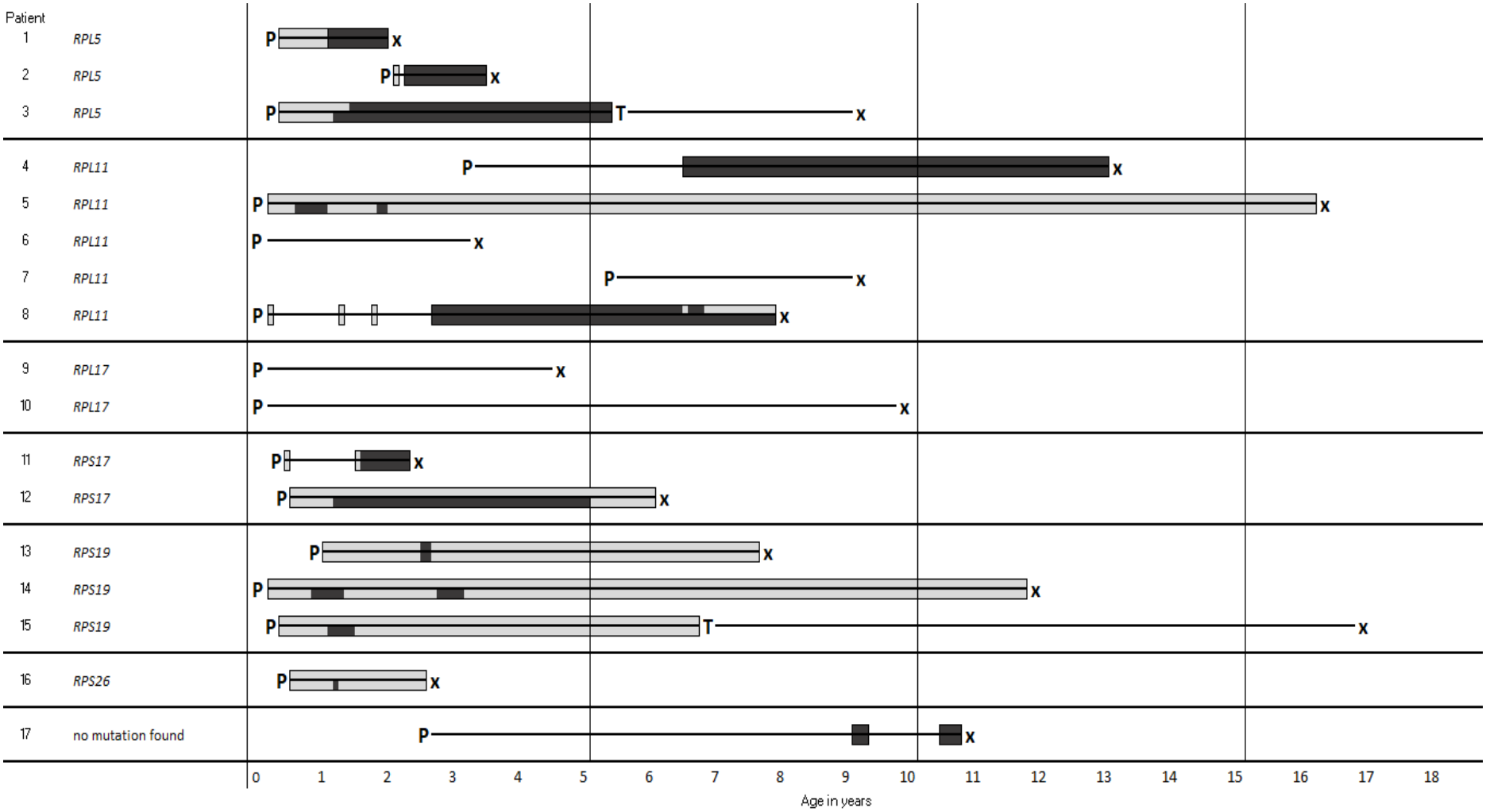
SUPPLEMENTARY FIGURE S2 Patient-specific timeline showing course of treatment according to genetic mutation in 17 Swiss pediatric Diamond Blackfan anemia patients.



Legend: *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

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

Therapy	Dosing and Duration	Number of patients	Effect
Leucine enriched diet	8 months and 10 months respectively	2 (12%)	No clinical effect on blood counts
Metoclopramide	5 months; 0.2mg/kg/day	1 (6%)	No clinical effect on blood counts
Traditional Chinese medicine (not further specified herbal mixture)	In combination with corticosteroid therapy	1 (6%)	No clinical effect on blood counts
Erythropoietin (EPO)	100 U/kg twice a week for 2 months, followed by 200 U/kg twice a week for 2 months and finally a dose of 130U/kg with subsequent tapering	1 (6%)	No clinical effect on blood counts