Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

*Pediatr Blood Cancer*. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

*Pediatr Blood Cancer*. 2015 September ; 62(9): 1597–1600. doi:10.1002/pbc.25534.

# Clinical and genomic heterogeneity of Diamond Blackfan anemia in the Russian Federation

**Natalia S. Smetanina, MD**[1,2], **Irina V. Mersiyanova, PhD**[1], **Maria A. Kurnikova, MD**[3], **Galina S. Ovsyannikova, MD**[1], **Lili A. Hachatryan, MD**[1], **Vlasta O. Bobrynina, PhD**[1], **Michael A. Maschan, MD**[1], **Galina. A. Novichkova, MD**[1], **Jeffrey M. Lipton, MD, PhD**[4,5,6], and **Alexey A. Maschan, MD**[1]

[1]Dmitry Rogachev Federal Clinical Research Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russia

[2]Pirogov Russian National Research Medical University, Moscow, Russia

[3]Evrogen, Moscow, Russia

[4]Hofstra North Shore-LIJ School of Medicine, Hempstead, NY, USA

[5]Feinstein Institute for Medical Research (FIMR); Manhasset, NY, USA

[6]Division of Hematology/Oncology and Stem Cell Transplantation, CCMC, New Hyde Park, NY, USA

## Abstract

**Background—**Diamond Blackfan anemia (DBA) is a genetically and clinically heterogeneous ribosomopathy and inherited bone marrow failure syndrome characterized by anemia, reticulocytopenia and decreased erythroid precursors in the bone marrow with an increased risk of malignancy and in approximately 50%, physical abnormalities.

**Methods—**We retrospectively analyzed clinical data from 77 patients with DBA born in the Russian Federation from 1993–2014. In 74 families there was one clinically affected individual; in only three instances a multiplex family was identified. Genomic DNA from 57 DBA patients and their first-degree relatives was sequenced for mutations in *RPS19, RPS10, RPS24, RPS26, RPS7, RPS17, RPL5, RPL11, RPL35a* and *GATA1*.

**Results—**Severe anemia presented before 8 months of age in all 77 patients; before 2 months in 61 (78.2%); before 4 months in 71 (92.2%). Corticosteroid therapy was initiated after 1 year of age in the majority of patients. Most responded initially to steroids, while 5 responses were transient. Mutations in *RP* genes were detected in 35 of 57 patients studied: 15 in *RPS19*, 6 in *RPL5*, 3 in RPS7, 3 each in *RPS10, RPS26* and *RPL11* and 1 each in *RPS24 and RPL35a*; 24 of

***Corresponding author and person to whom reprint requests should be addressed:*** Jeffrey M. Lipton, MD, PhD, Division of Hematology/Oncology and Stem Cell Transplantation, Cohen Children's Medical Center, 269-01 76th Ave, New Hyde Park, NY 11040, **Phone**: 718-470-3470 / **Fax**: 718-343-4642 / jlipton@nshs.edu.

***Disclosure statement:*** The Authors have nothing to disclose.

Conflict of Interest: The authors have nothing to disclose

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

which having not been previously reported. In one patient a balanced chromosomal translocation involving *RPS19* was found. No mutations in *GATA1* were found.

**Conclusion—**In our cohort from an ethnically diverse population the distribution of mutations among *RP* genes was approximately the same as was reported by others, although within genotypes most of the mutations had not been previously reported.

### Keywords

DBA; Diamond Blackfan anemia; ribosomal proteins

## Introduction

Diamond Blackfan anemia (DBA) is an inherited bone marrow failure syndrome characterized, in classical cases, by a moderate to severe hypoproliferative anemia that usually presents during infancy or early childhood [1]. Although red cell aplasia is the most prominent feature of DBA the disease is also characterized by growth retardation and congenital malformations, in particular craniofacial defects and defects of the upper limbs, heart, and genitourinary system present in ~30%–50% of patients [2–4] as well as a predisposition to cancer [5].

DBA is a clinically heterogeneous disease: laboratory findings, such as macrocytosis, elevated erythrocyte adenosine deaminase (eADA) activity and fetal hemoglobin (HbF) are observed in a majority of, but not in all, patients with DBA [6–8]. The incidence of DBA is estimated to be between 5–10 per million live births [2, 8–10]. It appears that approximately 40% of cases are familial, with disease inherited predominantly as an autosomal-dominant; the remaining cases are sporadic new dominants. The incidence is equal in both genders [8].

Boria *et al*. reviewed the molecular basis of Diamond Blackfan anemia, emphasizing that it is a disorder of defective ribosome biosynthesis [11]. DBA is genetically heterogeneous with confirmed mutations or deletions in 6 small subunit associated ribosomal protein genes (*RP*); *RPS7, RPS10, RPS17, RPS19, RPS24, RPS26* and as well as 5 large subunit associated *RP*s; *RPL5, RPL11, PRL19, RPL26 and RPL35a* in approximately 65–70% of DBA patients [12], Other *RP* gene mutations, in *RPS27, RPS28, RPS29* and *RPL15, RPL19, RPL27, RPL31* are less firmly established [13–17] indicating that DBA is a disorder of ribosomal biogenesis and/or function [18–24]. Recently, additional kindreds have been identified harboring mutations in the erythroid transcription regulator *GATA1* [25].

The aim of our study was to evaluate the presence of causative mutations in a genetically distinct and somewhat diverse cohort of patients from the Russian Federation and to compare our findings to those described in other cohorts.

## Methods

### Patients

We analyzed retrospective data from seventy-seven cases of DBA (41 male, 36 female) born in the Russian Federation over a 20-year period (1993–2014). The diagnosis of DBA in all

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

probands and their family members was established according to the criteria of the DBA Working Group of the European Society for Paediatric Haematology and Immunology [2] based solely upon modified classical criteria; the presence of normochromic, often macrocytic, anemia; reticulocytopenia; a low number or lack of erythroid precursors in the bone marrow; a normal chromosome fragility test (diepoxybutane) and; in some patients, congenital malformations. Using these relatively stringent criteria, only three families were multiplex and seventy-four had only one clinically affected individual. Furthermore the ascertainment of only seventy-seven cases over a 20 year time period (3.85 cases/year) is below the number of cases expected [8] for the population size of the Russian Federation with approximately 1.9 million births/year suggesting incomplete case ascertainment.

### Genetic studies

Genomic DNA was isolated from peripheral blood samples using the nucleic acid isolation kit AmpliPrime DNA-sorb B (Central Research Institute for Epidemiology, Moscow, Russia) according to the manufacturer's instructions. We amplified genomic DNA samples from fifty-seven unrelated DBA probands enrolled in the study and when available their first degree relatives by PCR and sequenced the products on an AB 3130xl Genetic Analyzer (Applied Biosystem, USA) for mutations in nine *RP* genes (*RPS19, RPS10, RPS24, RPS26, RPS7, RPS17, RPL5, RPL11, RPL35a*) and *GATA1*. All exons and exon-intron junction sites were studied. There was no ascertainment of copy number variants, thus it is probable that deletions of DBA-associated *RP* genes were undetected in this study (26). When sequence changes were found, independent PCR products were sequenced to confirm the mutations. In support that these sequence changes were not polymorphic variations we verified that none was reported in the Single Nucleotide Polymorphism database (dbSNP at www.nchi.nlm.nih.gov/SNP) or in the Ensembl database (www.ensembl.org).

## Results

Severe normochromic anemia with low reticulocyte and normal platelet count manifested before the age of two months in sixty-one cases (78.2%). Twenty-eight of these cases presented at birth. Ten cases (12.8%) presented at the age of 3–4 months and six cases (7.7%) at the age of 5–8 months. One 3 month old female patient presented, in addition to anemia, with thrombocytopenia and granulocytopenia, both resolving spontaneously at 6 months of age. Congenital malformations, predominantly craniofacial defects and short stature, were observed in forty-six patients (59.1 %). Table 1 describes the spectrum of malformations observed in our patients. Per consensus guidelines (8) corticosteroids were withheld from the majority (N=56) of patients for up to or greater than 1 year of age with patients receiving regular red blood cell transfusions to keep their hemoglobin level in the 9.5–12.0 g/dL range. Steroid therapy was initiated at the age of 5–8 months in fourteen cases. Five of thirty-five patients who initially responded to steroid therapy had only a transient response. Two non-responders, male and female, achieved remission at the ages of 15 and 18 years, respectively. The female patient relapsed within 6 months. Two patients were successfully transplanted from an HLA-identical sibling at the ages of 1 and 2 years, respectively.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

At the time of the study twenty-two patients (33%) were steroid-dependent, three (4%) achieved complete hematologic recovery after long-term low-dose steroid therapy, two (3%) became transfusion-independent after bone marrow transplantation and forty patients (60%) still required regular transfusions.

Fifty-seven families were available for DNA analyses. Their data are shown in Table 2. Mutations in different *RP* genes were detected in thirty-five probands (15 in *RPS19*, 3 in RPS7, 3 in *RPS26*, 3 in *RPS10*, 1 in *RPS24*, 6 in *RPL5*, 3 in *RPL11 and* 1 in *RPL35a*). There were no patients with mutations in *RPS17* or *GATA1*. Thirty-one cases among them appeared to be sporadic and only four were familial. This low number of familial cases is likely a consequence of incomplete genetic ascertainment and small family size. One patient (P2) showed a balanced chromosomal translocation involving *RPS19* [t (8; 19) (q24; q13)]. Thirty-five percent of patients did not have any mutations in the studied genes.

A database of DBA-associated mutations, curated by Boria and Ramenghi, in the Leiden Open Variation Database (http://www.dbagenes.unito.it/) was used to ascertain the presence of novel mutations in known DBA-associated *RP* genes. The predominant mutations, as described in numerous cohorts were present in *RPS19* (Table 2). Our patients were typical with regard to age at presentation, response to steroids and the nature of congenital anomalies; numbers being too small for a comparison to published data. Of note there were ten mutations previously not described and four known mutations associated with DBA. The mutations were predominantly frameshift mutations affecting the sequence of exons 2, 3, 4 and 5 (6 cases), five amino acid substitutions, three missense-mutations and one affecting the donor splice site of intron 4. In one case a balanced chromosomal translocation (t (8; 19) (q24; q13) c [13]) was found. *De novo* balanced reciprocal translocations have been described previously [27, 28].

Mutations in the *RPS26* gene were identified in three probands. All of them were sporadic and were not described previously. Two mutations affected the sequence of the second exon (amino acid substitution (Arg10Gly) associated with multiple congenital malformations and a frameshift mutation with typical craniofacial dysmorphism). The two carrying these mutations responded to steroid therapy and remain dependent on low-dose corticosteroids. The third patient had a donor splice site mutation at the third intron associated with craniofacial dysmorphism and cryptorchidism. He remains transfusion-dependent.

Mutations in the *RPL5* gene were identified in six probands. Five of them were sporadic, one familial. Five mutations were not described previously. These patients had craniofacial dysmorphism, webbed neck and poor hair growth in one case. All affected patients had their disease presentation during the first two months of life and were unresponsive to steroid therapy. Two probands were found to have a frameshift mutation. Two mutations affecting the splice sites were found: one (P4) was a *de novo* insertion near the donor splice site after exon 3 and the second one (P66) was acceptor splice site complex deletion-insertion mutation at the junction of intron 4 and exon 5 (c.325-4_c.332delATAGCTTCTCAAinsTT). This complex mutation was familial (father shares the same mutation, but he is not clinically affected). Two mutations were nonsense mutations at exon 2 and 3, respectively.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Two different mutations in *RPS10* were identified in three unrelated probands. A nonsense mutation at exon 4 (Arg113Stop) was previously described [18]. This mutation was identified in two unrelated probands. Interestingly, one patient's father bears the same nonsense mutation at exon 4 and has a MCV (99 fL) at the upper limit of normal. The other demonstrated this mutation *de novo*. In both cases, a response to initial steroid therapy was achieved. The other mutation is a new sporadic frameshift mutation at exon 3 associated with craniofacial dysmorphism. That patient initially responded to steroid therapy at 2 years of age but lost his response at 7 years of age and remains transfusion-dependent since then.

Three new mutations in the *RPS7* gene were identified in three cases. All of them had not been described previously and affect the donor splice site at intron 1, one sporadic, and the other two familial. In all cases, these mutations were associated with craniofacial dysmorphism and response to initial steroid therapy. In one, at the age of 16.5 years, osteogenic sarcoma was diagnosed, representing the first case of DBA-associated osteogenic sarcoma associated with a mutation in *RPS7*. Following the initiation of cyclophosphamide chemotherapy the patient developed resistant aplasia and became transfusion-dependent for the remainder of his treatment. After discontinuation of chemotherapy, steroid therapy was initiated, which was successful, and his hemoglobin level and reticulocyte count were sustained on prednisolone (0.6 mg/kg/day). The patient's affected brother and father shared the *RPS7* mutation. The father had a low normal hemoglobin level (12 mg/dL) with a MCV (98 fL) at the upper limit of normal. The father's parents did not share the mutation strongly suggesting that the father had a sporadic new dominant mutation.

The sporadic splice site mutation also affecting the donor site at intron 1 was detected in unrelated female proband (P18). Clinical manifestations appeared at the age of 1 month and until age 10 months she received regular blood transfusions. Subsequently, steroid therapy was initiated. The patient responded and remains on low dose prednisolone (0.5 mg/kg every other day).

An intronic deletion of seven nucleotides affecting the donor splice site of exon 2 was found in proband P75 who presented with mild anemia at birth and became transfusion dependent at the age of 2.5 years. His father bears the same mutation and has a MCV of 98 fL; his grandparents were not tested.

A mutation in the *RPS24* gene was identified in one case. It had not been described previously. The patient presented with a normochromic are generative anemia at the age of 4 months. Steroid therapy was initiated twice at the age of 2 and 7 years old but was ineffective. The patient remained on regular packed red cell transfusions until at the age of 17 years. Spontaneous recovery occurred which lasted for 6 months, after which regular blood transfusions were again required. This patient carried a sporadic mutation - donor splicing site mutation at intron 1 – associated with craniofacial dysmorphism.

A previously described frameshift mutation in exon 2 of the *RPL11* gene was identified in one patient and was associated with craniofacial dysmorphism and unresponsiveness to steroid therapy. Two of the other mutations (newly described frameshift mutation at exon 2

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and a nonsense mutation at exon 3), previously described, were associated with craniofacial dysmorphism and cardiac malformation.

A novel frameshift mutation in exon 3 of the *RPL35a* gene was identified in one patient. No congenital malformations were observed in this patient. Initial steroid therapy was effective, and this patient continues to receive low-dose prednisolone (0.2 mg/kg/day).

## Discussion

This report of patients with DBA from the Russian Federation is consistent with observations from previous reports and offers some new information describing novel mutations and a previously undescribed DBA-associated osteogenic sarcoma genotype (*RPS7*). Nonsense, splicing site and frameshift mutations all together were found in approximately 85% of cases, and missense mutations in 15% cases. We studied nine *RP* genes (*RPS19, RPS10, RPS24, RPS26, RPL5, RPL11, RPL35a, RPS7* and *RPS17*) and found twenty five novel mutations in eight. Two mutations affecting the splice site after the first non-coding exon of the *RPS7* gene were found to be pathogenic. One was found in two affected brothers and their father (mother and grandparents had normal DNA sequence of the *RPS7* gene), the other appeared *de novo* (both parents being normal). This observation shows the importance of noncoding regions for ribosomal protein synthesis. The majority of our DBA cases were sporadic, as observed by Willig and colleagues (7) (10–25% of cases familial) and Gazda et al. (29) (40–50% of the cases familial) with autosomal dominant inheritance. Our four familial cases also demonstrated autosomal dominant inheritance. Twelve cases were sporadic with confirmed *de novo* mutations (both parents were studied). However the prevalence of familial cases is most certainly underestimated as a consequence of bias introduced in a retrospective study, the very restrictive definition of DBA employed and incomplete family data; only one parent, primarily the mother, was available for eight probands, and no genetic abnormalities were found in only that parent.

The mean age at disease presentation was 1 month, and approximately 69% of patients had associated physical anomalies, including craniofacial dysmorphism, webbed neck, atrial septal defect, patent foramen ovale, membranous subaortic stenosis, consistent with previous observations (reviewed in 30).

Also consistent with prior observations the majority of our DBA cases (16 probands) were associated with a mutation in the *RPS19* gene and the frequency of other mutations in *RP* genes for our cohort of DBA patients is approximately the same as reported by others, save the Japanese registry which reports a *RPS19* mutation frequency of only 11% (reviewed in 31).

Vlachos and colleagues (5) reported an increased risk of malignant disease in patients with DBA, with a likelihood of developing cancer of 22% by age 46 years. Among the tumors described osteogenic sarcoma was identified as a DBA-associated malignancy with an observed to expected ratio of 32.6. Unfortunately the paucity of genotypic data did not permit the identification of any osteogenic sarcoma associated mutations. Of note in the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Russian DBA cohort, while only one patient developed osteosarcoma at age 17 years, a novel germ line mutation in *RPS7* was demonstrated.

The Russian cohort demonstrates that additional important information can be garnered from the study of diverse international cohorts of patients with Diamond Blackfan anemia. It is of interest that the RP genes involved in DBA in Russia are proportionally similar to other cohorts, while the actual mutations vary significantly within each genotype.

## Acknowledgments

**Grant support:**

This work is supported by grants from Charitable “Grand Life” Fund of Russian Federation (NSS, IVM, MAK, GSO, LAH, VOB, MAM, GAN, AAM) and the National Heart Lung and Blood Institute (R01HL079571), the Pediatric Cancer Foundation, the Diamond Blackfan Anemia Foundation and the Daniella Maria Arturi Foundation (JML).

## Literature

1. Alter, BP.; Young, NS. The bone marrow failure syndromes. In: Nathan, DG.; Orkin, HS., editors. Hematology of Infancy and Childhood. Vol. 1. Philadelphia: Saunders; 1998. p. 237-335.
2. Willig TN, Niemeyer CM, Leblanc T, Tiemann C, Robert A, Budde J, Lambiliotte A, Kohne E, Souillet G, Eber S, Stephan JL, Girot R, Bordigoni, Cornu G, Blanche S, Guillard JM, Mohandas N, Tchernia G. Identification of new prognosis factors from the clinical and epidemiologic analysis of a registry of 229 Diamond-Blackfan anemia patients. DBA group of Société d'Hématologie et d'Immunologie Pédiatrique (SHIP), Gesellshaft für Pädiatrische Onkologie und Hämatologie (GPOH), and the European Society for Pediatric Hematology and Immunology (ESPHI). Pediatr Res. 1999; 46:553–561. [PubMed: 10541318]
3. Orfali KA, Ohene-Abuakwa Y, Ball SE. Diamond Blackfan anaemia in the UK: clinical and genetic heterogeneity. Br J Haematol. 2004; 125:243–252. [PubMed: 15059149]
4. Lipton JM, Atsidaftos E, Zyskind I, Vlachos A. Improving clinical care and elucidating the pathophysiology of Diamond-Blackfan anemia: an update from the Diamond-Blackfan Anemia Registry. Pediatr Blood Cancer. 2006; 46:558–564. [PubMed: 16317735]
5. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. Blood. 2012; 119:3815–3819. [PubMed: 22362038]
6. Glader BE, Backer K. Elevated red cell adenosine deaminase activity: a marker of disordered erythropoiesis in Diamond-Blackfan anaemia and other haematologic diseases. Br J Haematol. 1998; 68:165–168. [PubMed: 3348976]
7. Willig TN, Draptchinskaia N, Dianzani I, Ball S, Niemeyer C, Ramenghi U, Orfali K, Gustavsson P, Garelli E, Brusco A, Tiemann C, Pérignon JL, Bouchier C, Cicchiello, Dahl N, Mohandas N, Tchernia G. Mutations in ribosomal protein S19 gene and Diamond Blackfan anemia: wide variations in phenotypic expression. Blood. 1999; 94:4294–4306. [PubMed: 10590074]
8. Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, Meerpohl J, Karlsson S, Liu JM, Leblanc T, Paley C, Kang EM, Leder EJ, Atsidaftos E, Shimamura A, Bessler M, Glader B, Lipton JM. Diagnosing and treating Diamond-Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol. 2008; 142:859–876. [PubMed: 18671700]
9. Ball SE, McGuckin CP, Jenkins G, Gordon-Smith EC. Diamond-Blackfan anaemia in the U.K.: analysis of 80 cases from a 20-year birth cohort. Br J Haematol. 1996; 94:645–653. [PubMed: 8826887]
10. Campagnoli MF, Garelli E, Quarello P, Carando A, Varotto S, Nobili B, Longoni D, Pecile V, Zecca M, Dufour C, Ramenghi U, Dianzani I. Molecular basis of Diamond-Blackfan anemia: new findings from the Italian registry and a review of the literature. Haematologica. 2004; 89:480–489. [PubMed: 15075082]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


11. Boria I, Garelli E, Gazda HT, Aspesi A, Quarello P, Pavesi E, Ferrante D, Meerpohl JJ, Kartal M, Da Costa L, Proust A, Leblanc T, Simansour M, Dahl N, Fröjmark AS, Pospisilova D, Cmejla R, Beggs AH, Sheen MR, Landowski M, Buros CM, Clinton CM, Dobson LJ, Vlachos A, Atsidaftos E, Lipton JM, Ellis SR, Ramenghi U, Dianzani I. The ribosomal basis of Diamond–Blackfan anemia: mutation and database update. Hum Mutat. 2010; 31:1269–1279. [PubMed: 20960466]
12. Vlachos A, Dahl N, Dianzani I, Lipton JM. Clinical utility gene card for: Diamond Blackfan anemia-update 2013. Euro J Hum Genet.
13. Wang R, Yoshida K, Toki T, Sawada T, Uechi T, Okuno Y, Sato-Otsubo A, Kudo K, Kamimaki I, Kanezaki R, Shiraishi Y, Chiba K, Tanaka H, Terui K, Sato T, Iribe Y, Ohga S, Kuramitsu M, Hamaguchi I, Ohara A, Hara J, Goi K, Matsubara K, Koike K, Ishiguro A, Okamoto Y, Watanabe K, Kanno H, Kojima S, Miyano S, Kenmochi N, Ogawa S, Ito E. Loss of function mutations in RPL27 and RPS27 identified by whole-exome sequencing in Diamond-Blackfan anaemia. Br J Haematol. 2014 Nov 25.
14. Gripp KW, Curry C, Olney AH, Sandoval C, Fisher J, Chong JX, UW Center for Mendelian Genomics. Pilchman L, Sahraoui R, Stabley DL, Sol-Church K. Diamond-Blackfan anemia with mandibulofacial dystostosis is heterogeneous, including the novel DBA genes TSR2 and RPS28. Am J Med Genet A. 2014 Sep; 164A(9):2240–2249. [PubMed: 24942156]
15. Mirabello L, Macari ER, Jessop L, Ellis SR, Myers T, Giri N, Taylor AM, McGrath KE, Humphries JM, Ballew BJ, Yeager M, Boland JF, He J, Hicks BD, Burdett L, Alter BP, Zon L, Savage SA. Whole-exome sequencing and functional studies identify RPS29 as a novel gene mutated in multicase Diamond-Blackfan anemia families. Blood. 2014; 124(1):24–32. 3. [PubMed: 24829207]
16. Landowski M, O'Donohue MF, Buros C, Ghazvinian R, Montel-Lehry N, Vlachos A, Sieff CA, Newburger PE, Niewiadomska E, Matysiak M, Glader B, Atsidaftos E, Lipton JM, Beggs AH, Gleizes PE, Gazda HT. Novel deletion of RPL15 identified by array-comparative genomic hybridization in Diamond-Blackfan anemia. Hum Genet. 2013 Nov; 132(11):1265–1274. [PubMed: 23812780]
17. Farrar JE, Quarello P, Fisher R, O'Brien KA, Aspesi A, Parrella S, Henson AL, Seidel NE, Atsidaftos E, Prakash S, Bari S, Garelli E, Arceci RJ, Dianzani I, Ramenghi U, Vlachos A, Lipton JM, Bodine DM, Ellis SR. Exploiting pre-rRNA processing in Diamond Blackfan anemia gene discovery and diagnosis. Am J Hematol. 2014 Oct; 89(10):985–991. [PubMed: 25042156]
18. Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, Ball S, Tchernia G, Klar J, Matsson H, Tentler D, Mohandas N, Carlsson B, Dahl N. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. Nat Genet. 1999; 21:169–175. [PubMed: 9988267]
19. Gazda HT, Grabowska A, Merida-Long LB, Latawiec E, Schneider HE, Lipton JM, Vlachos A, Atsidaftos E, Ball SE, Orfali KA, Niewiadomska E, Da Costa L, Tchernia G, Niemeyer C, Meerpohl JJ, Stahl J, Schratt G, Glader B, Backer K, Wong C, Nathan DG, Beggs AH, Sieff CA. Ribosomal protein S24 gene is mutated in Diamond-Blackfan anemia. Am J Hum Genet. 2006; 79:1110–1118. [PubMed: 17186470]
20. Cmejla R, Cmejlova J, Handrkova H, Petrak J, Pospisilova D. Ribosomal protein S17 gene (RPS17) is mutated in Diamond-Blackfan anemia. Hum Mutat. 2007; 28:1178–1182. [PubMed: 17647292]
21. Farrar JE, Nater M, Caywood E, McDevitt MA, Kowalski J, Takemoto CM, Talbot CC Jr, Meltzer P, Esposito D, Beggs AH, Schneider HE, Grabowska A, Ball SE, Niewiadomska E, Sieff CA, Vlachos A, Atsidaftos E, Ellis SR, Lipton JM, Gazda HT, Arceci RJ. Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia. Blood. 2008; 112:1582–1592. [PubMed: 18535205]
22. Gazda HT, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Schneider H, Darras N, Hasman C, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Zaucha JM, Glader B, Niemeyer C, Meerpohl JJ, Atsidaftos E, Lipton JM, Gleizes PE, Beggs AH. Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. Am J Hum Genet. 2008; 83:769–780. [PubMed: 19061985]
23. Doherty L, Sheen MR, Vlachos A, Choesmel V, O'Donohue M-F, Clinton C, Schneider HE, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Glader B, Arceci RJ, Farrar JE,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Atsidaftos E, Lipton JM, Gleizes P-E, Gazda HT. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. Am J Hum Genet. 2010; 86:222–228. [PubMed: 20116044]

24. Yazaki M, Kamei M, Ito Y, Konno Y, Wang R, Toki T, Ito E. A novel mutation of ribosomal protein S10 gene in a Japanese patient with Diamond-Blackfan anemia. J Pediatr Hematology/Oncology. 2012; 34(4):293–295.
25. Sankaran VG, Ghazvinian R, Do R, Thiru P, Vergilio J-A, Beggs AH, Sieff CA, Orkin SH, Nathan DG, Lander ES, Gazda HT. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. J Clin Invest. 2012; 122:2439–2443. [PubMed: 22706301]
26. Farrar JE, Vlachos A, Atsidaftos E, Carlson-Donohoe H, Markello TC, Arceci RJ, Ellis SR, Lipton JM, Bodine DM. Ribosomal protein gene deletions in Diamond Blackfan anemia. Blood. 2011; 118:6943–6951. [PubMed: 22045982]
27. Gustavsson P, Skeppner G, Johansson B, Berg T, Gordon L, Kreuger A, Dahl N. Diamond-Blackfan anaemia in a girl with a *de novo* balanced reciprocal X;19 translocation. J Med Genet. 1997; 34:779–782. [PubMed: 9321770]
28. Bugge M, Bruun-Petersen G, Brøndum-Nielsen K, Friedrich U, Hansen J, Jensen G, Jensen PKA, Versson UK, Lundsteen C, Niebuhr E, Rasmussen KR, Rasmussen K, Tommerup N. Disease associated balanced chromosome rearrangements: a resource for large scale genotype-phenotype delineation in man. J Med Genet. 2000; 37:858–865. [PubMed: 11073540]
29. Gazda HT, Preti M, Sheen MR, O'Donohue M-F, Vlachos A, Davies SM, Kattamis A, Doherty L, Landowski M, Buros C, Ghazvinian R, Sieff CA, Newburger PE, Niewiadomska E, Matysiak M, Glader B, Atsidaftos E, Lipton JM, Gleizes P-E, Beggs AH. Frameshift mutation of p53 regulator RPL26 is associated with multiple physical abnormalities and a specific pre-ribosomal RNA processing defect in Diamond-Blackfan anemia. Hum Mutat. 2012; 33:1037–1044. [PubMed: 22431104]
30. Vlachos A, Blanc L, Lipton JM. Diamond Blackfan anemia: a model for the translational approach to understanding human disease. Expert Review of Hematol. 2014; 7:359–372.
31. Farrar JE, Dahl N. Untangling the phenotypic heterogeneity of Diamond Blackfan anemia. Semin Hematol. 2012; 48:124–135. [PubMed: 21435509]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

**Table I**

Spectrum of physical malformations among 77 DBA patients

| Malformation | Number of patients | % |
|---|---|---|
| Craniofacial dysmorphism* | 23 | 29.9 |
| Craniofacial dysmorphism, webbed neck | 12 | 15.6 |
| Craniofacial dysmorphism, cardiac malformation** | 7 | 9.0 |
| Craniofacial dysmorphism, cryptorchidism | 1 | 1.3 |
| Craniofacial dysmorphism, cardiac malformation, malformation of the gastrointestinal tract***, single kidney | 2 | 2.6 |
| Multiple malformation (craniofacial, heart, hypospadias, bone dysplasia, microcephaly, absent left ear) | 1 | 1.3 |
| None | 31 | 40.3 |

* Craniofacial defects observed in our cohort of patients includes micrognathia, high arched palate, ocular hypertelorism, epicanthus, broad flat nasal bridge, microtia, low-set ears and in two cases low anterior hairline.

** Cardiac malformations included aortic septal defect, membranous subaortic stenosis, pulmonary stenosis, patent foramen ovale, ventricular septal defect, patent ductus arteriosus.

*** Malformation of the gastrointestinal tract – malrotation, megacolon.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



**Table II**

*RP* gene mutations, congenital anomalies and treatment response in DBA patients

| Gene | Proband's ID (gender), inheritance | Exon or intron | DNA mutation | Predicted amino acid change | Family member DNA results | Age of disease onset | Malformation status | Response at first steroid therapy/ age | Present therapy | Previously reported[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| **RPS19** | P30(M) sporadic | Ex 2 | c.3G>T | Met1>Ile | Parents not tested | 1 mo | craniofacial dysmorphism*, webbed neck | Unresponsive at 12 mo | Trs (RBC transfusions) | Yes |
| **RPS19** | P74 (M) sporadic | Ex 2 | c.3G>A | Met1>Ile | mother – normal, father not tested | At birth | None | Response at 18 mo, lost response at 38 mo | Trs | Yes |
| **RPS19** | P57(F) sporadic | Ex 2 | c.29_30insA | Frameshift at codon 10; stop at 50 p.Asn10Lys_fs*41 | Parents not tested | 1 mo | craniofacial dysmorphism* | Steroid naive | Trs | No |
| **RPS19** | P60(M) sporadic | Ex 2 | c.31C>T | Gln11* | Parents normal, *de novo* | At birth | craniofacial dysmorphism* | Unresponsive at 3 mo | Trs | Yes |
| **RPS19** | P25(M) sporadic | Ex 2 | c.33delG | Frameshift at codon 11; stop at 28 p.Gln11His_fs*18 | Parents normal, *de novo* | 2 mo | craniofacial dysmorphism*, cardiomyopathy | Response at 4 mo | Steroid therapy | No |
| **RPS19** | P11 (F) sporadic | Ex 3 | c.77_79del GGAinsTG | Frameshift at codon 27; stop at 28 p.Lys27Ser_fs*2 | mother – normal, father not tested | 1 mo | None | Response at 18 mo | Steroid therapy | No |
| **RPS19** | P24 (M) sporadic | Ex 3 | c.114G>C | Lys38Asn | Parents normal, *de novo* | At birth | craniofacial dysmorphism* | Response at 3 mo | Steroid therapy | No |
| **RPS19** | P14 (F) sporadic | Ex 3 | c.156G>A | Trp52* | Parents normal, *de novo* | At birth | None | Unresponsive at 23 mo | Trs | Yes |
| **RPS19** | P32(M) sporadic | Ex 4 | c.184C>T | Arg62Trp | Parents not tested | 2 mo | None | Unresponsive at 12 mo | Trs | Yes |
| **RPS19** | P22(M) sporadic | Ex 4 | c.242_243 insA | Frameshift at codon 82; stop at 153 p.Arg82Thr_fs*72 | Parents not tested | 1 mo | craniofacial dysmorphism*, aortic septal defect, neutropenia | Response at 4 mo | Steroid therapy | No |
| **RPS19** | P17(M) sporadic | Ex 4 | c.248_249 insA | Frameshift at codon 84; stop at 153 p.Arg84Glu_fs*70 | Parents not tested | At birth | None | Response at 2 mo | Steroid therapy | No |
| **RPS19** | P16(M) sporadic | Int 4 | c.356+1 G>C | Donor splice site mutation | Parents not tested | 1 mo | None | Response at 18 mo | Steroid therapy | No |

*Pediatr Blood Cancer*. Author manuscript; available in PMC 2016 September 01.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



| Gene | Proband's ID (gender), inheritance | Exon or intron | DNA mutation | Predicted amino acid change | Family member DNA results | Age of disease onset | Malformation status | Response at first steroid therapy/ age | Present therapy | Previously reported[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| **RPS19** | P54(M) sporadic | Ex 5 | c.386_387 delGA | Frameshift at codon 130; stop at 152 p.Asp130Ser_fs*23 | Parents normal, *de novo* | 1 mo | craniofacial dysmorphism* | Response at 5 y | Steroid therapy Remission at 20 years | No |
| **RPS19** | P62(M) sporadic | Ex 5 | c.392T>G | Leu131Arg | mother normal, father not tested | At birth | High arched palate, ocular hypertelorism, microtia, low-set ears pulmonary stenosis, ventricular septal defect | Unresponsive at 6 mo | Trs | Yes |
| **RPS19** | P71(M) sporadic | Ex 5 | c.406 G>T | Gly136* | Parents normal, *de novo* | At birth | None | Response at 26 | Steroid therapy | No |
| **RPS19** | P2(M) sporadic | t(8;19)(q24;q13)c[13] | | | Parents normal | 1 mo | craniofacial dysmorphism*, webbed neck | Response at 14 mo | Steroid therapy | No |
| **RPS7** | P8 (M) Familial | Int 1 | c.-19+1 G>T | Donor splice site mutation | father and brother – same mutation, mother and grandparents - normal | 8 mo | Craniofacial dysmorphism* | Response at 36 mo | Steroid therapy | No |
| **RPS7** | P18 (F) sporadic | Int 1 | c.19+2T >C | Donor splice site mutation | Parents normal, *de novo* | 1 mo | Craniofacial dysmorphism* | Response at 10 mo | Steroid therapy | No |
| **RPS7** | P75 (M) Familial | Int 2 | c.75+2_75+8delTGAGAGG | Donor splice site mutation | father– same mutation, mother - normal | 1 mo | None | Unresponsive at 36 mo | Trs | No |
| **RPS10** | P21 (M) sporadic | Ex 3 | c.292_293insTACGGCC | Frameshift at codon 98; stop at 109 p.Arg98Leu_fs*12 | Parents not tested | 2 mo | Craniofacial dysmorphism* | Response at 24 mo | Trs at 7 y | No |
| **RPS10** | P67 (M) sporadic | Ex 4 | c.337C>T | Arg113* | Parents normal, *de novo* | At birth | None | Response at 6 mo | Steroid therapy | Yes |
| **RPS10** | P64 (F) familial | Ex 4 | c.337C>T | Arg113* | father – same mutation, mother - normal | At birth | None | Response at 10 mo | Steroid therapy | Yes |
| **RPS24** | P7 (F) sporadic | Int 1 | c.3+1 G>T | Donor splice site mutation | Parents normal, *de novo* | 8 mo | Craniofacial dysmorphism* | Unresponsive at 24 mo | Trs | No |
| **RPS26** | P1 (F) sporadic | Ex 2 | c.8_11delAGAA | Frameshift at codon 4; stop at 43 p.Lys4Glu_fs*40 | Parents not tested | 3 mo | Craniofacial dysmorphism* | Response at 12 mo | Steroid therapy | No |

*Pediatr Blood Cancer*. Author manuscript; available in PMC 2016 September 01.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



| Gene | Proband's ID (gender), inheritance | Exon or intron | DNA mutation | Predicted amino acid change | Family member DNA results | Age of disease onset | Malformation status | Response at first steroid therapy/ age | Present therapy | Previously reported[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| **RPS26** | P3 (F) sporadic | Ex 2 | c.28C>G | Arg10Gly | Parents normal, *de novo* | 1 mo | Craniofacial dysmorphism*, ventricular septic defect, unfinished gut's turn | Response at 6 mo | Steroid therapy | No |
| **RPS26** | P5 (M) sporadic | Int 3 | c.312+3-c.312+6delGAGT | Donor splice site mutation | mother - normal, father not tested | 2 mo | Craniofacial dysmorphism*, cryptorchidism | Unresponsive at 4 mo | Trs | No |
| **RPL5** | P77 (F) sporadic | Ex 2 | c.47delA | Frameshift at codon 16; stop at 18 | Parents not tested | At birth | None | Steroid naive | Trs | No |
| **RPL5** | P76 (F) sporadic | Ex 2 | c.67C>T | Arg23* | Mother and sister – normal, father not tested | 11 days | None | Response at 12 | Steroid therapy | Yes |
| **RPL5** | P37 (M) sporadic | Ex 3 | c.187C>T | Gln63* | Parents normal, *de novo* | 1 mo | Craniofacial dysmorphism*, webbed neck | Unresponsive at 18 mo | Trs | No |
| **RPL5** | P4 (F) sporadic | Int 3 | c.189+3insC | Donor splice site mutation | Parents normal, *de novo* | 2 mo | Craniofacial dysmorphism*, low anterior hairline, webbed neck, | Unresponsive at 6 mo | Trs | No |
| **RPL5** | P66 (F) familial | Int 4 – Ex 5 | c.325-4_c.332del ATAGCTTCTCAA insTT | Acceptor splice site mutation | mother and sister - normal, father – the same mutation | At birth | Craniofacial dysmorphism* | Unresponsive at 12 mo | Trs | No |
| **RPL5** | P40 (M) sporadic | Ex 5 | c.351delG | Frameshift at codon 118; stop at 125 p.Ile118Ser_fs*8 | mother - normal, father not tested | 7 mo | Craniofacial dysmorphism* | Unresponsive at 24 mo | Trs | No |
| **RPL11** | P35 (M) sporadic | Ex 2 | c.60_61delCT | Frameshift at codon 21; stop at 53 | mother - normal, father not tested | 1 mo | Craniofacial dysmorphism* | Unresponsive at 6 years | Trs | Yes |
| **RPL11** | P72 (F) sporadic | Ex 2 | c.65delT | Frameshift at codon 22; stop at 33 p.Cys21Ser_fs*33 | mother - normal, father not tested | 1 mo | High arched palate, microtia, ocular hypertelorism, patent foramen ovale, patent ductus arteriosus | Steroid naive | Trs | No |
| **RPL11** | P73 (F) sporadic | Ex 3 | c.223C>T | Arg75* | Parents not tested | At birth | High arched palate, microtia, ocular | Steroid naive | Trs | Yes |

Author Manuscript Author Manuscript Author Manuscript Author Manuscript



| Gene | Proband's ID (gender), inheritance | Exon or intron | DNA mutation | Predicted amino acid change | Family member DNA results | Age of disease onset | Malformation status | Response at first steroid therapy/ age | Present therapy | Previously reported[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | hypertelorism, patent foramen ovale,, duplicated left kidney | | | |
| **RPL35a** | P55 (F) sporadic | Ex 3 | c.90delT | Frameshift at codon 30; stop at 74 p. Ile30Met_fs*45 | Parents not tested | At birth | None | Response at 11 mo | Steroid therapy | No |

[1] http://www.dbagenes.unito.it/

* Non-specific syndromic facies, not specified