



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

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



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



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

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**ABSTRACT**

Diamond-Blackfan Anemia (DBA) is characterized by a defect of erythroid progenitors and, clinically, by anemia and malformations. DBA exhibits an autosomal dominant pattern of inheritance with incomplete penetrance. Currently nine genes, all encoding ribosomal proteins (RP), have been found mutated in approximately 50% of patients. Experimental evidence supports the hypothesis that DBA is primarily the result of defective ribosome synthesis. By means of a large collaboration among six centers, we report here a mutation update that includes nine genes and 220 distinct mutations, 56 of which are new. The DBA Mutation Database now includes data from 355 patients. Of those where inheritance has been examined, 125 patients carry a *de novo* mutation and 72 an inherited mutation. Mutagenesis may be ascribed to slippage in 65.5% of indels, whereas CpG dinucleotides are involved in 23% of transitions. Using bioinformatic tools we show that gene conversion mechanism is not common in RP genes mutagenesis, notwithstanding the abundance of RP pseudogenes. Genotype-phenotype analysis reveals that malformations are more frequently associated with mutations in *RPL5* and *RPL11* than in the other genes. All currently reported DBA mutations together with their functional and clinical data are included in the DBA Mutation Database.

Key words: Diamond-Blackfan anemia; ribosomal protein; erythropoiesis; ribosome biogenesis.

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

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

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

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

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

## VARIANTS

### RPS19

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

Eighty-seven distinct mutations have been previously described in *RPS19* gene [most reviewed in Campagnoli et al., 2008]. We here report 42 additional mutations: 11 missense, 3 nonsense, 18 deletions and/or insertions, 10 splice-site defects (Table 1). Overall 129 distinct



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

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

**RPL5**

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

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

Ninety-five polymorphisms are listed in NCBI SNP database. There are multiple processed pseudogenes of *RPL5* gene dispersed through the genome: three of them are annotated as 'validated' in NCBI Gene database.

**RPL11**

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

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

Fifty-seven polymorphisms are reported in NCBI SNP database and five pseudogenes are annotated in NCBI Gene database as 'inferred'.

### **RPL35A**

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

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

The *RPL35A* gene has 45 polymorphisms listed in NCBI SNP database and only one pseudogene annotated as 'validated' in NCBI Gene database.

### **RPS24**

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

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

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

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

**RPS17**

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

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

**RPS7**

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

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

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

### **RPS26**

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

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

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

### **RPS10**

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

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

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

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**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*.

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

## BIOLOGICAL RELEVANCE

Several studies have addressed the effects of DBA mutations showing that they can lead either to a reduction of RP mRNA or to the production of ribosomal proteins with defective stability and/or localization. In all these cases, mutations cause haploinsufficiency which in turn interferes with the biogenesis of the large or the small ribosomal subunit. These defects are due to aberrant rRNA maturation at different steps, depending on the affected RP [Flygare et al., 2007; Choesmel et al., 2007; Idol et al., 2008; Gazda et al., 2008; Farrar et al., 2008; Doherty et al., 2010]. Here, we briefly revisit the biological function of each DBA RP and the effects of their mutations.

***RPS19.*** RPS19 mutations are associated with a defect in the maturation of 18S rRNA resulting in the accumulation of 21S pre-rRNA precursors [Flygare et al., 2007; Choesmel et al., 2007; Idol et al., 2008]. All of these mutations cause loss of function and some have been functionally characterized. Extensive functional data were recently reviewed by Campagnoli et al. in this journal [Campagnoli et al., 2008]. Furthermore Crétien and collaborators reported the study of the subcellular localization of several RPS19 mutants fused to green fluorescent protein (GFP) [Crétien et al., 2008]. They observed impaired nucleolar localization and a marked decrease in levels of protein expression for the following mutants: p.Leu131Pro, p.Trp33X, p.Tyr48X, p.Arg56X, p.Met75X, p.Arg94X, p.Glu13ArgfsX17, p.Arg82ThrfsX72, p.Leu131GlyfsX22. In contrast, p.Trp52Cys, p.Val9\_Phe14del and p.Gly120Ser mutants exhibited normal expression and localization. Proteasome inhibitors improved both the expression level and the nucleolar localization of p.Val15Phe, p.Gly127Glu, p.Leu131Pro, p.Arg94X, p.Arg82ThrfsX72 and p.Leu131GlyfsX22 mutants, but had no effect on

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

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

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



activity with ribosomal biogenesis [Dai et al., 2007]. RPL11 also cooperates with RPL5 to inhibit the E3 ubiquitin ligase activity of MDM2, thus resulting in the accumulation of transcriptionally active p53 [Zhang et al., 2003]. Fumagalli et al. recently showed that RPL11-mediated p53 induction is a general response to defective 40S or 60S ribosome biogenesis in human cell lines [Fumagalli et al. 2009]. The ubiquitin-like molecule NEDD8, that controls RPL11 stability and subcellular localization, plays an important role in the regulation of RPL11 signaling to p53 [Sundqvist et al., 2009]. In zebrafish *rpl11* knockdown activates the p53 pathway and disrupts the normal embryonic development through a p53-mediated apoptotic response [Chakraborty et al., 2009]. Gazda and collaborators showed that mutations in *RPL11*, c.314\_315delTT, IVS1+2t>c, IVS2-1g>a, IVS4+1g>t, lead to accumulation of the precursors of 28S and 5.8S rRNAs, similar to that of mutations in *RPL5* [Gazda et al., 2008].

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

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



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

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

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

***RPS10.*** Analysis of *RPS10* depleted HeLa cells and *RPS10* mutated lymphoblastoid cells (c.260\_261insC and c.337C>T in three different probands) revealed a pre-rRNA processing phenotype similar to *RPS26* [Doherty et al., 2010].

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

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

The gene currently associated with genitourinary malformations is *RPL35A*. Of 5 patients with mutations in *RPL35A*, 3 have genitourinary malformations. In contrast, only 16 of 249 patients with mutations in other RP genes have genitourinary malformations (Table 3). This difference in frequencies towards is statistically significant. Conversely, patients with mutations in *RPS19* are less likely to have malformations of any type when compared with the other patients (OR < 1). This finding is also true when each type of malformation is considered independently.

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

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

#### *Variable expressivity*

Variable expressivity is shown for all RP gene mutations. Possible mechanisms underlying variable expressivity include an influence of modifier genes and environmental factors.

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

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missense variant may be a silent mutation. Variations in the promoter or other regions have also been hypothesized to be phenotype modifiers [Crétien et al., 2010].

**DATABASE**

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

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

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

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

HUGO Mutation Database Initiative/HGVS (Supplemental Material). Mutation nomenclature has been checked with Mutalyzer program [Wildeman et al., 2008].

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

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

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

## DIAGNOSTIC RELEVANCE

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

patient with these types of malformations or with multiple malformations should be screened first for these genes.

**FUTURE PROSPECTS**

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

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

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

**Accession Numbers**

The GenBank accession number for human RPs are: NM\_001022.3 for *RPS19*, NM\_000969.3 for *RPL5*, NM\_000975.2 for *RPL11*, NM\_000996.2 for *RPL35A*, NM\_033022.3 for *RPS24*, NM\_001021.3 for *RPS17*, NM\_001011.3 for *RPS7*, NM\_001029.3 for *RPS26* and NM\_001014.3 for *RPS10*.

## 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/>

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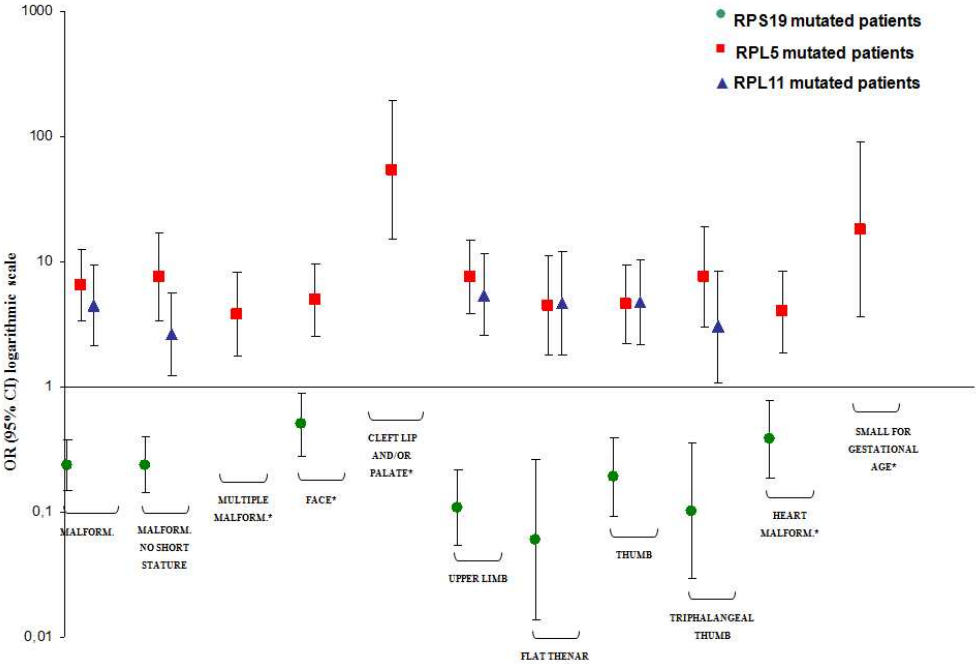
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**Figure Legends.**

FIGURE 1. Malformation status of patients with *RPS19*, *RPL5* and *RPL11* mutations. Associations between malformations and RP gene mutations are assessed with odds ratio (OR) and 95% CI from logistic regression; OR are drawn on a logarithmic scale. (\*) Non significant in *RPS19* and/or *RPL11*.



122x84mm (200 x 200 DPI)



**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](http://www.hgvs.org/mutnomen)). The initiation codon is codon 1.

Mutated Gene	Patient (Gender)	Exon/Intron	cDNA Mutation	Predicted amino acid change	Mutation Type	Malformations	Growth Retardation	Steroid Response	Inheritance
RPS19	Ps19_1(F)	Ex 2	c.2T>A	p.Met?	Missense	na	na	na	familial
	Ps19_2(F)	Ex 2	c.10_13delGTTA	p.Val4LeufsX2	Deletion	none	no	yes	sporadic
	Ps19_3(F)	Ex 2	c.14delC	p.Thr5MetfsX2	Deletion	na	na	na	unknown
	Ps19_4(M)	Ex 2	c.28_29insT	p.Asn10IlefsX41	Insertion	na	na	na	sporadic
	Ps19_5(F)	Ex 2	c.34_47del	p.Gln12SerfsX34	Deletion	Dystrophy	na	na	unknown
	Ps19_6(M)	Ex 2	c.49G>C	p.Ala17Pro	Missense	na	na	na	sporadic
	Ps19_7(NA)	Ex 2	c.58G>C	p.Ala20Pro	Missense	na	na	na	unknown
	Ps19_8(F)	Ex 3	c.83T>G	p.Leu28Arg	Missense	none	no	na	unknown
	Ps19_9(M)	Ex 3	c.88delG	p.Val30SerfsX46	Deletion	Microcephaly, micro-retrognathia, hypertelorism, cafe au lait spots	na	na	familial
	Ps19_10(M)	Ex 3	c.93delC	p.Glu32AsnfsX44	Deletion	Thumb	no	no	unknown
	Ps19_11(M)	Ex 3	c.103dupG	p.Asp35GlyfsX16	Insertion	none	no	no	unknown
	Ps19_12(M)	Ex 3	c.112A>T	p.Lys38X	Nonsense	none	no	no	sporadic
	Ps19_13(M)	Ex 3	c.156G>A	p.Trp52X	Nonsense	Low hairline, cafe au lait spots	na	na	unknown
	Ps19_14(M)	Ex 3	c.172G>C	p.Ala58Pro	Missense	none	yes	no	de novo
	Ps19_15(F)	Ex 4	c.178A>C	p.Thr60Pro	Missense	none	no	na	sporadic
	Ps19_16(M)	Ex 4	c.187_189insCAC	p.His63dup	Insertion	Flat nose, low hairline, mitral valve and tricuspid valve insufficiency	na	na	sporadic
	Ps19_17(NA)	Ex 4	c.195C>G	p.Tyr65X	Nonsense	na	na	na	unknown
	Ps19_18(NA)	Ex 4	c.195C>G	p.Tyr65X	Nonsense	na	na	na	unknown
	Ps19_19(F)	Ex 4	c.203_204insG	p.Gly69TrpfsX85	Insertion	na	na	na	sporadic
	Ps19_20(M)	Ex 4	c.212G>A	p.Gly71Glu	Missense	na	na	na	sporadic
	Ps19_21(F)	Ex 4	c.281G>T	p.Arg94Leu	Missense	none	na	na	sporadic
	Ps19_22(F)	Ex 4	c.284delG	p.Gly95AlafsX16	Deletion	ASD	na	na	unknown
	Ps19_23(M)	Ex 4	c.289_290insAGGC	p.Lys97ArgfsX58	Insertion	Dysplastic aortic valve	na	na	unknown
	Ps19_24(F)	Ex 4	c.296_297delTG	p.Val99GlyfsX54	Deletion	na	na	na	unknown
	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
24									
25	RPL5								
26	PI5_1(F)	Ex 1	c.1A>G	p.Met1?	Missense	Triphalangeal thumb	na	na	sporadic
27	PI5_2(F)	Ex 1	c.2T>G	p.Met1Arg	Missense	Duplicated ureter	yes	yes	familial
28	father (M)	Ex 1	c.2T>G	p.Met1Arg	Missense	Heart Murmur	yes	yes	familial
29	PI5_3(M)	Ex 2	c.48C>A	p.Tyr16X	Nonsense	Cleft palate, abnormal right thumb	yes	yes	de novo
30	PI5_4(F)	Ex 3	c.91delT	p.Tyr31MetfsX7	Deletion	Triphalangeal thumb	na	na	de novo
31	PI5_6(M)	Ex 3	c.172_173insA	p.Arg58LysfsX55	Insertion	Cleft lip and palate, triphalangeal thumb, short stature	na	na	de novo
32									
33	PI5_7(F)	Ex 4	c.191_204ins14	p.Ile64LeufsX10	Insertion	na	na	na	de novo
34	PI5_8(F)	Ex 4	c.208G>T	p.Glu70X	Nonsense	na	na	na	sporadic
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2	PI5_9(M)	Ex 4	c.283delT	p.Tyr95MetfsX31	Deletion	na	na	na	sporadic
3	PI5_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									
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6	PI5_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	PI5_12(F)	Ex 6	c.535C>T	p.Arg179X	Nonsense	na	na	na	sporadic
9									
10									
11	RPL11	PI11_1(F)	Ex 2	c.100_101dupA	p.Thr34AsnfsX21	VSD, ASD, abnormal thumbs	no	na	sporadic
12		PI11_2(M)	Ex 5	c.475_476ins11	p.Lys159ThrfsX39	Insertion	none	no	yes
13		PI11_3(M)	Int 2	c.158-2A>C	p.0?	Acceptor splice site	none	yes	yes
14									sporadic
15	RPS24	Ps24_1(M)	Int 4	c.390+1G>A	p.0?	Donor splice site	na	na	na
16									sporadic

*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)
<b>RPS19</b>	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)
<b>RPL5</b>	c.535C>T	Nonsense	Ex 6	CCAAACGATTC	CCAAATGATTC	CCAAATGATTC	RPL5P34 (22q13.2)*
<b>RPL11</b>	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
<b>Total</b>	<b>129</b>	<b>8</b>	<b>6</b>	<b>3</b>	<b>3</b>	<b>1</b>	<b>39</b>	<b>26</b>	<b>5</b>	<b>220</b>	<b>355</b>

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

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**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 at al., 2009; Quarello at 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.


**Diamond-Blackfan Anemia**  
 ribosomal protein S19 (RPS19)   
 Curators: [I. Boria](#) and [U. Ramenghi](#)

[Home](#)
[Variants](#)
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[Documentation](#)

[RPS19 homepage](#)
[Switch gene](#)

### LOVD Gene homepage

General information	
Gene name	ribosomal protein S19
Gene symbol	<b>RPS19</b>
Chromosome Location	19q13.2
Database location	<a href="#">www.dbagenes.unito.it</a>
Curator	<a href="#">I. Boria</a> and <a href="#">U. Ramenghi</a>
Database reference for citations	Boria et al (2008). <a href="#">A new database for ribosomal protein genes which are mutated in Diamond-Blackfan Anemia</a> . Hum. Mutat.29(11), E263-70
PubMed references	View all (unique) <a href="#">PubMed references</a> in the RPS19 database
Date of creation	November 12, 2007
Last update	June 11, 2010
Version	<b>RPS19 100611</b>
Add sequence variant	<a href="#">Submit a sequence variant</a>
First time submitters	<a href="#">Register here</a>
Reference sequence	<a href="#">coding DNA reference sequence</a> for describing sequence variants
GenBank reference	<a href="#">NM_001022.3</a>
Total number of unique DNA variants reported	<b>129</b>
Total number of individuals with variant(s)	<b>219</b>
Total number of variants reported	<b>219</b>
Subscribe to updates of this gene	
NOTE	<p>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.</p> <p><a href="#">Link to the location of the pathogenic variants in relation to the gene</a></p> <p><a href="#">Link to the location of the pathogenic variants in relation to the protein structure</a></p>

Graphical displays and utilities	
<a href="#">Summary tables</a>	Summary of all sequence variants in the RPS19 database, sorted by type of variant (with graphical displays and statistics)

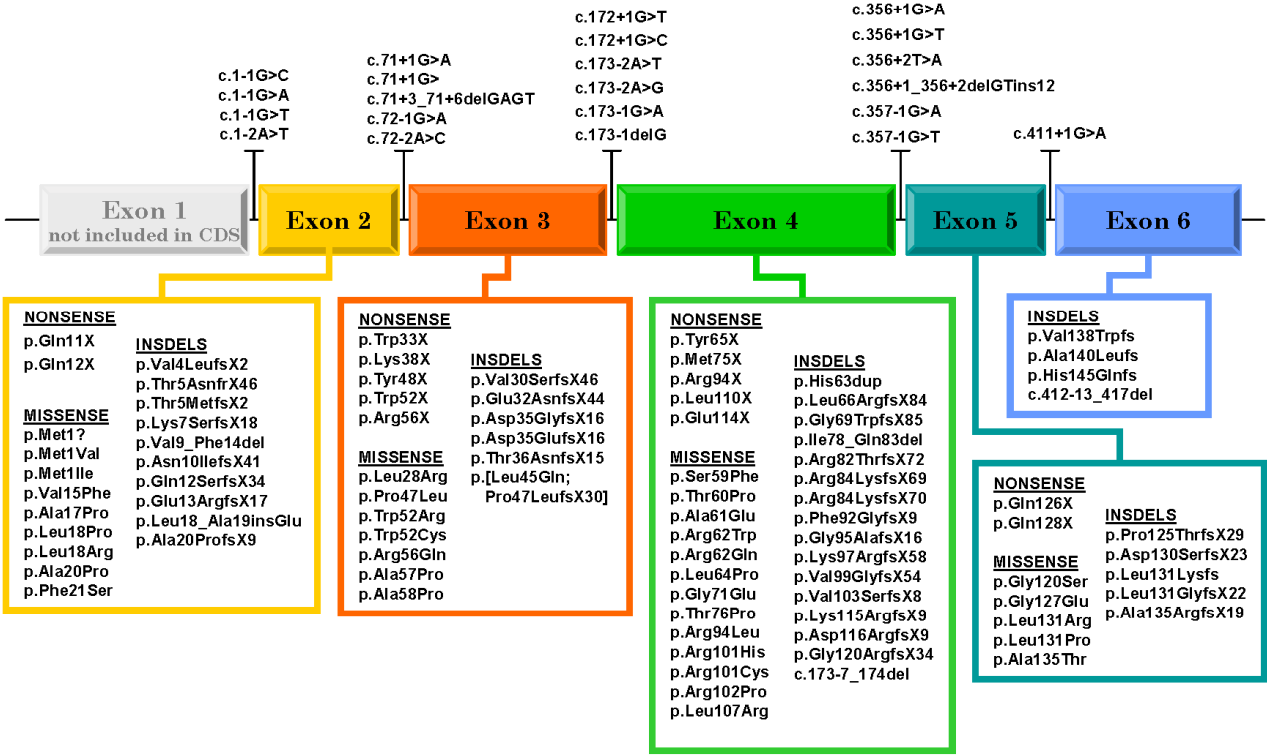
Sequence variant tables	
<a href="#">Unique sequence variants</a>	Listing of all unique sequence variants in the RPS19 database, without patient data
<a href="#">Complete sequence variant listing</a>	Listing of all sequence variants in the RPS19 database
<a href="#">Variants with no known pathogenicity</a>	Listing of all RPS19 variants reported to have no noticeable phenotypic effect (note: excluding variants of unknown effect)

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

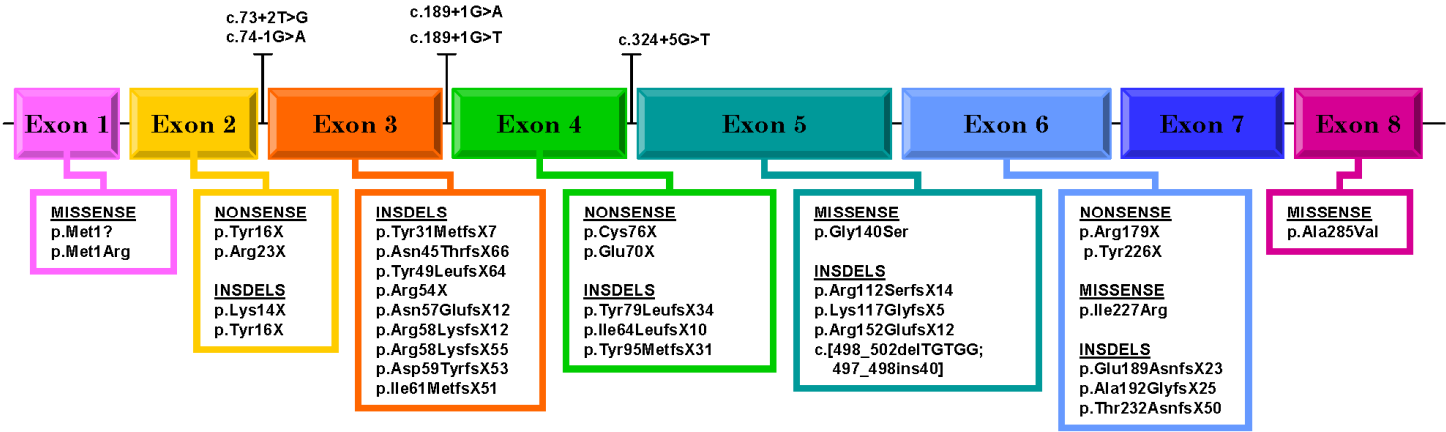
Links to other resources	
External link	<a href="#">dbSNP</a>
Entrez Gene	<a href="#">6223</a>
OMIM - Gene	<a href="#">603474</a>
OMIM - Disease	<a href="#">105650 (DBA1)</a>
HGMD	<a href="#">RPS19</a>

SUPPLEMENTAL FIGURE S2.

RPS19 MUTATION MAP

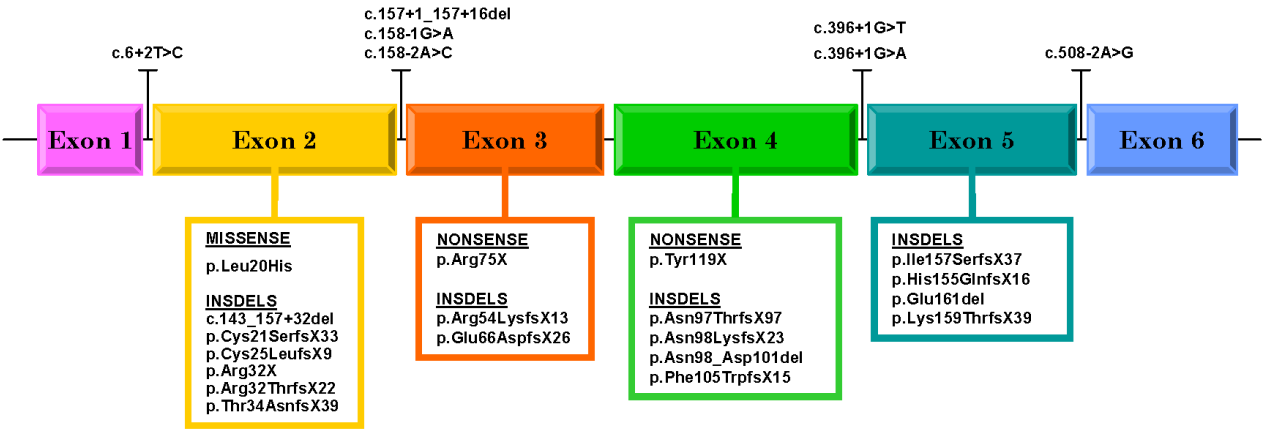


RPL5 MUTATION MAP



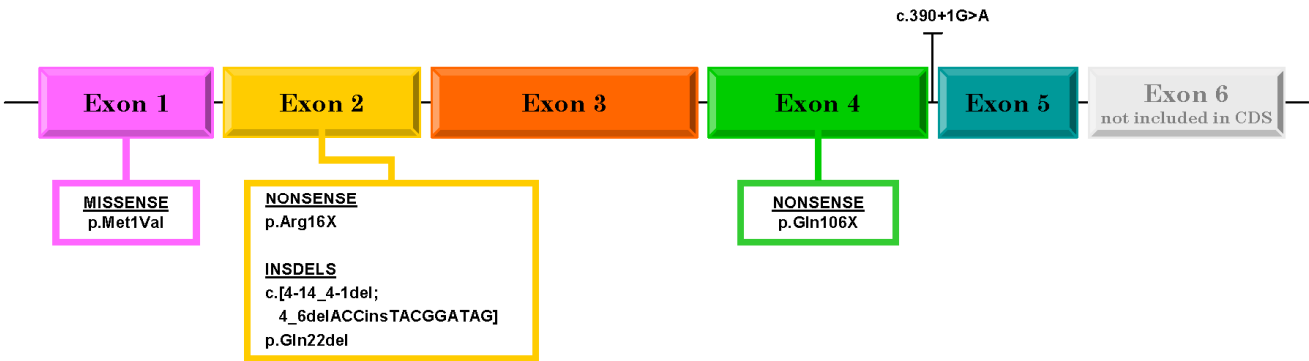


RPL11 MUTATION MAP



*RPS24* MUTATION MAP

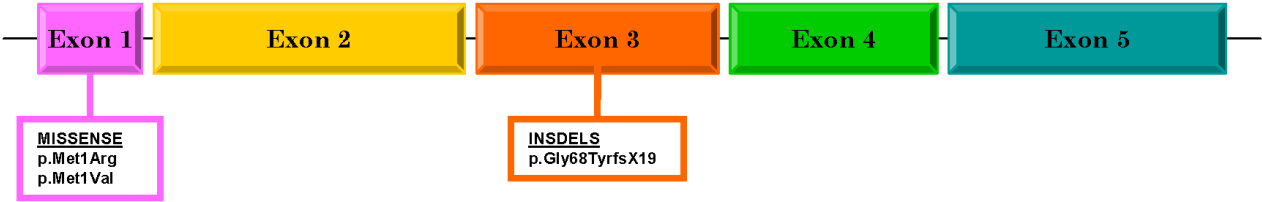
NM\_033022.3, (variant a)



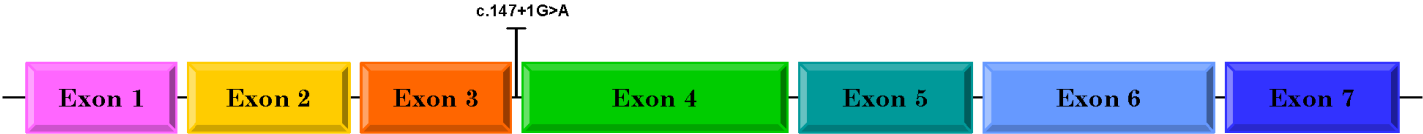
RPL35A MUTATION MAP



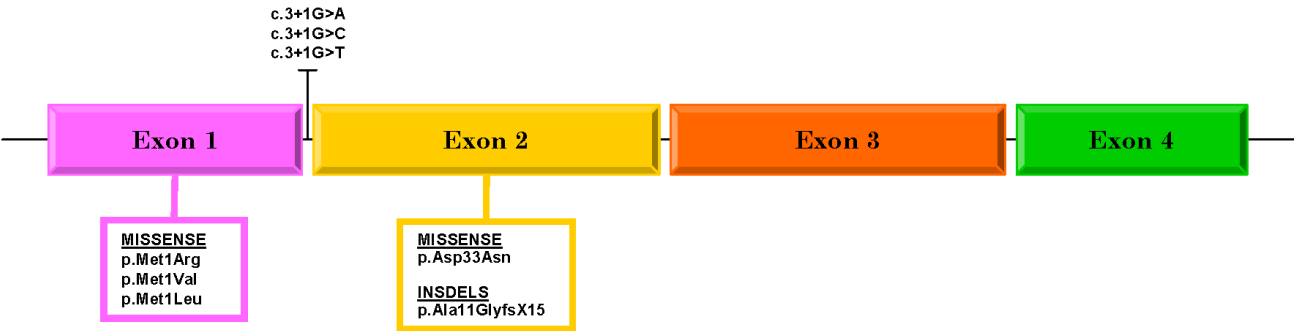
*RPS17* MUTATION MAP



*RPS7* MUTATION MAP



RPS26 MUTATION MAP



*RPS10* MUTATION MAP

