



HAL
open science

THE RIBOSOMAL BASIS OF DIAMOND-BLACKFAN ANEMIA: MUTATION AND DATABASE UPDATE

Ilenia Boria, Emanuela Garelli, Hanna T. Gazda, Anna Aspesi, Paola Quarello, Elisa Pavesi, Daniela Ferrante, Joerg J. Meerpohl, Mutlu Kartal, Lydie da Costa, et al.

► **To cite this version:**

Ilenia Boria, Emanuela Garelli, Hanna T. Gazda, Anna Aspesi, Paola Quarello, et al.. THE RIBOSOMAL BASIS OF DIAMOND-BLACKFAN ANEMIA: MUTATION AND DATABASE UPDATE. Human Mutation, 2010, 31 (12), pp.1269. 10.1002/humu.21383 . hal-00594911

HAL Id: hal-00594911

<https://hal.science/hal-00594911>

Submitted on 22 May 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**THE RIBOSOMAL BASIS OF DIAMOND-BLACKFAN ANEMIA:
MUTATION AND DATABASE UPDATE**

| | |
|-------------------------------|---|
| Journal: | <i>Human Mutation</i> |
| Manuscript ID: | humu-2010-0291.R1 |
| Wiley - Manuscript type: | Mutation Update |
| Date Submitted by the Author: | 10-Sep-2010 |
| Complete List of Authors: | <p>Boria, Ilenia; Università del Piemonte Orientale "A. Avogadro", Dept. Medical Sciences, Garelli, Emanuela; Università di Torino, Dept. of Pediatrics, Gazda, Hanna; Children's Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research,; Harvard Medical School, Aspesi, Anna; Università del Piemonte Orientale "A. Avogadro", Dept. Medical Sciences, Quarello, Paola; Università di Torino, Dept. of Pediatrics, Pavesi, Elisa; Università del Piemonte Orientale "A. Avogadro", Dept. Medical Sciences, Ferrante, Daniela; Università del Piemonte Orientale "A. Avogadro", Dept. Medical Sciences, Meerpohl, Joerg; University Medical Center, Pediatric Hematology & Oncology, Dept. of Pediatric and Adolescent Medicine, Kartal, Mutlu; University Medical Center, Pediatric Hematology & Oncology, Dept. of Pediatric and Adolescent Medicine, Da Costa, Lydie; Hôpital Robert-Debré, AP-HP, Service d'Hématologie Biologique,; INSERM Unit 1009; Université Paris VII-Denis Diderot Proust, Alexis; Hôpital Bicêtre, Hematology Laboratory, Leblanc, Thierry; Hôpital Robert-Debré, AP-HP, Service d'Hématologie Biologique, Simansour, Maud; Hôpital Robert-Debré, AP-HP, Service d'Hématologie Biologique, Dahl, Niklas; Uppsala University Children's Hospital, Dept. of Genetics and Pathology, Section of Clinical Genetics, The Rudbeck laboratory, Fröjmark, Anne-Sophie; Uppsala University Children's Hospital, Dept. of Genetics and Pathology, Section of Clinical Genetics, The Rudbeck laboratory, Pospisilova, Dagmar; Palacky University, Dept. of Pediatrics, Cmejla, Radek; Institute of Hematology and Blood Transfusion, Dept. of Cell Physiology,</p> |

| | |
|------------|--|
| | <p>Beggs, Alan; Children's Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research,; Harvard Medical School</p> <p>Sheen, Mee; Children's Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research,</p> <p>Landowski, Michael; Children's Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research,</p> <p>Buros, Christopher; Children's Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research,</p> <p>Clinton, Catherine; Children's Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research,</p> <p>Dobson, Lori; Children's Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research,</p> <p>Vlachos, Adrianna; The Feinstein Institute for Medical Research,; Cohen Children's Medical Center of New York, Hofstra University School of Medicine,</p> <p>Atsidaftos, Eva; The Feinstein Institute for Medical Research,; Cohen Children's Medical Center of New York, Hofstra University School of Medicine,</p> <p>Lipton, Jeffrey; The Feinstein Institute for Medical Research,; Cohen Children's Medical Center of New York, Hofstra University School of Medicine,</p> <p>Ellis, Steven; University of Louisville,</p> <p>Ramenghi, Ugo; Università di Torino, Dept. of Pediatrics,</p> <p>Dianzani, Irma; Università del Piemonte Orientale "A. Avogadro", Dept. Medical Sciences,</p> |
| Key Words: | Diamond-Blackfan anemia, ribosomal protein, erythropoiesis, ribosome biogenesis |
| | |

SCHOLARONE™
Manuscripts

1
2
3 **THE RIBOSOMAL BASIS OF DIAMOND-BLACKFAN ANEMIA: MUTATION AND**
4
5 **DATABASE UPDATE**
6
7
8
9

10 I.Boria^a, E.Garelli^b, H.T.Gazda^{c,d}, A.Aspesia^a, P.Quarello^b, E.Pavesia^a, D.Ferrante^a,
11 J.J.Meerpohl^{e,f}, M.Kartale^e, L.Da Costag^{h,i}, A.Proust^j, T.Lebanc^g, M.Simansour^g, N.Dahl^k,
12 A-S. Fröjmark^k, D.Pospisiloval^l, R.Cmejlam^m, A.H.Beggs^{c,d}, M.R.Sheenc^c, M.Landowski^c,
13 C.Burosc^c, C.Clinton^c, L.Dobson^c, A.Vlachos^{n,o}, E.Atsidaftos^{n,o}, J.M.Lipton^{n,o}, S.R.Ellisq^p,
14 U.Ramenghi^b, I.Dianzani^a.
15
16
17
18
19
20
21
22
23
24
25

26 ^a Dept. Medical Sciences, Università del Piemonte Orientale “A. Avogadro” - Alessandria,
27 Novara, Vercelli, Italy; ^b Dept. Pediatrics, Università di Torino, Torino, Italy; ^c Children's
28 Hospital Boston, Division of Genetics and Program in Genomics, The Manton Center for
29 Orphan Disease Research, Boston, MA, USA; ^d Harvard Medical School, Boston, MA, USA;
30 ^e Pediatric Hematology & Oncology, Dept. of Pediatric and Adolescent Medicine, University
31 Medical Center, Freiburg, Germany; ^f German Cochrane Center, Institute of Medical
32 Biometry and Medical Informatics, University Medical Center Freiburg, Freiburg, Germany;
33 ^g AP-HP, Service d'Hématologie Biologique, Hôpital Robert-Debré, Paris, France; ^h
34 INSERM Unit 1009, Villejuif, France; ⁱ Université Paris VII-Denis Diderot, Paris, France; ^j
35 Hematology Laboratory, Bicêtre Hospital, Le-Kremlin-Bicêtre, France; ^k Dept. of Genetics
36 and Pathology, Section of Clinical Genetics, The Rudbeck laboratory, Uppsala University
37 Children's Hospital, Uppsala, Sweden; ^l Dept. of Pediatrics, Palacky University, Olomouc,
38 Czech Republic; ^m Dept. of Cell Physiology, Institute of Hematology and Blood Transfusion,
39 Prague, Czech Republic; ⁿ The Feinstein Institute for Medical Research, Manhasset, NY,
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 USA; o Cohen Children's Medical Center of New York, Hofstra University School of
4
5
6 Medicine, New Hyde Park, NY, USA, p University of Louisville, KY, USA.
7
8
9

10 11 12 **ABSTRACT**

13
14
15 Diamond-Blackfan Anemia (DBA) is characterized by a defect of erythroid progenitors and,
16
17 clinically, by anemia and malformations. DBA exhibits an autosomal dominant pattern of
18
19 inheritance with incomplete penetrance. Currently nine genes, all encoding ribosomal proteins
20
21 (RP), have been found mutated in approximately 50% of patients. Experimental evidence
22
23 supports the hypothesis that DBA is primarily the result of defective ribosome synthesis.
24
25

26
27 By means of a large collaboration among six centers, we report here a mutation update that
28
29 includes nine genes and 220 distinct mutations, 56 of which are new. The DBA Mutation
30
31 Database now includes data from 355 patients. Of those where inheritance has been
32
33 examined, 125 patients carry a *de novo* mutation and 72 an inherited mutation. Mutagenesis
34
35 may be ascribed to slippage in 65.5% of indels, whereas CpG dinucleotides are involved in
36
37 23% of transitions. Using bioinformatic tools we show that gene conversion mechanism is not
38
39 common in RP genes mutagenesis, notwithstanding the abundance of RP pseudogenes.
40
41 Genotype-phenotype analysis reveals that malformations are more frequently associated with
42
43 mutations in *RPL5* and *RPL11* than in the other genes. All currently reported DBA mutations
44
45 together with their functional and clinical data are included in the DBA Mutation Database.
46
47
48
49
50

51
52
53
54
55 Key words: Diamond-Blackfan anemia; ribosomal protein; erythropoiesis; ribosome
56
57 biogenesis.
58
59
60

BACKGROUND

Diamond Blackfan anemia (DBA, OMIM 105650) is a rare inherited disease characterized by severe normochromic macrocytic anemia and reticulocytopenia, typically presenting in the first year of life. Patients generally show a decreased number of erythroid progenitors in their bone marrow [Campagnoli et al., 2004]. The other bone marrow cell lineages are only rarely suppressed. Erythrocytes in DBA patients frequently express fetal hemoglobin (HbF) and erythrocyte adenosine deaminase (eADA) activity, a crucial enzyme of the purine salvage pathway, is elevated in 85% of cases [Glader and Backer, 1988]. DBA is associated with an increased risk of malignancies, especially hematopoietic neoplasms and osteogenic sarcomas [Vlachos et al., 2008]. In 30% to 47% of cases patients show physical malformations involving head, thumb, heart and urogenital system [Lipton, 2006]. Prenatal or postnatal growth retardation independent of steroid therapy is also often present.

The incidence of DBA is around 6 per 1 million live births [Campagnoli et al., 2004]. Most cases are sporadic, but the disease can be inherited with an autosomal dominant pattern. Penetrance is incomplete and expressivity widely variable, even in patients from the same family [Campagnoli et al., 2004]. First-line therapy in DBA patients is steroid treatment. Although 80% of patients have an initial steroid response, less than half the patients can be maintained on a safe and effective dose. Thus many of these initial responders may experience temporary or definitive steroid-resistance or dose limiting toxicity [Vlachos et al., 2008]. Patients who do not respond to steroids undergo chronic blood transfusions and need iron chelation to avoid secondary hemochromatosis. Preliminary data suggest that patients with DBA are more likely to develop iron overload than patients with thalassemia, another disease treated with chronic transfusions [Roggero et al., 2009]. Twenty percent of patients inexplicably achieve remission [Lipton, 2006]. DBA can be treated successfully by allogeneic bone marrow or stem cell transplantation, but the mortality from infections, graft-versus-host

1
2
3 disease and graft failure is significant, especially for unrelated donor transplants [Roy et al.,
4
5
6 2005; Vlachos et al., 2008].

7
8 The first DBA gene, ribosomal protein (RP) *S19*, was identified in 1999 [Draptchinskaia et
9
10 al., 1999] and is mutated in about 25% of patients [Willig et al., 1999; Campagnoli et al.,
11
12 2008]. Mutations in an increasing number of other genes encoding RPs of the small (*RPS24*,
13
14 *RPS17*, *RPS7*, *RPS10*, *RPS26*) and large (*RPL35A*, *RPL5*, *RPL11*) ribosomal subunits have
15
16
17 been recently described in DBA patients [Gazda et al., 2006; Cmejla et al., 2007; Farrar et al.,
18
19 2008; Gazda et al., 2008; Doherty et al., 2010]. All mutations are present on a single allele,
20
21 pointing to autosomal dominant inheritance haploinsufficiency. DBA is unquestionably a
22
23 ribosomopathy, a term initially proposed for dyskeratosis congenita [Luzzatto and
24
25 Karadimitris, 1998].
26
27

28
29 In eukaryotes, the ribosome is composed of four different ribosomal RNAs (rRNAs) and 79
30
31 ribosomal proteins. While 5S rRNA is transcribed by RNA polymerase III, 28S, 5.8S and 18S
32
33 rRNAs are processed from a 45S precursor transcribed by RNA polymerase I. The maturation
34
35 of pre-rRNA occurs in the nucleolus through a complex pathway involving both endo- and
36
37 exonucleases that remove external and internal transcribed sequences (ETS and ITS). During
38
39 these steps, the 45S pre-rRNA associates with ribosomal proteins, ribonucleases, RNA
40
41 helicases, small nucleolar RNPs (snoRNPs) and other accessory factors, to form 90S pre-
42
43 ribosomes. During the maturation process, the 90S pre-ribosome is separated into pre-40S and
44
45 pre-60S subunits that are exported to the cytoplasm where maturation is completed
46
47 [Tschochner and Hurt, 2003]. Mature 40S subunits include 18S rRNA and 33 ribosomal
48
49 proteins whereas mature 60S subunits contain 28S, 5.8S and 5S rRNAs and 46 ribosomal
50
51 proteins. In humans there are several loci containing rRNA genes, but only one gene for each
52
53
54
55
56
57
58 of the 79 ribosomal proteins.
59
60

1
2
3 Molecular mechanisms underlying the causal effect between RP haploinsufficiency and
4 anemia have not been elucidated. A generally recognized pathogenetic hypothesis implies
5 defective ribosome biogenesis leading to apoptosis in erythroid progenitors. This mechanism
6 has been named “ribosomal stress” and there are indications that it may be signalled through
7 p53 [Lipton and Ellis, 2009]. Several RPs have a second function different from their roles as
8 structural components of the ribosome. Defects in these extra-ribosomal functions might also
9 contribute to the overall complexity of DBA phenotypes.

10
11 Mutations in DBA genes, along with their functional consequences and genotype-phenotype
12 correlations, have been catalogued in the DBA Mutation Database, created by our group in
13 2008 and available via www.dbagenes.unito.it [Boria et al., 2008]. Here we report an update
14 of the DBA Mutation Database. The updated database contains nine DBA genes (*RPS19*,
15 *RPS24*, *RPS17*, *RPS7*, *RPS10*, *RPS26*, *RPL5*, *RPL11*, *RPL35A*) and 220 distinct mutations. It
16 now includes information on molecular mechanisms involved in RP mutagenesis and more
17 detailed information about inheritance. This update arises from the collaboration of Czech,
18 French, German, Swedish, American and Italian DBA clinical and research groups.

41 VARIANTS

43 RPS19

44 The *RPS19* gene (**OMIM 603474**; locus 19q13.2) was the first DBA gene that was discovered
45 and is the most frequently mutated in patients. It comprises 6 exons and spans 11 kb. The first
46 exon (372 bp) is not included in the coding DNA sequence (CDS) region, while the other five
47 (435 bp) encode a protein of 145 amino acids (MW ~16 kDa).

48 Eighty-seven distinct mutations have been previously described in *RPS19* gene [most
49 reviewed in Campagnoli et al., 2008]. We here report 42 additional mutations: 11 missense, 3
50 nonsense, 18 deletions and/or insertions, 10 splice-site defects (Table 1). Overall 129 distinct
51
52
53
54
55
56
57
58
59
60

1
2
3 *RPS19* mutations are reported and they are carried by 219 patients: 82 of these are *de novo*
4
5 and 45 are inherited. The inheritance was not ascertained in the remaining cases.
6

7
8 At least 163 polymorphisms are listed in NCBI SNP database. We have identified 9
9
10 unpublished intronic allelic variants: c.71+174A>G, c.71+24A>G, c.356+166G>T,
11
12 c.411+6G>T, c.412-75A>G, c.356+153G>A, c.356+29T>C, c.356+229G>A and c.412-
13
14 131T>C. Seven pseudogenes are annotated in the NCBI Gene database as 'inferred'.
15
16

17 **RPL5**

18
19 The human *RPL5* gene (OMIM 603634; locus 1p22.1) consists of eight exons and spans 9.8
20
21 kb. The primary transcript is 1031 nt long and encodes a 297-aa protein (MW ~34.2 kDa)
22
23 component of the 60S ribosomal subunit.
24
25

26
27 Heterozygous mutations in *RPL5* gene have been reported in DBA patients [Gazda et al.,
28
29 2008; Cmejla et al., 2009; Quarello et al., 2010]. Gazda et al. showed mutations in 18 of 196
30
31 DBA probands (9%) and in six additional family members [Gazda et al., 2008], for a total of
32
33 24 individuals; Cmejla et al. studied 28 Czech families and identified sequence changes in 8
34
35 DBA patients from 6 families (21.4%) [Cmejla et al., 2009]; Quarello et al. reported
36
37 mutations in 12 out of 92 (13%) unrelated Italian probands [Quarello et al., 2010]. In this
38
39 paper we are adding 10 new mutations found in 12 patients (Table 1) and two patients
40
41 carrying two previously described mutations. The total number of patients with mutations in
42
43 *RPL5* is 58: 21 have a *de novo* mutation, 10 are familial cases. Thirty-nine mutations are
44
45 distinct and are distributed as follows: 6 missense, 7 nonsense, 21 small deletions and/or
46
47 insertions, 5 splice-site defects.
48
49
50
51

52
53 Ninety-five polymorphisms are listed in NCBI SNP database. There are multiple processed
54
55 pseudogenes of *RPL5* gene dispersed through the genome: three of them are annotated as
56
57 'validated' in NCBI Gene database.
58
59

60 **RPL11**

1
2
3 The *RPL11* gene is located on chromosome 1 (OMIM 604175; locus 1p36.1-p35) and
4
5 encompasses 6 exons spanning 4.6 kb. The *RPL11* mRNA is 609 bp long and encodes a 178
6
7 amino acid protein (MW ~20.1 kDa).
8
9

10 Twenty-three distinct mutations in 34 DBA patients have been previously described [Gazda et
11
12 al., 2008; Cmejla et al., 2009; Quarello et al., 2010]; three new mutations are reported here for
13
14 the first time (Table 1). The 26 mutations are classified as follows: 1 missense, 2 nonsense, 17
15
16 small deletions and/or insertions, and 6 splice-site defects. The total number of patients with
17
18 mutations is 37: 12 carry a *de novo* mutation, whereas 7 are familial cases.
19
20

21 Fifty-seven polymorphisms are reported in NCBI SNP database and five pseudogenes are
22
23 annotated in NCBI Gene database as 'inferred'.
24
25

26 **RPL35A**

27
28 The *RPL35A* gene (OMIM 180468) is located on chromosome 3q29-qter and comprises 6
29
30 exons spanning 5.6 kb. The predicted size of the primary transcript is 511 bp. The first exon
31
32 (41 bp) is not included in the CDS region, while the other five (470 bp) encode a 110-aa
33
34 protein (MW ~12.4 kDa).
35
36

37
38 Farrar et al. [2008] reported five mutations: one missense, one nonsense, one small deletion
39
40 and two deletions of a complete allele. They are all considered pathogenic. The missense
41
42 mutation c.97G>A, which creates a cryptic splice donor site within exon 3, was also found in
43
44 the proband's father and sister, both showing isolated macrocytosis. Inheritance was not tested
45
46 in the remaining cases [Farrar et al., 2008].
47
48

49
50 The *RPL35A* gene has 45 polymorphisms listed in NCBI SNP database and only one
51
52 pseudogene annotated as 'validated' in NCBI Gene database.
53
54

55 **RPS24**

56
57 The *RPS24* (OMIM 602412; locus 10q22-q23) gene comprises 6 exons and spans 8 kb. Its
58
59 three isoforms are expressed as splice variants: S24a or variant 1 (615 bp), S24c or variant 2
60

1
2
3 (593 bp) and S24b or variant 3 (633 bp), encoding proteins of 130, 133 and 131 amino acids,
4
5 respectively. The different isoforms show a tissue-specific pattern of expression [Xu and
6
7 Roufa, 1996; Gazda et al., 2006].
8
9

10 Three *RPS24* mutations in a total of 8 DBA patients [Gazda et al., 2006] have been previously
11 reported in the DBA Mutation Database. Recently Quarello et al. and Badhai et al. showed
12 two further changes in *RPS24* gene: a small deletion and a missense mutation [Quarello et al.,
13 2010; Badhai et al., 2009]. Additionally, we identified an unpublished splice donor variant
14 (Table 1). In total 6 mutations are reported for this gene: one missense, two nonsense, one
15 small deletion and two splice-site defects. In total, 12 patients were found mutated in this
16 gene: only one has a *de novo* mutation, whereas 5 carry an inherited mutation.
17
18
19
20
21
22
23
24
25
26

27 Eighty-two polymorphisms are listed in NCBI SNP database and only one *RPS24* pseudogene
28 is annotated as 'validated' in NCBI Gene database.
29
30

31 **RPS17**

32
33 The *RPS17* gene (OMIM 180472) is located on chromosome 15 (*locus* 15q). It encompasses
34 five exons and spans 3.7 kb. The *RPS17* mRNA is 562 nt long and encodes for a 135-amino
35 acid protein (MW ~15.5 kDa). Two different sequence changes eliminating the natural start
36 site for protein synthesis were found in this gene by Cmejla et al. in 2007 and Song et al. in
37 2010. Gazda et al. [2008] identified a new mutation among 196 tested probands; it is a
38 deletion of two nucleotides causing a frameshift. All mutations are *de novo*.
39
40
41
42
43
44
45
46
47

48 The *RPS17* gene has 30 polymorphisms listed in NCBI SNP database and has two 'validated'
49 pseudogenes in NCBI Gene database.
50
51

52 **RPS7**

53
54 The *RPS7* gene (OMIM 603658; *locus* 2p25) consists of 7 exons spanning 5.6 kb. The
55 predicted size of the primary transcript is 745 bp encoding a 194-aa protein (MW ~22 kDa).
56
57
58
59
60

1
2
3 A donor splice-site mutation in intron 2 was found in a single DBA patient by Gazda and
4 collaborators [Gazda et al. 2008]. The inheritance was not tested.

5
6
7
8 Eighty-three polymorphisms are listed in NCBI SNP database for this gene and only one
9
10 pseudogene is annotated as 'validated' in NCBI Gene database.

11 12 **RPS26**

13
14
15 The *RPS26* gene (OMIM 603701) is located on chromosome 12 (*locus* 12q13) and has four
16
17 exons spanning 2.32 kb. It results in a transcript of 699 bp encoding a 115-amino acid protein
18
19 (MW ~12.9 kDa).

20
21
22 Recently, Doherty and collaborators identified 8 distinct mutations in 13 DBA patients: 5
23
24 carry a *de novo* mutation, whereas 3 are familial cases [Doherty et al., 2010]. Mutations in
25
26 this gene were identified in about 6.4% of the overall DBA population and are distributed as
27
28 follows: 4 missense, one insertion and 3 splice-site defects.

29
30
31 The *RPS26* gene has 64 polymorphisms listed in NCBI SNP database and 4 pseudogenes
32
33 annotated as 'validated' in NCBI Gene database.

34 35 36 **RPS10**

37
38
39 The *RPS10* gene (OMIM 603632; *locus* 6p21.31) encompasses 6 exons spanning 8.65 kb. The
40
41 predicted size of the primary transcript is 636 bp encoding a 165-aa protein (MW ~18.8 kDa).

42
43
44 Mutations in this gene were identified in about 2.6% of the overall DBA population [Doherty
45
46 et al., 2010]. Specifically three distinct sequence changes in 5 DBA probands have been
47
48 reported: one missense, one nonsense and one insertion. One mutation is *de novo*, whereas in
49
50 the other cases the inheritance was not ascertained [Doherty et al., 2010].

51
52
53 The *RPS10* gene has 88 polymorphisms reported in NCBI SNP database and 3 'validated'
54
55 pseudogenes listed in NCBI Gene database.

MUTAGENESIS MECHANISMS

Common mutagenesis mechanisms that generate point mutations include slippage, that causes small indels, and cytosine-guanine (CpG) dinucleotide methylation followed by spontaneous deamination, which causes G>A or C>T transitions. We evaluated the involvement of these mechanisms in DBA by comparing each mutant sequence to the wild-type gene sequence and by observing the context in which a mutation occurred. We found that 57 out of 87 indels in DBA patients are consistent with a slippage mechanism. The frequency of the different substitution classes is 64 transitions versus 50 transversions. Out of 64 transitions, we identified 15 mutations occurring within CpG dinucleotides.

A rare mechanism of mutagenesis is interlocus gene conversion arising from transfer of genetic information between highly homologous genes. Well-known diseases caused by this process are steroid 21-hydroxylase deficiency [Morel et al., 1989] and Shwachman-Diamond syndrome [Boocock et al., 2003]. In these cases the donor sequence is a nearby pseudogene resulting from a recent duplication. To investigate the occurrence of pseudogene-mediated gene conversion in DBA, we retrieved pseudogene sequences of the most frequently mutated RPs (*RPS19*, *RPL5* and *RPL11*) annotated as 'validated' and/or 'inferred' in NCBI Gene database; all of them were intronless. We aligned them with their respective RP gene sequences and looked at 5bp on each side of the mutation, searching for a correspondence between the pseudogene and the mutated sequence. We found only 6 mutations possibly due to gene conversion: 4 in *RPS19*, 1 in *RPL5* and 1 in *RPL11* (Table 2). The pseudogenes are all located on different chromosomes, as compared to the respective genes. Two mutations in *RPS19* are identical to the corresponding sequence of pseudogene *RPS19P2* located on chromosome 1. Obviously, we could not exclude that the same changes arose independently in the gene and in the pseudogene. In any case, our results show that gene conversion does not play a major role in generating mutations in *RPS19*, *RPL5* and *RPL11*.

1
2
3 In conclusion, the most frequent mutagenic mechanism observed in DBA patients appears to
4 be slippage, followed by transitions occurring at CpG dinucleotides.
5
6
7
8
9

10 **BIOLOGICAL RELEVANCE**

11
12 Several studies have addressed the effects of DBA mutations showing that they can lead
13 either to a reduction of RP mRNA or to the production of ribosomal proteins with defective
14 stability and/or localization. In all these cases, mutations cause haploinsufficiency which in
15 turn interferes with the biogenesis of the large or the small ribosomal subunit. These defects
16 are due to aberrant rRNA maturation at different steps, depending on the affected RP [Flygare
17 et al., 2007; Choismel et al., 2007; Idol et al., 2008; Gazda et al., 2008; Farrar et al., 2008;
18 Doherty et al., 2010]. Here, we briefly revisit the biological function of each DBA RP and the
19 effects of their mutations.
20
21
22
23
24
25
26
27
28
29
30

31 ***RPS19***. RPS19 mutations are associated with a defect in the maturation of 18S rRNA
32 resulting in the accumulation of 21S pre-rRNA precursors [Flygare et al., 2007; Choismel et
33 al., 2007; Idol et al., 2008]. All of these mutations cause loss of function and some have been
34 functionally characterized. Extensive functional data were recently reviewed by Campagnoli
35 et al. in this journal [Campagnoli et al., 2008]. Furthermore Crétien and collaborators reported
36 the study of the subcellular localization of several RPS19 mutants fused to green fluorescent
37 protein (GFP) [Crétien et al., 2008]. They observed impaired nucleolar localization and a
38 marked decrease in levels of protein expression for the following mutants: p.Leu131Pro,
39 p.Trp33X, p.Tyr48X, p.Arg56X, p.Met75X, p.Arg94X, p.Glu13ArgfsX17, p.Arg82ThrfsX72,
40 p.Leu131GlyfsX22. In contrast, p.Trp52Cys, p.Val9_Phe14del and p.Gly120Ser mutants
41 exhibited normal expression and localization. Proteasome inhibitors improved both the
42 expression level and the nucleolar localization of p.Val15Phe, p.Gly127Glu, p.Leu131Pro,
43 p.Arg94X, p.Arg82ThrfsX72 and p.Leu131GlyfsX22 mutants, but had no effect on
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 p.Glu13ArgfsX17, p.Trp33X, p.Tyr48X, p.Arg56X, and p.Met75X RPS19 proteins [Crétien
4
5 et al., 2008]. Another mutation was recently investigated for its functional consequences by
6
7 Badhai and collaborators, who reported that primary fibroblasts from a DBA patient with a
8
9 *RPS19* acceptor splice-site mutation (c.72-2A>C) showed reduced proliferative capacity due
10
11 to G1 arrest [Badhai et al., 2009].
12
13

14
15 ***RPL5***. *RPL5* has been implicated in nucleocytoplasmic transport of 5S rRNA prior to its
16
17 assembly into the large ribosomal subunit [Steitz et al., 1988]. *RPL5* specifically binds to this
18
19 rRNA through the domains located at both the amino terminus and the carboxyl terminus
20
21 [Michael and Dreyfuss, 1996]. It has been shown that the perturbation of ribosomal
22
23 biogenesis by impaired rRNA synthesis, processing, or ribosome assembly, triggers the direct
24
25 binding of *RPL5* along with *RPL11* and possibly *RPL23* to MDM2. These interactions inhibit
26
27 MDM2-mediated p53 ubiquitination and degradation, resulting in p53 activation [Zhang and
28
29 Lu, 2009]. The pathogenic effect of *RPL5* haploinsufficiency on ribosome biogenesis has
30
31 been studied by Gazda and collaborators both in a knockdown cell model and in patient cells
32
33 that harbored the following mutations: c.67C>T, c.173delG, c.175_176delGA,
34
35 c.[498_502delTGTGG;497_498ins40] [Gazda et al., 2008]. HeLa cells expressing small
36
37 interfering RNAs (siRNAs) against *RPL5* show decreased production of 28S and 5.8S mature
38
39 rRNAs and accumulation of their precursors, in particular 32S and 12S. The same defect was
40
41 observed in lymphoblastoid cells established from DBA patients. Moreover, *RPL5*
42
43 knockdown induces reduction of free 60S subunit and formation of half-mer polysomes
44
45 [Gazda et al., 2008].
46
47
48
49
50
51

52
53 ***RPL11***. In yeast, Rpl11 forms a subcomplex with Rpl5 and 5S rRNA that is recruited into
54
55 nascent ribosomes at an early step [Zhang et al., 2007]. In human cells, *RPL11* appears to
56
57 have a similar role in ribosome biogenesis but also functions to suppress the transcriptional
58
59 activity of c-Myc and plays a feedback regulatory role in coordinating c-Myc level and
60

1
2
3 activity with ribosomal biogenesis [Dai et al., 2007]. RPL11 also cooperates with RPL5 to
4
5 inhibit the E3 ubiquitin ligase activity of MDM2, thus resulting in the accumulation of
6
7 transcriptionally active p53 [Zhang et al., 2003]. Fumagalli et al. recently showed that
8
9 RPL11-mediated p53 induction is a general response to defective 40S or 60S ribosome
10
11 biogenesis in human cell lines [Fumagalli et al. 2009]. The ubiquitin-like molecule NEDD8,
12
13 that controls RPL11 stability and subcellular localization, plays an important role in the
14
15 regulation of RPL11 signaling to p53 [Sundqvist et al., 2009]. In zebrafish *rpl11* knockdown
16
17 activates the p53 pathway and disrupts the normal embryonic development through a p53-
18
19 mediated apoptotic response [Chakraborty et al., 2009]. Gazda and collaborators showed that
20
21 mutations in *RPL11*, c.314_315delTT, IVS1+2t>c, IVS2-1g>a, IVS4+1g>t, lead to
22
23 accumulation of the precursors of 28S and 5.8S rRNAs, similar to that of mutations in *RPL5*
24
25 [Gazda et al., 2008].
26
27
28
29
30

31
32 ***RPL35A***. Farrar et al. studied RPL35A deficiency in UT-7/Epo and TF-1 cells by
33
34 transduction with three different small hairpin RNAs (shRNAs) against Rpl35A mRNA. They
35
36 observed decreased proliferation, increased apoptosis and reduced biogenesis of 60S subunits.
37
38 Metabolic rRNA labeling and Northern blot analysis revealed accumulation of 45S and 41S
39
40 early precursors and decreased 12S and 7S pre-RNAs. An EBV-transformed lymphoblastoid
41
42 cell line from a DBA patient with deletion of a complete allele also showed reduced 12S
43
44 rRNA compared to healthy controls [Farrar et al., 2008].
45
46
47

48
49 ***RPS24***. RPS24, like RPS19, is essential for the production of the small ribosomal subunit, as
50
51 displayed by the reduction of 40S subunits and 80S monosomes in polysomal profiles of
52
53 RPS24-depleted cells [Choismel et al., 2008]. Lymphoblastoid cells from three patients with
54
55 mutations in RPS24 (p.Gln106X, p.Arg16X, deletion N2-Q22) showed delayed maturation of
56
57 30S pre-rRNA with a corresponding decrease in 21S and 18S-E pre-rRNAs. Accumulation of
58
59 the 30S pre-rRNA suggests that RPS24 is required for the maturation of both the 5' and 3'
60

1
2
3 ends of 18S rRNA. Primary fibroblasts obtained from a DBA patient with an *RPS24* start
4
5 codon mutation (c.1A>G) showed reduced proliferation and abnormal expression of cell cycle
6
7 regulatory proteins [Badhai et al., 2009]. Moreover, Quarello et al. expressed FLAG-tagged
8
9 *RPS24* protein carrying the mutation p.Gln22del in HEK293 cells to study its subcellular
10
11 localization. Although this mutant protein is less stable than the wild-type, it was able to reach
12
13 the nucleolus [Quarello et al., 2010].
14
15

16
17 ***RPS7.*** *RPS7*, like other RPs discussed above, interacts with MDM2 and regulates its E3
18
19 ligase activity on p53 [Chen et al., 2007]. *RPS7* is itself a substrate of MDM2 and *RPS7*
20
21 ubiquitination enhances p53 response and facilitates cell death triggered by different stress
22
23 signals [Zhu et al., 2009]. The pathogenic effect of the *RPS7* mutation c.147+1G>A was
24
25 studied in lymphoblastoid cells derived from the one patient. These cells show accumulation
26
27 of 45S and 30S pre-rRNAs when compared to an unaffected sibling. Depletion of *RPS7* by
28
29 siRNA in HeLa cells confirmed a defect in 5'-ETS processing [Gazda et al., 2008].
30
31

32
33 ***RPS26.*** *RPS26* can regulate its own expression by binding its pre-mRNA and suppressing its
34
35 splicing [Ivanov et al., 2005]. Northern blot analysis showed that depletion of *RPS26* in HeLa
36
37 cells leads to the accumulation of 43S, 26S and 18S-E pre-rRNAs, pointing to defective
38
39 cleavage at both ends of 18S. The same phenotype was present in lymphoblastoid cells
40
41 derived from *RPS26* mutated patients (c.1A>T, c.1A>G in two different probands, c.97G>A
42
43 and IVS1+1g>c) [Doherty et al., 2010].
44
45
46

47
48 ***RPS10.*** Analysis of *RPS10* depleted HeLa cells and *RPS10* mutated lymphoblastoid cells
49
50 (c.260_261insC and c.337C>T in three different probands) revealed a pre-rRNA processing
51
52 phenotype similar to *RPS26* [Doherty et al., 2010].
53
54
55
56
57
58
59
60

CLINICAL RELEVANCE

For many years DBA was considered to be rarely inherited since most patients presented without any family history. Mutational analyses have clarified this observation: in our study, 125 of 197 patients whose family history was ascertained had de novo mutations, whereas the other 72 were familial. Thus, it is likely that the majority of DBA mutations arise spontaneously. This pattern may be due to reduced reproductive fitness of mutated patients. Difficulties in completing pregnancy and an increase in stillborn offspring have been reported for those women with DBA who have conceived [Faivre et al., 2006].

Genotype-Phenotype Correlation

Genotype-phenotype correlations were evaluated in all patients reported in the DBA Mutation Database. They represent approximately 50% of the total number of DBA patients. Clinical description was not available for a few patients thus each analysis was calculated only for patients that had the specific clinical information.

The statistical analysis was performed by considering the following parameters: growth retardation, craniofacial malformation (other than cleft lip and palate), cleft lip and/or palate, upper limb abnormalities, flat thenar muscle, malformed thumb, triphalangeal thumb, heart anomalies, genitourinary anomalies, presence of any type of malformation including short stature, any type of malformation with the exclusion of short stature, multiple malformations, mental retardation, small for gestational age (SGA), response to steroids (Table 3). Hematological information was not considered because clinical data reported in old publications were not updated. The risk connected with carrying a mutation in a specific RP relative to patients carrying mutations in the other eight RP genes studied was obtained by using logistic regression and odds ratio (OR) and 95% CI were calculated. P values less than 0.05 were considered statistically significant. Data were analyzed with the SAS software

1
2
3 version 8.01. Data were not informative for some rarely mutated RP genes, namely *RPS7*,
4
5
6 *RPS17*, *RPS24* and *RPS10* (Table 3). Results for *RPS19*, *RPL5* and *RPL11* are presented in
7
8 Fig. 1.

9
10 As previously shown [Gazda et al., 2008; Cmejla et al., 2009; Quarello et al., 2010], patients
11
12 carrying mutations in *RPL5* or *RPL11* present more frequently with malformations. The risk
13
14 of malformations of any type, including or excluding short stature, is 6.5 or 7.6-fold higher in
15
16 *RPL5*-mutated and 4.5 or 2.7-fold higher in *RPL11*-mutated patients, respectively, than in
17
18 patients with mutations in other genes. *RPL5*-mutated patients have a statistically higher risk
19
20 of multiple malformations (OR 3.8). Each type of malformation evaluated in this study, with
21
22 the exception of those of the genitourinary tract, is more frequent in patients with mutations in
23
24 *RPL5*. Specifically, 21 out of 24 patients with cleft have mutations in *RPL5*. A cleft was
25
26 shown in two patients who carried mutations in *RPL11* and in one patient who carried a
27
28 mutation in *RPS26*. Clefts have never been found in *RPS19* patients. *RPL5*-mutated patients
29
30 are SGA more frequently than patients with other mutations. Of 9 patients with SGA, 7 carry
31
32 a *RPL5* and 2 a *RPL11* mutation. Although mutations in *RPL11* are associated with an
33
34 increased risk of any type of malformations, most of these are hand abnormalities (Table 3).

35
36 The gene currently associated with genitourinary malformations is *RPL35A*. Of 5 patients
37
38 with mutations in *RPL35A*, 3 have genitourinary malformations. In contrast, only 16 of 249
39
40 patients with mutations in other RP genes have genitourinary malformations (Table 3). This
41
42 difference in frequencies towards is statistically significant. Conversely, patients with
43
44 mutations in *RPS19* are less likely to have malformations of any type when compared with
45
46 the other patients (OR < 1). This finding is also true when each type of malformation is
47
48 considered independently.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Patients mutated in *RPS26* exhibit the lowest response to steroids (4 out of 10), whereas most
4
5 DBA patients with mutations in other known RP genes respond to steroids at a higher
6
7 frequency (92/125) (CI 0.06-0.90).
8
9

10 Interestingly, mental retardation is shown in only 9/270 patients. Eight of these have
11
12 mutations in *RPS19*: four have large deletions at the *RPS19* locus, two have translocations
13
14 associated with deletions, one has a deletion of exons 1-3 and one has a splice-site defect
15
16 (c.72-1G>A). The last patient has a frameshift mutation in *RPL5* (c.169_172delAACA) and a
17
18 complex malformation phenotype that includes myelomeningocele, cleft palate, and facial
19
20 dysmorphism. Patients with mental retardation and large deletions/rearrangements in *RPS19*
21
22 are likely to show a contiguous gene syndrome [Tentler et al., 2000]. In conclusion, we can
23
24 say that mental retardation is not typically associated with mutations in ribosomal protein
25
26 genes and when found in association with other clinical features of DBA is probably linked to
27
28 contiguous genes.
29
30
31
32
33

34 35 36 *Variable expressivity*

37
38 Variable expressivity is shown for all RP gene mutations. Possible mechanisms underlying
39
40 variable expressivity include an influence of modifier genes and environmental factors.
41
42

43 Stochastic factors are invoked in the case of non concordance for malformations in
44
45 monozygous twins [Campagnoli et al., 2004]. Potential modifier genes could be genes
46
47 involved in modulating the level of expression of RP genes or other genes involved in
48
49 ribosome biogenesis. A patient harboring mutations in two different RP genes, *RPL5* and
50
51 *RPS24*, was reported by Quarello et al. (2010). In this case, the malformation phenotype was
52
53 likely due to *RPL5*, since the patient carried hand malformation, often associated with *RPL5*
54
55 mutations. Moreover *RPL5* mutation was *de novo* and the parent carrier of the *RPS24*
56
57 missense mutation did not show malformations. These aspects suggest that the *RPS24*
58
59
60

1
2
3 missense variant may be a silent mutation. Variations in the promoter or other regions have
4
5 also been hypothesized to be phenotype modifiers [Crétien et al., 2010].
6
7
8
9

10 DATABASE

11
12 The need for a comprehensive collection of all mutations in DBA genes, as well as of their
13 functional consequences and clinical phenotypes, prompted us to create and maintain the
14
15 DBA Mutation Database (<http://www.dbagenes.unito.it>) [Boria et al. 2008]. It is founded on
16
17 the Leiden Open (source) Variation Database (LOVD) system that was upgraded to the latest
18
19 version, LOVD 2.0 build 25, released in March 2010 [Fokkema et al., 2005].
20
21
22
23

24 The first version of the database included only three DBA genes, *RPS19*, *RPS24* and *RPS17*,
25
26 and 86 distinct disease-causing mutations. The database has been updated with the newly
27
28 described DBA genes: *RPL5*, *RPL11*, *RPL35A*, *RPS7*, *RPS26*, *RPS10*, and now comprises a
29
30 total of 220 distinct pathogenetic mutations, distributed as follows: 52 missense, 27 nonsense,
31
32 87 small deletions and insertions, 14 large deletion and rearrangements and 40 splice-site
33
34 defects (Table 4). Out of the 134 newly added sequence changes, 78 were previously
35
36 published while 56 are reported here for the first time. Overall, the database includes data for
37
38 355 patients, all carrying RP mutations.
39
40
41
42

43 According to the basic structure of the LOVD database scheme, each DBA gene has its own
44
45 homepage providing general gene and database information, access to allelic variant tables,
46
47 search tools for browsing data and links to external gene-related resources, such as NCBI
48
49 SNP database, MIM, NCBI Entrez, HGMD (Supp. Figure S1). Furthermore links to
50
51 schematic drawings showing the location of the pathogenetic variants in relation to the gene
52
53 (Supp. Figure S2) and, when available, to the protein structures are included.
54
55
56

57 All mutations are described according to the Human Variation Society (HGVS) nomenclature
58
59 [den Dunnen and Antonarakis 2000] and their pathogenicity was established according to the
60

1
2
3 HUGO Mutation Database Initiative/HGVS (Supplemental Material). Mutation nomenclature
4
5 has been checked with Mutalyzer program [Wildeman et al., 2008].
6
7

8 All available data relative to each mutation are provided in the “Variants” section and include
9
10 the exact molecular description at DNA and protein levels, the clinical features of the
11
12 corresponding patients, literature references and details on the detection methods. Consistent
13
14 with the functional classification proposed for RPS19 by Campagnoli et al. (2008),
15
16 information about functional consequences of mutations on mRNA and protein are reported.
17
18

19 Compared to the previous database version, it is now possible to specify the potential
20
21 molecular mechanism leading to each allelic variant (“Molecular Mechanisms” column) and
22
23 for each patient the clinical complications (“Complications” column). Furthermore, we
24
25 substituted the “Occurrence” column with “Variant Origin” that describes the inheritance of
26
27 the mutation in an exhaustive way. Further details can be found at the database web site.
28
29

30 A link to the DBA Mutation Database is provided for each gene in NCBI Gene database.
31
32
33

34 35 36 **DIAGNOSTIC RELEVANCE** 37

38 At the time of submission, 220 distinct mutations in 355 DBA patients have been identified in
39
40 nine genes, all encoding ribosomal proteins. The difficulties in clinical diagnosis and the
41
42 absence of biochemical assays make identification of the causative mutation clinically
43
44 important. Identification of non-symptomatic carriers is mandatory when potential donors of
45
46 hematological stem cells are evaluated within first degree relatives. Moreover, prenatal
47
48 diagnosis may be requested by families with severely affected children. The *RPS19* gene is
49
50 the most frequently involved being mutated in 25% of patients. Mutations in *RPL5* and
51
52 *RPL11* are frequently found in patients with malformations of upper limbs or face.
53
54 Malformations in general, also appear increased in patients with mutations in these genes. A
55
56
57
58
59
60

1
2
3 patient with these types of malformations or with multiple malformations should be screened
4
5 first for these genes.
6
7
8
9

10 **FUTURE PROSPECTS**

11
12 The ribosomal basis of DBA is evident. So far about 50% of DBA patients may be
13 characterized using the four most commonly mutated genes: *RPS19*, *RPL5*, *RPL11*, *RPS26*. It
14
15 is expected that other RP genes may be mutated in the remaining patients. For this reason the
16
17 DBA community has started a large project focused at sequencing each of the 79 RP genes in
18
19 every DBA patient.
20
21
22
23

24 However, sequencing is tedious and time-consuming. An easy and quick diagnostic assay
25
26 would be of great help to clinical hematologists: the perfect assay should be able to diagnose
27
28 all DBA patients, independently of the gene affected. A genetic or functional abnormality
29
30 shared by all patients may be exploited to generate a diagnostic assay.
31
32
33

34 The definition of the molecular basis of DBA has also opened the road to molecular therapy.
35
36 Gene therapy looks promising since even a small increase of RP expression may be helpful to
37
38 resolve the bone marrow failure thereby making this disease a reasonable target for this
39
40 treatment [Flygare et al., 2008]. Treatment with leucine has been proven helpful in rare cases
41
42 [Pospisilova et al., 2007] but large clinical trials are necessary to ascertain if its effect may be
43
44 general or gene specific.
45
46
47
48
49

50 **Accession Numbers**

51
52 The GenBank accession number for human RPs are: NM_001022.3 for *RPS19*,
53
54 NM_000969.3 for *RPL5*, NM_000975.2 for *RPL11*, NM_000996.2 for *RPL35A*,
55
56 NM_033022.3 for *RPS24*, NM_001021.3 for *RPS17*, NM_001011.3 for *RPS7*, NM_001029.3
57
58 for *RPS26* and NM_001014.3 for *RPS10*.
59
60

Web Resources

The URLs of resources cited in this work are the following:

Single Nucleotide Polymorphisms database, <http://www.ncbi.nlm.nih.gov/SNP/>

Entrez Gene database, <http://www.ncbi.nlm.nih.gov/gene/>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim>

The Human Gene Mutation Database, <http://www.hgmd.cf.ac.uk>

PolyPhen, <http://genetics.bwh.harvard.edu/pph/>

Acknowledgements

We would like to thank Dr. Ivo Fokkema for his support in improving the quality of the database. This work was partly funded by **the following grants:** Telethon (to ID), DBA Foundation (to ID, **to HTG**), Italian Ministry of University and Research (to ID and UR), Gruppo di Sostegno DBA Italia (to ID and UR), the National Institutes of Health (USA) **R01 AR044345 (to AHB)**, **R01 HL079571** and The Feinstein Institute for Medical Research General Clinical Research Center M01 RR018535 (to JML, AV, EA), the Pediatric Cancer Foundation (to JML), **the Ministry of Education, Czech Republic, grant MSM 6198959205**, **the Ministry of Health, Czech Republic grants 00023736 and NT11059 (to DP and RC)**, the **Federal Ministry of Education and Research, Germany, grants 01GM0312 and 01GM0847 (to JJM)**.

We also thank the Daniella Maria Arturi Foundation for supporting communication among DBA researchers.

REFERENCES

- Badhai J, Froejmark AS, Davey EJ, Schuster J, Dahl N. 2009. Ribosomal protein S19 and S24 insufficiency cause distinct cell cycle defects in Diamond-Blackfan anemia. *Biochim Biophys Acta* 1792:1036-42.
- Boocock GR, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, Rommens JM. 2003. Mutations in SBDS are associated with Shwachman-Diamond syndrome. *Nat Genet* 33:97-101.
- Boria I, Quarello P, Avondo F, Garelli E, Aspesi A, Carando A, Campagnoli MF, Dianzani I, Ramenghi U. 2008. A new database for ribosomal protein genes which are mutated in Diamond-Blackfan Anemia. *Hum Mutat* 29:E263-70.
- Campagnoli MF, Garelli E, Quarello P, Carando A, Varotto S, Nobili B, Longoni D, Pecile V, Zecca M, Dufour C, Ramenghi U, Dianzani I. 2004. Molecular basis of Diamond-Blackfan anemia: new findings from the Italian registry and a review of the literature. *Haematologica* 89:480-489.
- Campagnoli MF, Ramenghi U, Armiraglio M, Quarello P, Garelli E, Carando A, Avondo F, Pavesi E, Fribourg S, Gleizes PE, Loreni F, Dianzani I. 2008. RPS19 mutations in patients with Diamond-Blackfan anemia. *Hum Mutat* 29:911-20.
- Chakraborty A, Uechi T, Higa S, Torihara H, Kenmochi N. 2009. Loss of ribosomal protein L11 affects zebrafish embryonic development through a p53-dependent apoptotic response. *PLoS One* 4:e4152.
- Chen D, Zhang Z, Li M, Wang W, Li Y, Rayburn ER, Hill DL, Wang H, Zhang R. 2007. Ribosomal protein S7 as a novel modulator of p53-MDM2 interaction: binding to MDM2, stabilization of p53 protein, and activation of p53 function. *Oncogene* 26:5029-37.
- Choesmel V, Bacqueville D, Rouquette J, Noaillac-Depeyre J, Fribourg S, Crétien A, Leblanc T, Tchernia G, Da Costa L, Gleizes PE. 2007. Impaired ribosome biogenesis in Diamond-Blackfan anemia. *Blood* 109:1275-83.
- Choesmel V, Fribourg S, Aguisa-Touré AH, Pinaud N, Legrand P, Gazda HT, Gleizes PE. 2008. Mutation of ribosomal protein RPS24 in Diamond-Blackfan anemia results in a ribosome biogenesis disorder. *Hum Mol Genet* 17:1253-63.
- Cmejla R, Cmejlova J, Handrkova H, Petrak J, Pospisilova D. 2007. Ribosomal protein S17 gene (RPS17) is mutated in Diamond-Blackfan anemia. *Hum Mutat* 28:1178-1182.
- Cmejla R, Cmejlova J, Handrkova H, Petrak J, Petrylova K, Mihal V, Sary J, Cerna Z, Jabali Y, Pospisilova D. 2009. Identification of mutations in the ribosomal protein L5 (RPL5) and ribosomal protein L11 (RPL11) genes in Czech patients with Diamond-Blackfan anemia. *Hum Mutat* 30:321-7.
- Crétien A, Hurtaud C, Moniz H, Proust A, Marie I, Wagner-Ballon O, Choesmel V, Gleizes PE, Leblanc T, Delaunay J, Tchernia G, Mohandas N, Da Costa L. 2008. Study of the effects

1
2
3 of proteasome inhibitors on ribosomal protein S19 (RPS19) mutants, identified in patients
4 with Diamond-Blackfan anemia. *Haematologica* 93:1627-34.

6
7 Crétien A, Proust A, Delaunay J, Rincé P, Leblanc T, Ducrocq R, Simansour M, Marie I,
8 Tamary H, Meerpohl J, Niemeyer C, Gazda H, Sieff C, Ball S, Tchernia G, Mohandas N, Da
9 Costa L. 2010. Genetic variants in the noncoding region of RPS19 gene in Diamond-Blackfan
10 anemia: potential implications for phenotypic heterogeneity. *Am J Hematol* 85:111-6.

12
13 Dai MS, Sears R, Lu H. Feedback regulation of c-Myc by ribosomal protein L11. 2007. *Cell*
14 *Cycle* 6:2735-41.

16
17 den Dunnen JT, Antonarakis SE. 2000. Mutation nomenclature extensions and suggestions to
18 describe complex mutations: a discussion. *Hum Mutat.* 15:7-12.

19
20 Doherty L, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Clinton C, Schneider HE,
21 Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Glader B, Arceci RJ,
22 Farrar JE, Atsidaftos E, Lipton JM, Gleizes PE, Gazda HT. 2010. Ribosomal protein genes
23 RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. *Am J Hum Genet.*
24 86:222-8.

26
27 Faivre L, Meerpohl J, Da Costa L, Marie I, Nouvel C, Gnekow A, Bender-Götze C, Bauters
28 F, Coiffier B, Peaud PY, Rispal P, Berrebi A, Berger C, Flesch M, Sagot P, Varet B,
29 Niemeyer C, Tchernia G, Leblanc T. 2006. High-risk pregnancies in Diamond-Blackfan
30 anemia: a survey of 64 pregnancies from the French and German registries. *Haematologica*
31 91:530-3.

33
34 Farrar JE, Nater M, Caywood E, McDevitt MA, Kowalski J, Takemoto CM, Talbot CC Jr,
35 Meltzer P, Esposito D, Beggs AH, Schneider HE, Grabowska A, Ball SE, Niewiadomska E,
36 Sieff CA, Vlachos A, Atsidaftos E, Ellis SR, Lipton JM, Gazda HT, Arceci RJ. 2008.
37 Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia.
38 *Blood* 112:1582-92.

40
41 Flygare J, Aspesi A, Bailey JC, Miyake K, Caffrey JM, Karlsson S, Ellis SR. 2007. Human
42 RPS19, the gene mutated in Diamond-Blackfan anemia, encodes a ribosomal protein required
43 for the maturation of 40S ribosomal subunits. *Blood* 109:980-6.

45
46 Flygare J, Olsson K, Richter J, Karlsson S. 2008. Gene therapy of Diamond Blackfan anemia
47 CD34(+) cells leads to improved erythroid development and engraftment following
48 transplantation. *Exp Hematol* 36:1428-35.

50
51 Fokkema IF, den Dunnen JT, Taschner PE. 2005. LOVD: easy creation of a locus-specific
52 sequence variation database using an "LSDB-in-a-box" approach. *Hum Mutat* 26:63-8.

53
54 Fumagalli S, Di Cara A, Neb-Gulati A, Natt F, Schwemberger S, Hall J, Babcock GF,
55 Bernardi R, Pandolfi PP, Thomas G. 2009. Absence of nucleolar disruption after impairment
56 of 40S ribosome biogenesis reveals an rpL11-translation-dependent mechanism of p53
57 induction. *Nat Cell Biol* 11:501-8.

59
60 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,

1
2
3 Meerpohl JJ, Stahl J, Schrott G, Glader B, Backer K, Wong C, Nathan DG, Beggs AH, Sieff
4 CA. 2006. Ribosomal protein S24 is mutated in Diamond-Blackfan anemia. *Am J Hum Genet*
5 79:1110-1118.
6

7
8 Gazda HT, Sheen MR, Vlachos A, Choemmel V, O'Donohue MF, Schneider H, Darras N,
9 Hasman C, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Zaucha JM,
10 Glader B, Niemeyer C, Meerpohl JJ, Atsidaftos E, Lipton JM, Gleizes PE, Beggs AH. 2008.
11 Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal
12 thumbs in Diamond-Blackfan anemia patients. *Am J Hum Genet* 83:769-80.
13

14
15 Glader BE, Backer K. 1988. Elevated red cell adenosine deaminase activity: a marker of
16 disordered erythropoiesis in Diamond-Blackfan anaemia and other haematologic diseases. *Br*
17 *J Haematol* 68:165-168.
18

19
20 Idol RA, Robledo S, Du HY, Crimmins DL, Wilson DB, Ladenson JH, Bessler M, Mason PJ.
21 2007. Cells depleted for RPS19, a protein associated with Diamond Blackfan Anemia, show
22 defects in 18S ribosomal RNA synthesis and small ribosomal subunit production. *Blood Cells*
23 *Mol Dis* 39:35-43.
24

25
26 Ivanov AV, Malygin AA, Karpova GG. 2005. Human ribosomal protein S26 suppresses the
27 splicing of its pre-mRNA. *Biochim Biophys Acta* 1727:134-40.
28

29
30 Lipton JM. 2006. Diamond Blackfan anemia: new paradigms for a "not so pure" inherited red
31 cell aplasia. *Semin Hematol* 43:167-177.
32

33
34 Lipton JM and Ellis SR. 2009. Diamond-Blackfan anemia: diagnosis, treatment, and
35 molecular pathogenesis. *Hematol Oncol Clin North Am* 23:261-82.
36

37
38 Luzzatto L, Karadimitris A. 1998. Dyskeratosis and ribosomal rebellion. *Nat Genet* 19:6-7.
39

40
41 Michael WM and Dreyfuss G. 1996. Distinct domains in ribosomal protein L5 mediate 5 S
42 rRNA binding and nucleolar localization. *J Biol Chem* 271:11571-4.
43

44
45 Morel Y, David M, Forest MG, Betuel H, Hauptman G, Andre J, Bertrand J, Miller WL.
46 1989. Gene conversions and rearrangements cause discordance between inheritance of forms
47 of 21-hydroxylase deficiency and HLA types. *J Clin Endocrinol Metab* 68:592-9.
48

49
50 Pospisilova D, Cmejlova J, Hak J, Adam T, Cmejla R. 2007. Successful treatment of a
51 Diamond-Blackfan anemia patient with amino acid leucine. *Haematologica* 92:e66-e67.
52

53
54 Proust A, Da Costa L, Rince P, Landois A, Tamary H, Zaizov R, Tchernia G, Delaunay J;
55 SHIP Working Group on DBA. 2003. Ten novel Diamond-Blackfan anemia mutations and
56 three polymorphisms within the rps19 gene. *Hematol J.* 4:132-6.
57

58
59 Quarello P, Garelli E, Carando A, Brusco A, Calabrese R, Dufour C, Longoni D, Misuraca A,
60 Vinti L, Aspesi A, Biondini L, Loreni F, Dianzani I, Ramenghi U. 2010. Diamond-Blackfan
anemia: genotype-phenotype correlation in Italian patients with RPL5 and RPL11 mutations.
Haematologica 95:206-13.

1
2
3 Roggero S, Quarello P, Vinciguerra T, Longo F, Piga A, Ramenghi U. 2009. Severe iron
4 overload in Blackfan-Diamond anemia: a case-control study. *Am J Hematol* 84:729-32.
5

6
7 Roy V, Pérez WS, Eapen M, Marsh JCW, Pasquini M, Pasquini R, Mustafa MM, Bredeson
8 CN. 2005. Bone marrow transplantation for Diamond-Blackfan anemia. *Biol Blood Marrow*
9 *Transplant* 11:600-608.
10

11 Song MJ, Yoo EH, Lee KO, Kim GN, Kim HJ, Kim SY, Kim SH. 2010. A novel initiation
12 codon mutation in the ribosomal protein S17 gene (RPS17) in a patient with Diamond-
13 Blackfan anemia. *Pediatr Blood Cancer* 54:629-31.
14

15
16 Steitz JA, Berg C, Hendrick JP, La Branche-Chabot H, Metspalu A, Rinke J, Yario T. 1988.
17 A 5S rRNA/L5 complex is a precursor to ribosome assembly in mammalian cells. *J Cell Biol*
18 106:545-56.
19

20
21 Sundqvist A, Liu G, Mirsaliotis A, Xirodimas DP. 2009. Regulation of nucleolar signalling to
22 p53 through NEDDylation of L11. *EMBO Rep* 10:1132-9.
23

24
25 Tentler D, Gustavsson P, Elinder G, Eklöf O, Gordon L, Mandel A, Dahl N. 2000. A
26 microdeletion in 19q13.2 associated with mental retardation, skeletal malformations, and
27 Diamond-Blackfan anaemia suggests a novel contiguous gene syndrome. *J Med Genet*
28 37:128-31.
29

30
31 Tschochner H, Hurt E. 2003. Pre-ribosomes on the road from the nucleolus to the cytoplasm.
32 *Trends Cell Biol* 13:255-263.
33

34
35 Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, Meerpohl J, Karlsson S, Liu JM,
36 Leblanc T, Paley C, Kang EM, Leder EJ, Atsidaftos E, Shimamura A, Bessler M, Glader B,
37 Lipton JM; Participants of Sixth Annual Daniella Maria Arturi International Consensus
38 Conference. 2008. Diagnosing and treating Diamond Blackfan anaemia: results of an
39 international clinical consensus conference. *Br J Haematol* 142:859-76.
40

41
42 Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M,
43 Trotta CR, Hwang S, Wilde RG, Karp G, Takasugi J, Chen G, Jones S, Ren H, Moon YC,
44 Corson D, Turpoff AA, Campbell JA, Conn MM, Khan A, Almstead NG, Hedrick J, Mollin
45 A, Risher N, Weetall M, Yeh S, Branstrom AA, Colacino JM, Babiak J, Ju WD, Hirawat S,
46 Northcutt VJ, Miller LL, Spatrack P, He F, Kawana M, Feng H, Jacobson A, Peltz SW,
47 Sweeney HL. 2007. PTC124 targets genetic disorders caused by nonsense mutations. *Nature*
48 447:87-91.
49

50
51 Wildeman M, van Ophuizen E, den Dunnen JT, Taschner PEM. 2008. Improving sequence
52 variant descriptions in mutation databases and literature using the Mutalyzer sequence
53 variation nomenclature checker. *Hum Mutat* 29:6-13.
54

55
56 Willig TN, Draptchinskaia N, Dianzani I, Ball S, Niemeyer C, Ramenghi U, Orfali K,
57 Gustavsson P, Garelli E, Brusco A, Tiemann C, Pérignon JL, Bouchier C, Cicchiello L, Dahl
58 N, Mohandas N, Tchernia G. 1999. Mutations in ribosomal protein S19 gene and Diamond
59 Blackfan anemia: wide variations in phenotypic expression. *Blood* 94:4294-4306.
60

1
2
3 Xu WB, Roufa DJ. 1996. The gene encoding human ribosomal protein S24 and tissue-specific
4 expression of differentially spliced mRNAs. *Gene* 169:257-62.

5
6
7 Zhang Y, Wolf GW, Bhat K, Jin A, Allio T, Burkhart WA, Xiong Y. 2003. Ribosomal protein
8 L11 negatively regulates oncoprotein MDM2 and mediates a p53-dependent ribosomal-stress
9 checkpoint pathway. *Mol Cell Biol* 23:8902-12.

10
11 Zhang J, Harnpicharnchai P, Jakovljevic J, Tang L, Guo Y, Oeffinger M, Rout MP, Hiley SL,
12 Hughes T, Woolford JL Jr. 2007. Assembly factors Rpf2 and Rrs1 recruit 5S rRNA and
13 ribosomal proteins rpL5 and rpL11 into nascent ribosomes. *Genes Dev* 21:2580-92.

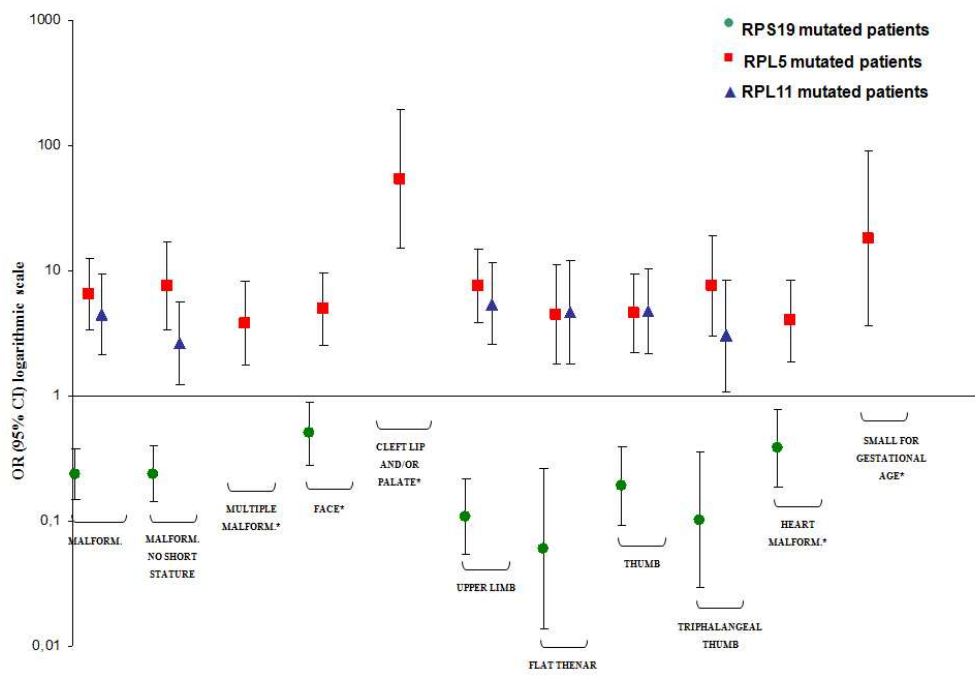
14
15
16 Zhang Y, Lu H. 2009. Signaling to p53: ribosomal proteins find their way. *Cancer Cell*
17 16:369-77.

18
19
20 Zhu Y, Poyurovsky MV, Li Y, Biderman L, Stahl J, Jacq X, Prives C. 2009. Ribosomal
21 protein S7 is both a regulator and a substrate of MDM2. *Mol Cell* 35:316-26.

22
23
24
25
26
27
28 **Figure Legends.**

29
30 **FIGURE 1.** Malformation status of patients with *RPS19*, *RPL5* and *RPL11* mutations.

31
32 Associations between malformations and RP gene mutations are assessed with odds ratio
33 (OR) and 95% CI from logistic regression; OR are drawn on a logarithmic scale. (*) Non
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000



122x84mm (200 x 200 DPI)

Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Newly reported mutations in *RPS19*, *RPL5*, *RPL11* and *RPS24*. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

| 7 Mutated Gene | 8 Patient (Gender) | Exon/Intron | cDNA Mutation | Predicted amino acid change | Mutation Type | Malformations | Growth Retardation | Steroid Response | Inheritance |
|----------------|--------------------|-------------|------------------|-----------------------------|---------------|---|--------------------|------------------|-------------|
| 9 RPS19 | Ps19_1(F) | Ex 2 | c.2T>A | p.Met? | Missense | na | na | na | familial |
| 10 | Ps19_2(F) | Ex 2 | c.10_13delGTTA | p.Val4LeufsX2 | Deletion | none | no | yes | sporadic |
| 11 | Ps19_3(F) | Ex 2 | c.14delC | p.Thr5MetfsX2 | Deletion | na | na | na | unknown |
| 12 | Ps19_4(M) | Ex 2 | c.28_29insT | p.Asn10IlefsX41 | Insertion | na | na | na | sporadic |
| 13 | Ps19_5(F) | Ex 2 | c.34_47del | p.Gln12SerfsX34 | Deletion | Dystrophy | na | na | unknown |
| 14 | Ps19_6(M) | Ex 2 | c.49G>C | p.Ala17Pro | Missense | na | na | na | sporadic |
| 15 | Ps19_7(NA) | Ex 2 | c.58G>C | p.Ala20Pro | Missense | na | na | na | unknown |
| 16 | Ps19_8(F) | Ex 3 | c.83T>G | p.Leu28Arg | Missense | none | no | na | unknown |
| 17 | Ps19_9(M) | Ex 3 | c.88delG | p.Val30SerfsX46 | Deletion | Microcephaly, micro-retrognathia, hypertelorism, cafe au lait spots | na | na | familial |
| 18 | Ps19_10(M) | Ex 3 | c.93delC | p.Glu32AsnfsX44 | Deletion | Thumb | no | no | unknown |
| 19 | Ps19_11(M) | Ex 3 | c.103dupG | p.Asp35GlyfsX16 | Insertion | none | no | no | unknown |
| 20 | Ps19_12(M) | Ex 3 | c.112A>T | p.Lys38X | Nonsense | none | no | no | sporadic |
| 21 | Ps19_13(M) | Ex 3 | c.156G>A | p.Trp52X | Nonsense | Low hairline, cafe au lait spots | na | na | unknown |
| 22 | Ps19_14(M) | Ex 3 | c.172G>C | p.Ala58Pro | Missense | none | yes | no | de novo |
| 23 | Ps19_15(F) | Ex 4 | c.178A>C | p.Thr60Pro | Missense | none | no | na | sporadic |
| 24 | Ps19_16(M) | Ex 4 | c.187_189insCAC | p.His63dup | Insertion | Flat nose, low hairline, mitral valve and tricuspid valve insufficiency | na | na | sporadic |
| 25 | Ps19_17(NA) | Ex 4 | c.195C>G | p.Tyr65X | Nonsense | na | na | na | unknown |
| 26 | Ps19_18(NA) | Ex 4 | c.195C>G | p.Tyr65X | Nonsense | na | na | na | unknown |
| 27 | Ps19_19(F) | Ex 4 | c.203_204insG | p.Gly69TrpfsX85 | Insertion | na | na | na | sporadic |
| 28 | Ps19_20(M) | Ex 4 | c.212G>A | p.Gly71Glu | Missense | na | na | na | sporadic |
| 29 | Ps19_21(F) | Ex 4 | c.281G>T | p.Arg94Leu | Missense | none | na | na | sporadic |
| 30 | Ps19_22(F) | Ex 4 | c.284delG | p.Gly95AlafsX16 | Deletion | ASD | na | na | unknown |
| 31 | Ps19_23(M) | Ex 4 | c.289_290insAGGC | p.Lys97ArgfsX58 | Insertion | Dysplastic aortic valve | na | na | unknown |
| 32 | Ps19_24(F) | Ex 4 | c.296_297delTG | p.Val99GlyfsX54 | Deletion | na | na | na | unknown |
| 33 | Ps19_25(M) | Ex 4 | c.301C>T | p.Arg101Cys | Missense | na | na | yes | unknown |

| | | | | | | | | | |
|----|----------------------|--------------|-------------------------|------------------|----------------------|--|-----|-----|----------|
| 1 | | | | | | | | | |
| 2 | Ps19_26(F) | Ex 4 | c.305G>C | p.Arg102Pro | Missense | none | no | na | unknown |
| 3 | Ps19_27(M) | Ex 4 | c.320T>G | p.Leu107Arg | Missense | na | na | na | unknown |
| 4 | Ps19_28(F) | Ex 4 | c.344delA | p.Lys115ArgfsX9 | Deletion | na | na | na | familial |
| 5 | mother (F) | Ex 4 | c.344delA | p.Lys115ArgfsX9 | Deletion | High palatine | na | na | unknown |
| 6 | Ps19_29(NA) | Ex 4 | c.356_357insG | p.Gly120ArgfsX34 | Donor splice site | na | na | na | unknown |
| 7 | Ps19_30(F) | Ex 5 | c.372_373insA | p.Pro125ThrfsX29 | Insertion | Hip subluxation on both sides | na | na | sporadic |
| 8 | Ps19_31(F) | Ex 5 | c.401_402insT | p.Ala135ArgfsX19 | Insertion | na | na | na | unknown |
| 9 | Ps19_32(F) | Ex 6 | c.418delG | p.Ala140Leufs | Deletion | na | na | na | sporadic |
| 10 | Ps19_33(M) | Int 1 | c.-1G>C | p.0? | Acceptor splice site | Short stature | na | yes | familial |
| 11 | Ps19_34(M) | Int 1 | c.1-2A>T | p.0? | Acceptor splice site | none | no | na | familial |
| 12 | brother (M) | Int 1 | c.1-2A>T | p.0? | Acceptor splice site | none | no | na | familial |
| 13 | Ps19_35(M) | Int 2 | c.71+1G>C | p.0? | Donor splice site | na | na | na | de novo |
| 14 | Ps19_36(M) | Int 2 | c.72-1G>A | p.0? | Acceptor splice site | Macrocephaly, mental retardation | na | na | unknown |
| 15 | Ps19_37(F) | Int 2 | c.72-2A>C | p.0? | Acceptor splice site | Thumb | yes | yes | sporadic |
| 16 | Ps19_38(M) | Int 3 | c.172+1G>T | p.0? | Donor splice site | na | na | no | de novo |
| 17 | Ps19_39(M) | Int 3 | c.172+1G>T | p.0? | Donor splice site | Triphalangeal thumbs | yes | yes | sporadic |
| 18 | Ps19_40(M) | Int 3 | c.172+1G>C | p.0? | Donor splice site | na | na | no | unknown |
| 19 | Ps19_41(F) | Int 3 | c.173-2A>G | p.0? | Acceptor splice site | Low set ears | yes | no | de novo |
| 20 | Ps19_42(F) | Int 3 / Ex 4 | c.173-7_174del | p.0? | Deletion | na | na | na | unknown |
| 21 | Ps19_43(M) | Int 4 | c.356+1G>T | p.0? | Donor splice site | none | no | yes | sporadic |
| 22 | Ps19_44(F) | Int 4 | c.356+1_356+2delGTins12 | p.0? | Donor splice site | na | yes | nd | unknown |
| 23 | Ps19_45(M) | Int 5 / Ex 6 | c.412-13_417del | p.0? | Deletion | none | no | na | de novo |
| 24 | | | | | | | | | |
| 25 | RPL5 P15_1(F) | Ex 1 | c.1A>G | p.Met1? | Missense | Triphalangeal thumb | na | na | sporadic |
| 26 | P15_2(F) | Ex 1 | c.2T>G | p.Met1Arg | Missense | Duplicated ureter | yes | yes | familial |
| 27 | father (M) | Ex 1 | c.2T>G | p.Met1Arg | Missense | Heart Murmur | yes | yes | familial |
| 28 | P15_3(M) | Ex 2 | c.48C>A | p.Tyr16X | Nonsense | Cleft palate, abnormal right thumb | yes | yes | de novo |
| 29 | P15_4(F) | Ex 3 | c.91delT | p.Tyr31MetfsX7 | Deletion | Triphalangeal thumb | na | na | de novo |
| 30 | P15_6(M) | Ex 3 | c.172_173insA | p.Arg58LysfsX55 | Insertion | Cleft lip and palate, triphalangeal thumb, short stature | na | na | de novo |
| 31 | P15_7(F) | Ex 4 | c.191_204ins14 | p.Ile64LeufsX10 | Insertion | na | na | na | de novo |
| 32 | P15_8(F) | Ex 4 | c.208G>T | p.Glu70X | Nonsense | na | na | na | sporadic |
| 33 | | | | | | | | | |
| 34 | | | | | | | | | |
| 35 | | | | | | | | | |
| 36 | | | | | | | | | |
| 37 | | | | | | | | | |
| 38 | | | | | | | | | |
| 39 | | | | | | | | | |
| 40 | | | | | | | | | |
| 41 | | | | | | | | | |
| 42 | | | | | | | | | |
| 43 | | | | | | | | | |
| 44 | | | | | | | | | |
| 45 | | | | | | | | | |
| 46 | | | | | | | | | |
| 47 | | | | | | | | | |

| | | | | | | | | | |
|----|------------------------|-------|----------------|------------------|----------------------|--|-----|-----|----------|
| 1 | | | | | | | | | |
| 2 | P15_9(M) | Ex 4 | c.283delT | p.Tyr95MetfsX31 | Deletion | na | na | na | sporadic |
| 3 | P15_10(M) | Ex 5 | c.454delA | p.Arg152GlufsX12 | Deletion | Dysmorphic face, VSD, cleft soft palate, triphalangeal thumbs, reflux of the left ureter | yes | yes | sporadic |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |
| 6 | P15_11(M) | Ex 6 | c.535C>T | p.Arg179X | Nonsense | Cleft lip, abnormality of bilateral second toe, aortic valve defect | yes | no | sporadic |
| 7 | | | | | | | | | |
| 8 | P15_12(F) | Ex 6 | c.535C>T | p.Arg179X | Nonsense | na | na | na | sporadic |
| 9 | | | | | | | | | |
| 10 | | | | | | | | | |
| 11 | RPL11 P111_1(F) | Ex 2 | c.100_101dupA | p.Thr34AsnfsX21 | Insertion | VSD, ASD, abnormal thumbs | no | na | sporadic |
| 12 | P111_2(M) | Ex 5 | c.475_476ins11 | p.Lys159ThrfsX39 | Insertion | none | no | yes | sporadic |
| 13 | P111_3(M) | Int 2 | c.158-2A>C | p.0? | Acceptor splice site | none | yes | yes | sporadic |
| 14 | | | | | | | | | |
| 15 | RPS24 Ps24_1(M) | Int 4 | c.390+1G>A | p.0? | Donor splice site | na | na | na | sporadic |
| 16 | | | | | | | | | |

F: female; M: male; na: not available; VSD: ventricular septal defect; ASD: atrial septal defect.

Table 2. Pathogenetic point mutations in RP genes that are homologous to pseudogene sequences. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

| Gene name | DNA Mutation | Mutation Type | Exon/Intron | Gene sequence | Mutated sequence | Pseudogene sequence | Pseudogene name (locus) |
|--------------|----------------|---------------|-------------|----------------|------------------|---------------------|-------------------------|
| RPS19 | c.384_385delAA | Deletion | Ex 5 | GGACAAAGAGAT | GGACA- -GAGAT | GGACA- -GAGAT | RPS19P2 (1p13.2) |
| | c.403G>A | Missense | Ex 5 | GAATCGCCGGA | GAATCACCGGA | GAATCACCGGA | RPS19P2 (1p13.2) |
| | c.191T>C | Missense | Ex 4 | GCACCTGTACC | GCACCCGTACC | GCACCCGTACC | RPS19P4 (5q11.2) |
| | c.166C>T | Nonsense | Ex 3 | ACACGCGAGCT | ACACGTGAGCT | ACACGTGAGCT | RPS19P7 (10q11.21) |
| RPL5 | c.535C>T | Nonsense | Ex 6 | CCAAACGATTC | CCAAATGATTC | CCAAATGATTC | RPL5P34 (22q13.2)* |
| RPL11 | c.94_97delAGAC | Deletion | Ex 2 | GAGACAGACTGACG | GAGAC----TGACG | GAGAC----TGACG | RPL11P5 (12q24.31) |

* Note that this variation is carried also by other pseudogenes, but in a slightly different context (data not shown).

Table 3. Clinical data relative to all DBA patients reported in the DBA Mutation Database.

| Gene Name | Patients with Mutations* | Patients with Malformations | Malformations no Short Stature | Face | Cleft Lip and/or Palate | Upper Limb | Flat Thenar | Thumb | Triphalangeal Thumb | Heart Malf. | Genitourinary Anomalies | Mental Retardation | Small for Gestational Age | Multiple Malf. | GR | SR |
|-----------|--------------------------|-----------------------------|--------------------------------|------|-------------------------|------------|-------------|-------|---------------------|-------------|-------------------------|--------------------|---------------------------|----------------|----|----|
| RPS19 | 166 | 55 | 53 | 27 | 0 | 12 | 2 | 11 | 3 | 14 | 7 | 8 | 0 | 15 | 41 | 39 |
| RPL5 | 50 | 42 | 41 | 24 | 21 | 28 | 10 | 18 | 12 | 15 | 3 | 1 | 7 | 14 | 6 | 19 |
| RPL11 | 36 | 26 | 24 | 5 | 2 | 19 | 8 | 14 | 6 | 6 | 3 | 0 | 2 | 2 | 2 | 19 |
| RPL35A | 5 | 4 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 1 | na | 4 |
| RPS26 | 10 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | na | 4 |
| RPS7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| RPS10 | 4 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | na | 3 |
| RPS17 | 3 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 |
| RPS24 | 10 | 3 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |

GR: growth retardation; SR: steroid response. () Total number of patients whose clinical data were available.*

Table 4. Summary of the pathogenic variants in DBA Mutation Database.

| Type of Mutation | Gene | | | | | | | | | | Patients* |
|--------------------------------|------------|----------|----------|----------|----------|----------|-----------|-----------|----------|------------|------------|
| | RPS19 | RPS26 | RPS24 | RPS17 | RPS10 | RPS7 | RPL5 | RPL11 | RPL35A | Total | |
| Missense | 36 | 4 | 1 | 2 | 1 | 0 | 6 | 1 | 1 | 52 | 110 |
| Nonsense | 14 | 0 | 2 | 0 | 1 | 0 | 7 | 2 | 1 | 27 | 56 |
| Small insertions and deletions | 44 | 1 | 1 | 1 | 1 | 0 | 21 | 17 | 1 | 87 | 121 |
| Splice site defects | 23 | 3 | 2 | 0 | 0 | 1 | 5 | 6 | 0 | 40 | 53 |
| Large deletions/rearrangements | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 14 | 15 |
| Total | 129 | 8 | 6 | 3 | 3 | 1 | 39 | 26 | 5 | 220 | 355 |

(*) We have here considered the association with an individual patient irrespective to the fact that a mutation could recur in the same family.

SUPPLEMENTARY MATERIAL

METHODS

MUTATION DETECTION AND PATHOGENICITY ASSESSMENT

Mutation detection has been performed using automatic sequencing. For each group details can be found on their previous papers [Gazda et al., 2008; Cmejla et al., 2009; Quarello et al., 2010; Proust et al., 2003].

Generally, the complete coding sequence and exon-intron boundaries have been sequenced to eliminate the possibility of the presence of other mutations. Verification has been performed in keeping with the journal's requirements. In particular, all mutations have been confirmed on a second sample and on a second PCR product. In absence of biochemical data, pathogenicity of missense mutations have been established by at least two methods. In turn, the following methods were used: linkage to disease in a family, concurrent appearance of the phenotype with a *de novo* mutation, or determination that the mutation is absent among at least 50 normal individuals (100 alleles) or involves a highly conserved amino acid. In some cases the Polyphen algorithm has been used. Otherwise pathogenicity is reported as uncertain in the Database.

FIGURES

SUPPLEMENTAL FIGURE S1. The main page of the RPS19 mutation database.

SUPPLEMENTAL FIGURE S2. Schematic drawings showing the location of the pathogenic variants in relation to the corresponding gene structure.

SUPPLEMENTAL FIGURE S1.

LOVD Leiden Open Variation Database

Diamond-Blackfan Anemia
ribosomal protein S19 (RPS19)

Curators: [I. Boria](#) and [U. Ramenghi](#)

Home Variants Submitters Submit Configuration Setup Documentation

RPS19 homepage Switch gene

LOVD Gene homepage

| General information | |
|--|--|
| Gene name | ribosomal protein S19 |
| Gene symbol | RPS19 |
| Chromosome Location | 19q13.2 |
| Database location | www.dbagenes.unito.it |
| Curator | I. Boria and U. Ramenghi |
| Database reference for citations | Boria et al (2008). A new database for ribosomal protein genes which are mutated in Diamond-Blackfan Anemia . Hum. Mutat.29(11), E263-70 |
| PubMed references | View all (unique) PubMed references in the RPS19 database |
| Date of creation | November 12, 2007 |
| Last update | June 11, 2010 |
| Version | RPS19 100611 |
| Add sequence variant | Submit a sequence variant |
| First time submitters | Register here |
| Reference sequence | coding DNA reference sequence for describing sequence variants |
| GenBank reference | NM_001022.3 |
| Total number of unique DNA variants reported | 129 |
| Total number of individuals with variant(s) | 219 |
| Total number of variants reported | 219 |
| Subscribe to updates of this gene |  |
| NOTE | The RPS19 gene comprises 6 exons and spans 11 Kb. The first exon (372 bp) is included in the 5' UTR region, while the other five (435 bp) encode for a 145 amino acid protein (MW 16 kDa). The predicted size of the whole RPS19 primary transcript, including the polyA stretch, is 872 bp. RPS19 has 3 annotated pseudogenes. Link to the location of the pathogenic variants in relation to the gene Link to the location of the pathogenic variants in relation to the protein structure |

| Graphical displays and utilities | |
|----------------------------------|--|
| Summary tables | Summary of all sequence variants in the RPS19 database, sorted by type of variant (with graphical displays and statistics) |

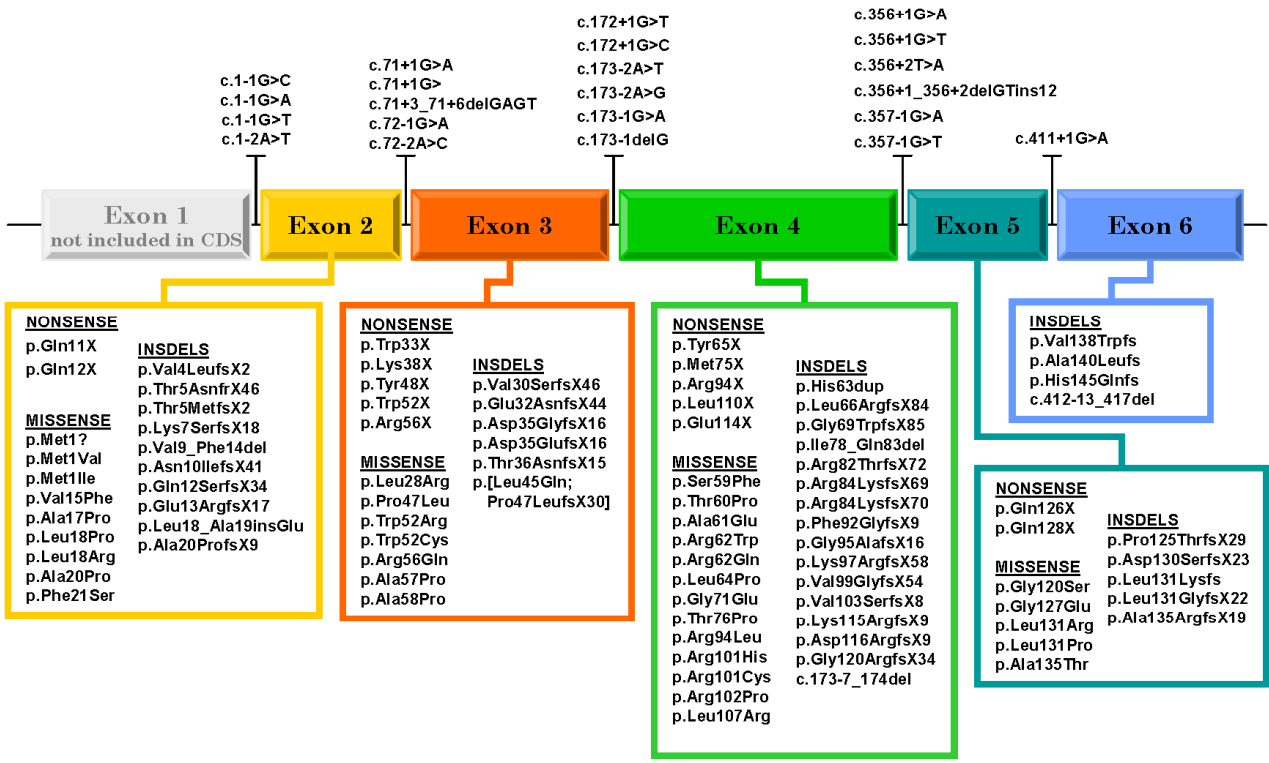
| Sequence variant tables | |
|--|---|
| Unique sequence variants | Listing of all unique sequence variants in the RPS19 database, without patient data |
| Complete sequence variant listing | Listing of all sequence variants in the RPS19 database |
| Variants with no known pathogenicity | Listing of all RPS19 variants reported to have no noticeable phenotypic effect (note: excluding variants of unknown effect) |

| Search the database | |
|---|--|
| By type of variant | View all sequence variants of a certain type |
| Simple search | Query the database by selecting the most important variables (exon number, type of variant, disease phenotype) |
| Advanced search | Query the database by selecting a combination of variables |
| Based on patient origin | View all variants based on your patient origin search terms |
| Search through hidden entries | Find the number of variant entries in the database (including hidden entries) matching your search terms. |

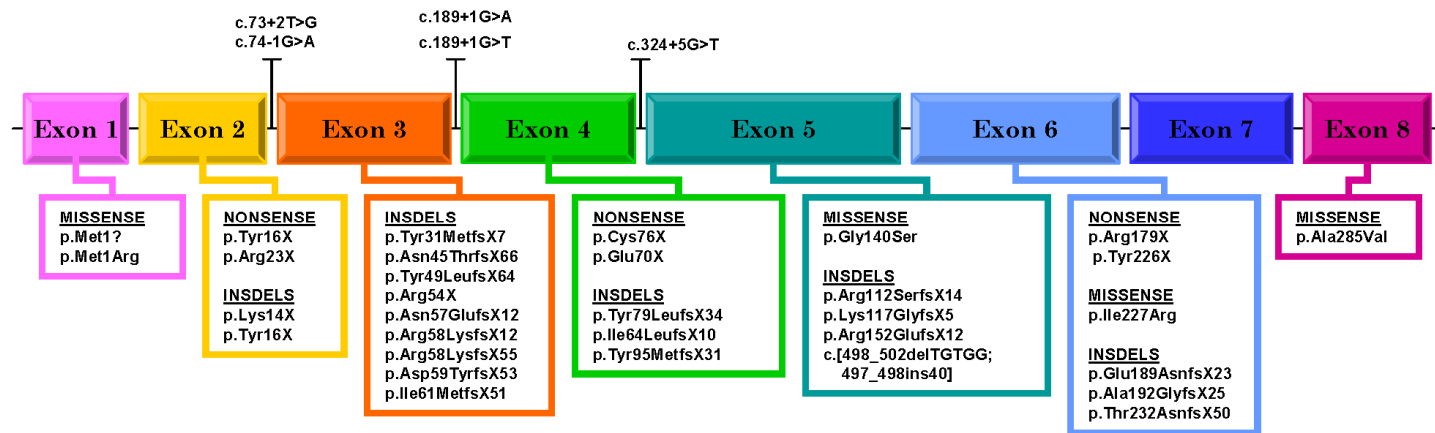
| Links to other resources | |
|--------------------------|-------------------------------|
| External link | dbSNP |
| Entrez Gene | 6223 |
| OMIM - Gene | 603474 |
| OMIM - Disease | 105650 (DBA1) |
| HGMD | RPS19 |

SUPPLEMENTAL FIGURE S2.

RPS19 MUTATION MAP

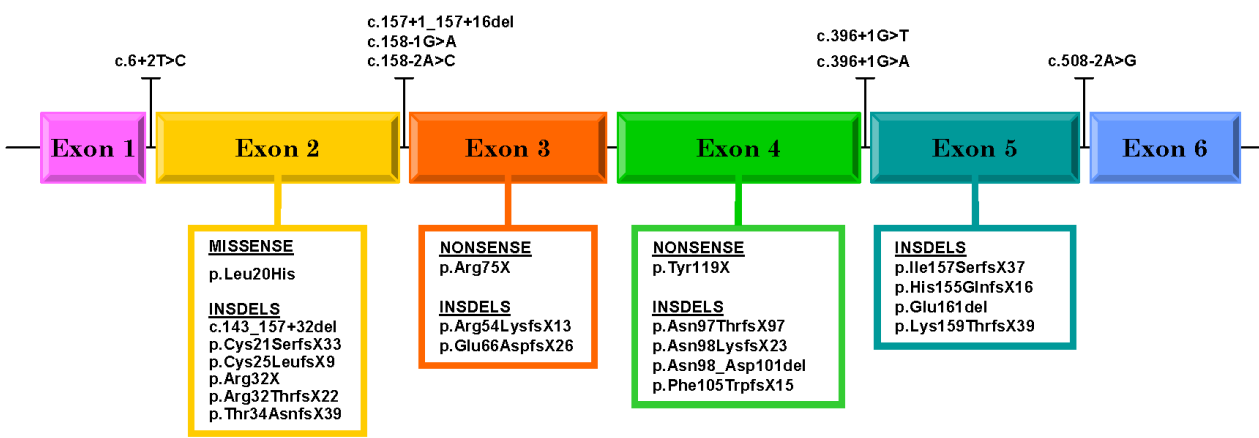


RPL5 MUTATION MAP



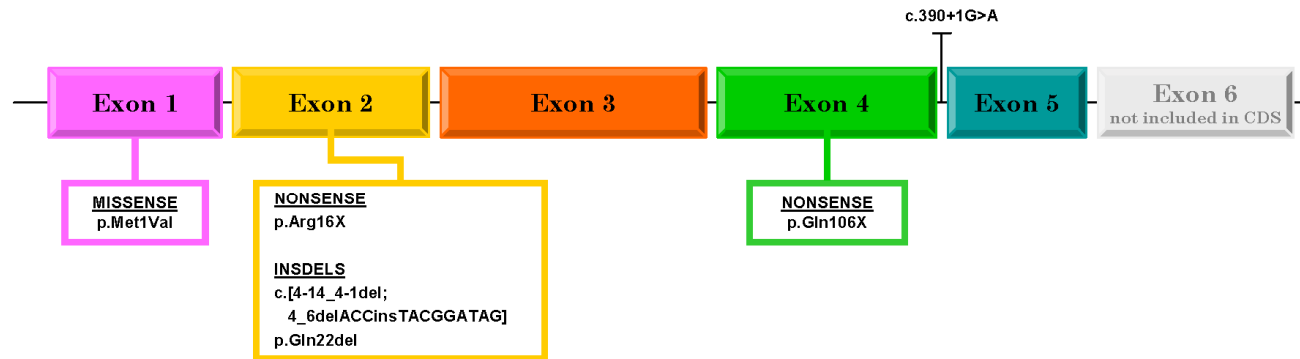
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

RPL11 MUTATION MAP



RPS24 MUTATION MAP

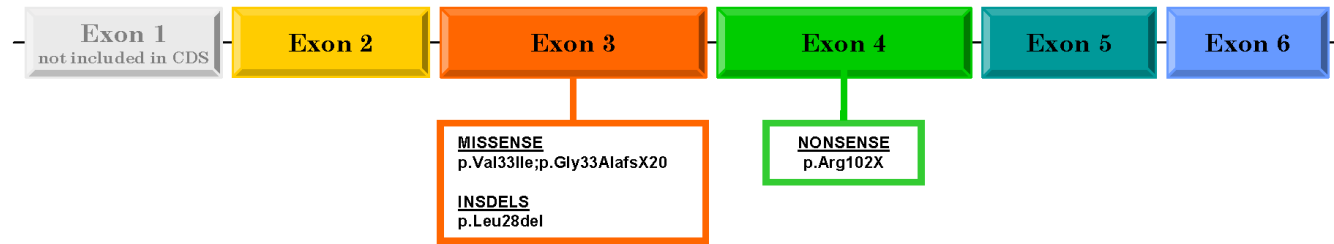
NM_033022.3, (variant a)



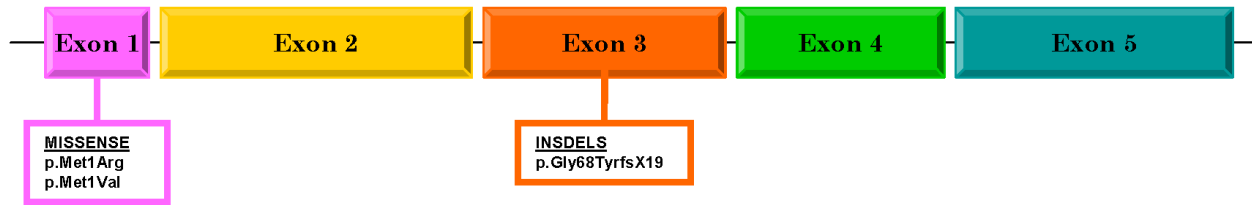
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

RPL35A MUTATION MAP



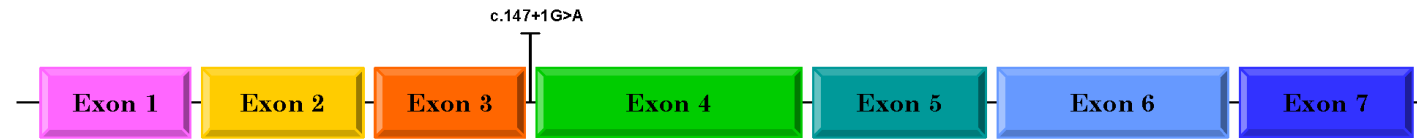
RPS17 MUTATION MAP

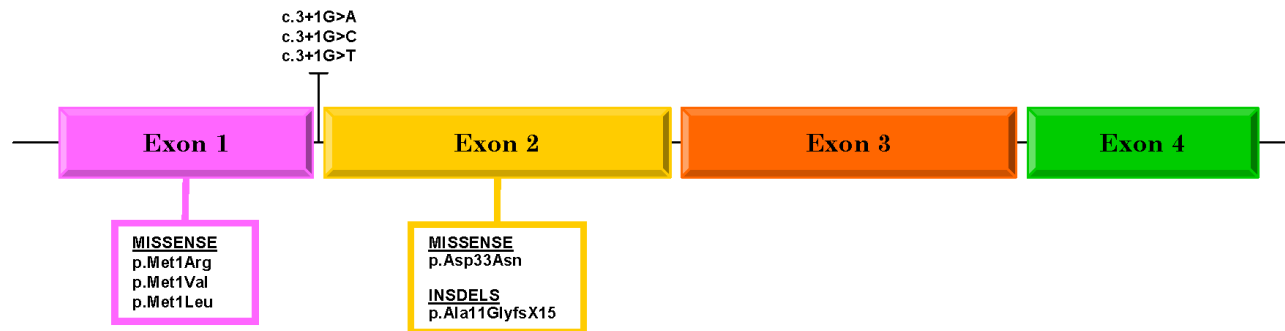


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

RPS7 MUTATION MAP



RPS26 MUTATION MAP

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

RPS10 MUTATION MAP

