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► **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

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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
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Key Words:	Diamond-Blackfan anemia, ribosomal protein, erythropoiesis, ribosome biogenesis

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3 **THE RIBOSOMAL BASIS OF DIAMOND-BLACKFAN ANEMIA: MUTATION AND**
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5 **DATABASE UPDATE**
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10 11 12 **ABSTRACT**

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15 Diamond-Blackfan Anemia (DBA) is characterized by a defect of erythroid progenitors and,
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17 clinically, by anemia and malformations. DBA exhibits an autosomal dominant pattern of
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19 inheritance with incomplete penetrance. Currently nine genes, all encoding ribosomal proteins
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21 (RP), have been found mutated in approximately 50% of patients. Experimental evidence
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23 supports the hypothesis that DBA is primarily the result of defective ribosome synthesis.
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27 By means of a large collaboration among six centers, we report here a mutation update that
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29 includes nine genes and 220 distinct mutations, 56 of which are new. The DBA Mutation
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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.
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55 Key words: Diamond-Blackfan anemia; ribosomal protein; erythropoiesis; ribosome
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57 biogenesis.
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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

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

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8 The first DBA gene, ribosomal protein (RP) *S19*, was identified in 1999 [Draptchinskaia et
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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*,
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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
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23 ribosomopathy, a term initially proposed for dyskeratosis congenita [Luzzatto and
24
25 Karadimitris, 1998].
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29 In eukaryotes, the ribosome is composed of four different ribosomal RNAs (rRNAs) and 79
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31 ribosomal proteins. While 5S rRNA is transcribed by RNA polymerase III, 28S, 5.8S and 18S
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33 rRNAs are processed from a 45S precursor transcribed by RNA polymerase I. The maturation
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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
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47 [Tschochner and Hurt, 2003]. Mature 40S subunits include 18S rRNA and 33 ribosomal
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49 proteins whereas mature 60S subunits contain 28S, 5.8S and 5S rRNAs and 46 ribosomal
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51 proteins. In humans there are several loci containing rRNA genes, but only one gene for each
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58 of the 79 ribosomal proteins.
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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
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3 *RPS19* mutations are reported and they are carried by 219 patients: 82 of these are *de novo*
4 and 45 are inherited. The inheritance was not ascertained in the remaining cases.
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8 At least 163 polymorphisms are listed in NCBI SNP database. We have identified 9
9 unpublished intronic allelic variants: c.71+174A>G, c.71+24A>G, c.356+166G>T,
10 c.411+6G>T, c.412-75A>G, c.356+153G>A, c.356+29T>C, c.356+229G>A and c.412-
11 131T>C. Seven pseudogenes are annotated in the NCBI Gene database as 'inferred'.
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15 16 17 **RPL5**

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19 The human *RPL5* gene (OMIM 603634; locus 1p22.1) consists of eight exons and spans 9.8
20 kb. The primary transcript is 1031 nt long and encodes a 297-aa protein (MW ~34.2 kDa)
21 component of the 60S ribosomal subunit.
22
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26 Heterozygous mutations in *RPL5* gene have been reported in DBA patients [Gazda et al.,
27 2008; Cmejla et al., 2009; Quarello et al., 2010]. Gazda et al. showed mutations in 18 of 196
28 DBA probands (9%) and in six additional family members [Gazda et al., 2008], for a total of
29 24 individuals; Cmejla et al. studied 28 Czech families and identified sequence changes in 8
30 DBA patients from 6 families (21.4%) [Cmejla et al., 2009]; Quarello et al. reported
31 mutations in 12 out of 92 (13%) unrelated Italian probands [Quarello et al., 2010]. In this
32 paper we are adding 10 new mutations found in 12 patients (Table 1) and two patients
33 carrying two previously described mutations. The total number of patients with mutations in
34 *RPL5* is 58: 21 have a *de novo* mutation, 10 are familial cases. Thirty-nine mutations are
35 distinct and are distributed as follows: 6 missense, 7 nonsense, 21 small deletions and/or
36 insertions, 5 splice-site defects.
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53 Ninety-five polymorphisms are listed in NCBI SNP database. There are multiple processed
54 pseudogenes of *RPL5* gene dispersed through the genome: three of them are annotated as
55 'validated' in NCBI Gene database.
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59 60 **RPL11**

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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
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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.
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21 Fifty-seven polymorphisms are reported in NCBI SNP database and five pseudogenes are
22
23 annotated in NCBI Gene database as 'inferred'.
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26 **RPL35A**

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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).
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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].
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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.
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55 **RPS24**

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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
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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
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7 Roufa, 1996; Gazda et al., 2006].
8
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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.
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27 Eighty-two polymorphisms are listed in NCBI SNP database and only one *RPS24* pseudogene
28 is annotated as 'validated' in NCBI Gene database.
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31 **RPS17**

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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*.
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48 The *RPS17* gene has 30 polymorphisms listed in NCBI SNP database and has two 'validated'
49 pseudogenes in NCBI Gene database.
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52 **RPS7**

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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).
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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.

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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**

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15 The *RPS26* gene (OMIM 603701) is located on chromosome 12 (*locus* 12q13) and has four
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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).

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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.

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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 **RPS10**

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

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

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

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3 In conclusion, the most frequent mutagenic mechanism observed in DBA patients appears to
4 be slippage, followed by transitions occurring at CpG dinucleotides.
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10 **BIOLOGICAL RELEVANCE**

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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; Choemmel 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.
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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; Choemmel 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
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3 p.Glu13ArgfsX17, p.Trp33X, p.Tyr48X, p.Arg56X, and p.Met75X RPS19 proteins [Crétien
4 et al., 2008]. Another mutation was recently investigated for its functional consequences by
5
6 Badhai and collaborators, who reported that primary fibroblasts from a DBA patient with a
7
8 *RPS19* acceptor splice-site mutation (c.72-2A>C) showed reduced proliferative capacity due
9
10 to G1 arrest [Badhai et al., 2009].
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13
14 ***RPL5***. *RPL5* has been implicated in nucleocytoplasmic transport of 5S rRNA prior to its
15
16 assembly into the large ribosomal subunit [Steitz et al., 1988]. *RPL5* specifically binds to this
17
18 rRNA through the domains located at both the amino terminus and the carboxyl terminus
19
20 [Michael and Dreyfuss, 1996]. It has been shown that the perturbation of ribosomal
21
22 biogenesis by impaired rRNA synthesis, processing, or ribosome assembly, triggers the direct
23
24 binding of *RPL5* along with *RPL11* and possibly *RPL23* to MDM2. These interactions inhibit
25
26 MDM2-mediated p53 ubiquitination and degradation, resulting in p53 activation [Zhang and
27
28 Lu, 2009]. The pathogenic effect of *RPL5* haploinsufficiency on ribosome biogenesis has
29
30 been studied by Gazda and collaborators both in a knockdown cell model and in patient cells
31
32 that harbored the following mutations: c.67C>T, c.173delG, c.175_176delGA,
33
34 c.[498_502delTGTGG;497_498ins40] [Gazda et al., 2008]. HeLa cells expressing small
35
36 interfering RNAs (siRNAs) against *RPL5* show decreased production of 28S and 5.8S mature
37
38 rRNAs and accumulation of their precursors, in particular 32S and 12S. The same defect was
39
40 observed in lymphoblastoid cells established from DBA patients. Moreover, *RPL5*
41
42 knockdown induces reduction of free 60S subunit and formation of half-mer polysomes
43
44 [Gazda et al., 2008].
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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
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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].
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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].
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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'
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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].
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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
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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].
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49 ***RPS10***. Analysis of *RPS10* depleted HeLa cells and *RPS10* mutated lymphoblastoid cells
50
51 (c.260_261insC and c.337C>T in three different probands) revealed a pre-rRNA processing
52
53 phenotype similar to *RPS26* [Doherty et al., 2010].
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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

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3 version 8.01. Data were not informative for some rarely mutated RP genes, namely *RPS7*,
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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
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12 carrying mutations in *RPL5* or *RPL11* present more frequently with malformations. The risk
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14 of malformations of any type, including or excluding short stature, is 6.5 or 7.6-fold higher in
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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
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30 are SGA more frequently than patients with other mutations. Of 9 patients with SGA, 7 carry
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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).
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41 The gene currently associated with genitourinary malformations is *RPL35A*. Of 5 patients
42
43 with mutations in *RPL35A*, 3 have genitourinary malformations. In contrast, only 16 of 249
44
45 patients with mutations in other RP genes have genitourinary malformations (Table 3). This
46
47 difference in frequencies towards is statistically significant. Conversely, patients with
48
49 mutations in *RPS19* are less likely to have malformations of any type when compared with
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51 the other patients (OR < 1). This finding is also true when each type of malformation is
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53 considered independently.
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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.
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34 35 36 *Variable expressivity*

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

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2
3 HUGO Mutation Database Initiative/HGVS (Supplemental Material). Mutation nomenclature
4
5 has been checked with Mutalyzer program [Wildeman et al., 2008].
6
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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.
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32
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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
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3 patient with these types of malformations or with multiple malformations should be screened
4
5 first for these genes.
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10 **FUTURE PROSPECTS**

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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.
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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.
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50 **Accession Numbers**

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52 The GenBank accession number for human RPs are: NM_001022.3 for *RPS19*,
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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*.
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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.

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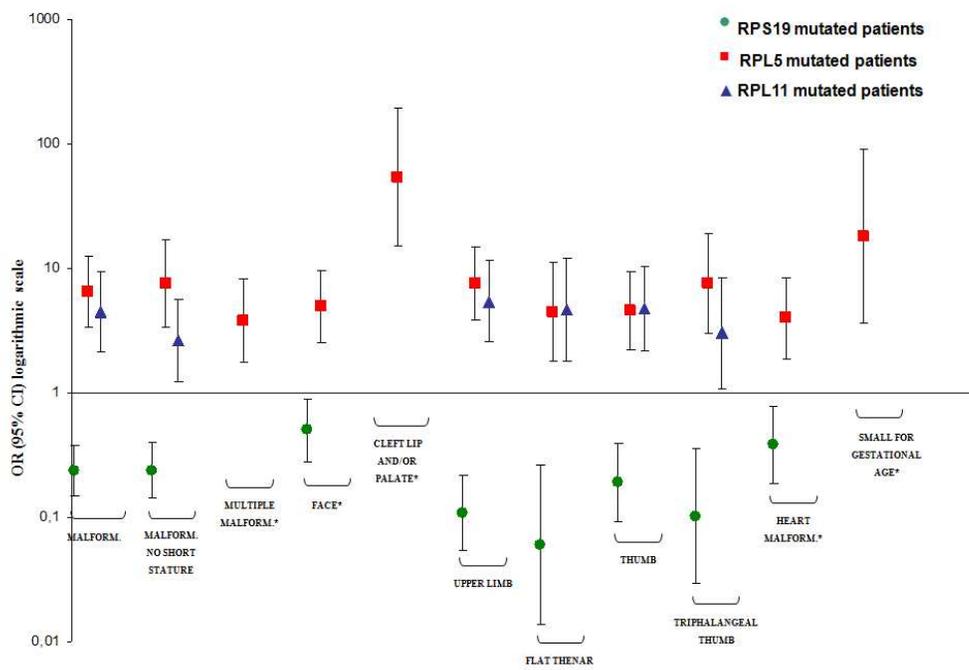
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28 **Figure Legends.**

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

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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
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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

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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
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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
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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.

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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	RSS
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)

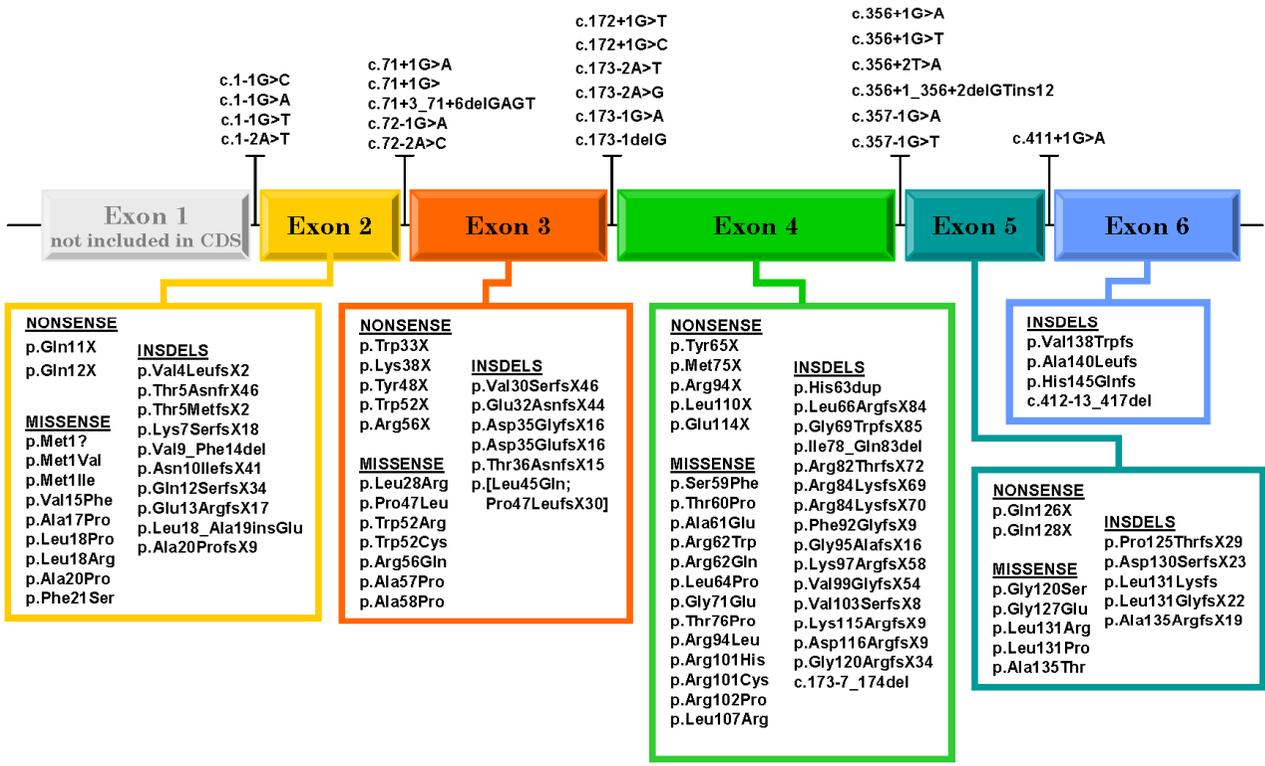
Sequence variant tables	
Unique sequence variants	Listing of all unique sequence variants in the RPS19 database, without patient data
Complete sequence variant listing	Listing of all sequence variants in the RPS19 database
Variants with no known pathogenicity	Listing of all RPS19 variants reported to 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.

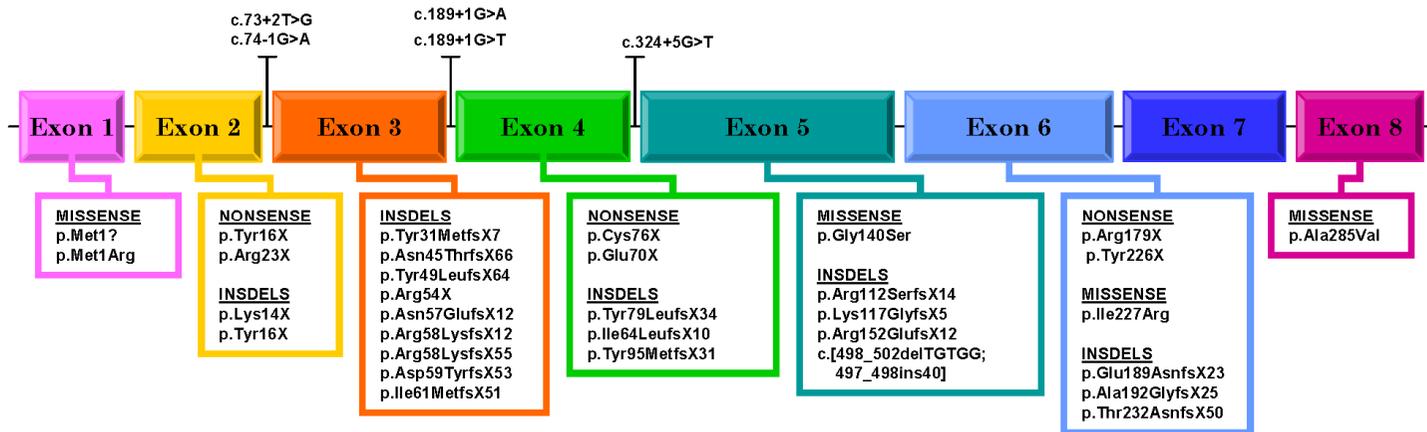
Links to other resources	
External link	dbSNP
Entrez Gene	6223
OMIM - Gene	603474
OMIM - Disease	105650 (DBA1)
HGMD	RPS19

SUPPLEMENTAL FIGURE S2.

RPS19 MUTATION MAP



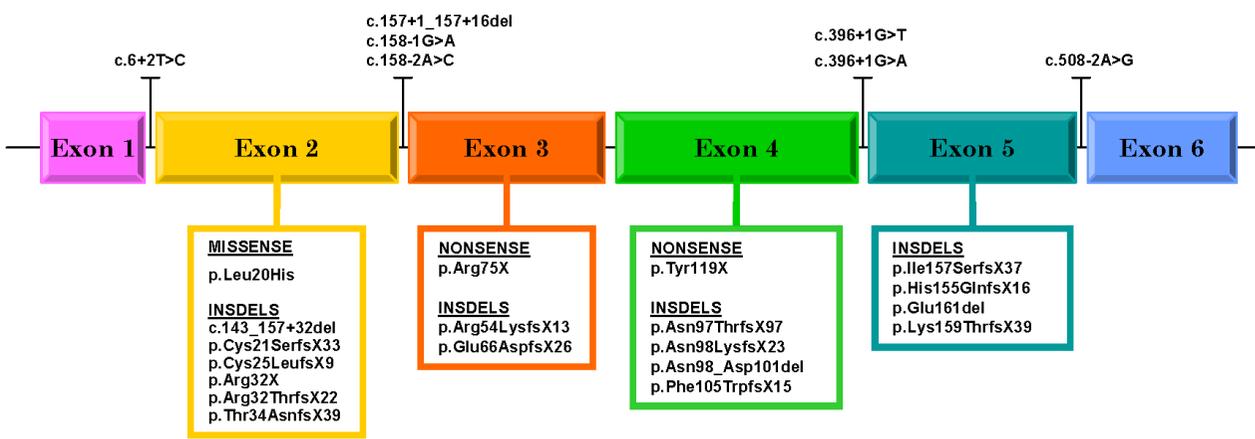
RPL5 MUTATION MAP



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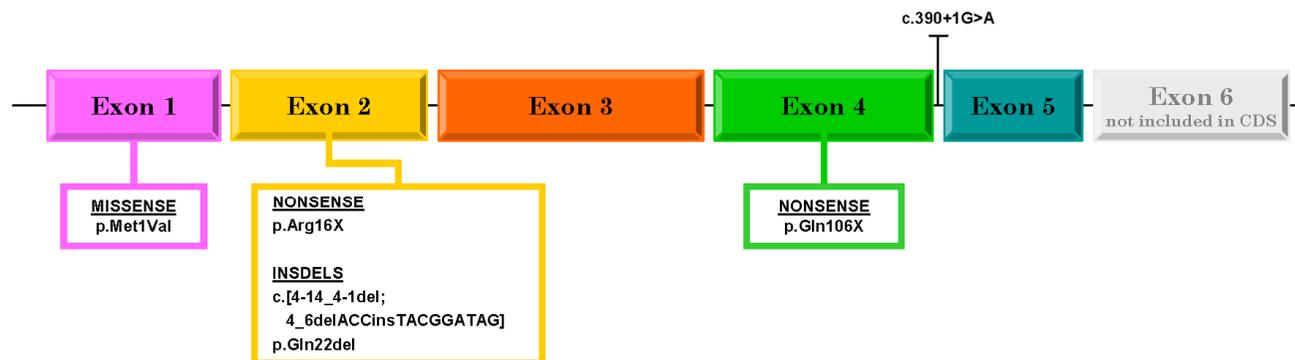
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RPL11 MUTATION MAP



RPS24 MUTATION MAP

NM_033022.3, (variant a)



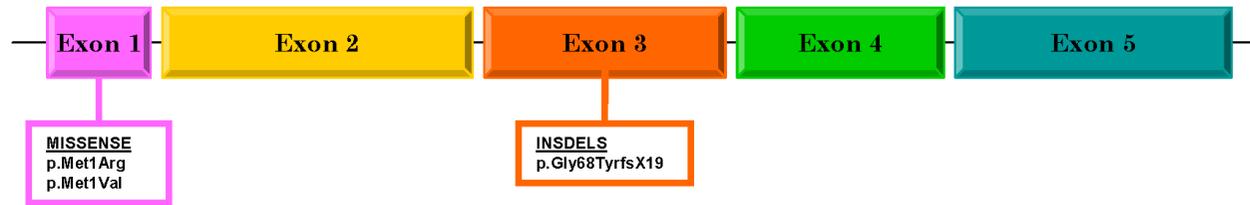
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RPL35A MUTATION MAP



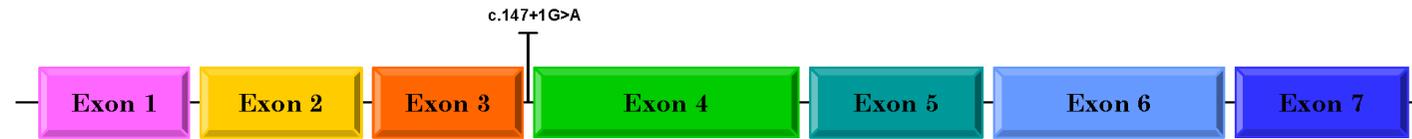
RPS17 MUTATION MAP



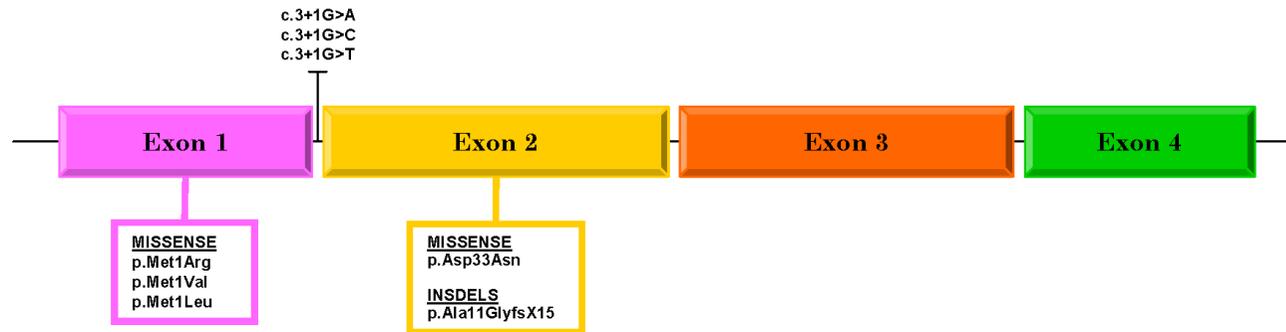
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RPS7 MUTATION MAP



RPS26 MUTATION MAP



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RPS10 MUTATION MAP

