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**A 20-year long term experience of the Italian Diamond-Blackfan Anaemia Registry: RPS and RPL genes, different faces of the same disease?**

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## ORIGINAL PAPER

### TITLE

A 20-year long experience of the Diamond-Blackfan Anaemia Italian Registry: *RPS* and *RPL* genes, different faces of the same disease?

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## **ABSTRACT**

Diamond-Blackfan anaemia (DBA) is a rare and heterogeneous disease characterized by hypoplastic anaemia, congenital anomalies, and a predisposition for malignancies. The aim of this paper is to report the findings from the Italian DBA Registry, and to discuss the registry's future challenges in tackling this disease.

Our 20-year long work allowed the connection of 50 AIEOP centres and the recruitment of 283 cases. Almost all patients have been characterized at a molecular level (96%, 271/283) finding a causative mutation in 68% (184/271). We confirm the importance of determination of eADA and of rRNA assay in the diagnostic pipeline and the characterization of a remission state. Patients with mutations in *RPL* genes were reported to show a significant correlation with the incidences of malformations, higher eADA levels and more severe outcomes, in comparison to patients with mutations in *RPS* genes.

Furthermore, as a consequence of our results, especially the incidence of malignancies and the high percentage of patients older than 18 years, we stressed the importance of collaboration with adult clinicians to guarantee a regular multi-specialist follow up.

In conclusion, this study highlights the importance of national registries to increase the knowledge and to improve the management of a such complex disease.

## Introduction

Diamond-Blackfan Anemia (DBA) is a rare genetic disorder being one of the group of inherited bone marrow failure syndromes (IBMFS). Onset of the disorder is usually in the first year of life with a pure erythroid aplasia that is also associated with the increase of erythrocyte adenosine deaminase (eADA) levels and to physical malformations (Vlachos and Muir 2010).

Sixty per cent of patients respond to the administration of steroids; non-responders and those that develop adverse effects to steroids require chronic transfusion. To date, the only curative treatment still remains haematopoietic stem cell transplant (HSCT). Interestingly, twenty per cent of patients demonstrate a progressive reduction in their requirement of treatment leading to clinical remission (Vlachos and Muir 2010). The risk of DBA patients developing cancer is higher than normal but lower than in other IBMFS such as Fanconi anaemia (Vlachos, *et al* 2012, Vlachos, *et al* 2018).

More than sixty per cent of patients carry loss of function mutations in one of 19 ribosomal proteins (RP), the most frequent being *RPS19*, *RPS26*, *RPL5* and *RPL11*, whereas rare cases carry mutations in the other RP genes (Ulirsch, *et al* 2018). Few cases are due to non-RP genes, such as *TSR2* and *GATA1*, while around thirty per cent of patients do not carry mutations in known genes (Gripp, *et al* 2014, Sankaran, *et al* 2012). Ribosomal RNA (rRNA) analysis is focused on the identification of long rRNA precursors that specifically accumulate when a RP is deficient. Furthermore, rRNA analysis is also a useful tool to confirm a DBA diagnosis and to direct sequencing towards a specific RP gene (Farrar, *et al* 2014, Quarello, *et al* 2016).

The objective difficulties in diagnosis, the great clinical variability among clinics, the rarity of the involvement of several DBA genes and the still conspicuous number of patients that do not carry a mutation in known genes, underline the need for a disease-based registry which can describe the natural history of this disease in a statistically significant number of patients. Several DBA registries were created more than twenty years ago. The first being the Czech Republic DBA Registry in 1992 (Pospisilova, *et al* 2012). This was shortly followed by registries in the USA (1993), Germany (1993), France (1995) and Italy (1995). (Da Costa, *et al* 2018, Vlachos, *et al* 2001). In Italy, children affected by oncologic, hematologic and immunodeficiency disorders are managed under a network of 50 centres of the Italian Association of Paediatric Haematology and Oncology (AIEOP). All data from these patients has been recorded in a national online database. The registry has become part of the European DBA consortium from its foundation in 2012 (Da Costa, *et al* 2018). These

efforts allowed for great strides to be made in the definition of DBA natural history and pathophysiology. The aim of this paper is, therefore, to report the findings from the Italian DBA Registry, the clinical profiles of the patients and to discuss the registry's future challenges in fighting this disease.

## **Methods**

### AIEOP DBA registry structure

The AIEOP DBA Registry is a platform linked to the existing web-based AIEOP national database for paediatric patients affected by oncologic, hematologic and immunodeficiency disorders (known as Mod.1.01 form). It is based on the AxMR® (Advanced eXtended Multicentre Research) IT solution and developed by CINECA (an Interuniversity Computing Center) (Pession and Rondelli 2008) (**Online supplement, Fig 1A**).

### Diagnostic criteria

A diagnosis of classical or non-classical DBA was made according to the diagnostic criteria reported in Vlachos et al. (Vlachos, *et al* 2008). Our laboratory located in Torino, offered both the evaluation of eADA activity and the molecular analyses for all Italian patients with suspected DBA. A multi-step approach that combines rRNA analysis with Sanger sequencing and deletion/duplication detection by MLPA was used (Quarello, *et al* 2012, Quarello, *et al* 2016) (**Online supplement, Fig 1B**).

### Statistical analyses

Patient-, disease- variables were expressed as medians and ranges, or as percentages, as appropriate. Patients were censored in the event of their death or at their last follow-up. OS was calculated according to the Kaplan-Meier method. The incidence of occurrence of remission and malignancies was expressed as cumulative incidence (CI) curves, in order to adjust the analysis for competing risks. Death from any cause and HSCT were competing risks to estimate the cumulative incidence of malignancies. Death by any cause, remission induced by steroids or after HSCT, and non-classical patients were competing risks to estimate the CI of spontaneous remission (**Online supplement**).

## **Results**

### Patients

On June 2019, the Italian DBA patient's registry included 283 cases reported by 37 AIEOP Centres. Clinically, 95% (270/283) were diagnosed with a classical form of DBA and 13 non-classical (5%). The male to female ratio was approximately equal (147/136). Diagnosis was reached within the first year of life in 80% (226/283) (median 3 months, range 0-58 years).

### Malformations

Clinical details on this phenotype were available for 244 cases. One half (50.5%, 123/244) had malformations. Multiple malformations, involving two or more organ systems, were present in 64 patients (26%). The most common malformations were skeletal, involving craniofacial (17%) or upper limbs anomalies (16%), followed by cardiac defects (15%).

### Evaluation of eADA

The eADA was tested in 125 patients without transfusions, revealing that it had increased in the 88% (110/125) of cases. Among 12 patients with normal eADA levels, 7 patients showed a *RPS* gene mutation. Furthermore, eADA was evaluated in 78 transfusion-dependent patients showing an increase in 41% (32/78). The eADA value was significantly higher in patients who carried a mutation in *RPL* genes in comparison to patients mutated in *RPS* genes ( $p < 0.0001$ ). Furthermore, in transfusion dependent patients higher eADA levels were observed in patients mutated in *RPL* genes ( $p = 0.01$ ) (**Fig 2**).

### Malignancies

A diagnosis of malignancies was reported in eight patients (3.2%, 8/244). The median age at diagnosis was 19 years (range 4-48 years) (**Table I**). Excluding post-HSCT neoplasm from analysis, the cumulative incidence for cancer was 1% (95% IC, 0.35-4%) at 20 years, 2% (95%, CI 0.6-7%) at 45 years and 13.5% (95%, CI 4.5-41%) at 50 years (**Fig 3A**). The patient affected by myelodysplastic syndrome underwent HSCT and he is currently in remission with good clinical conditions. A causative variant in RP genes was detected in six patients (**Table I**).

### Steroid treatment

Data on steroid treatments was available in 230 patients. 78% (180/230) were treated with steroids at some stage during the course of their disease; the majority after 1 year of age. In all cases the initial dose of prednisone was 2 mg/kg/d, followed by an attempt to decrease the dose to a minimal effective dose.

Upon treatment, 53% (96/180) showed a complete response reaching transfusion independence; 10% (18/180) showed a partial response, whereas 37% (66/180) never responded to therapy. Prednisone was discontinued in 29 out of 114 responders (25%).

### Follow up and survival

Data on follow-up was available for 260 patients. The median duration of follow-up was 12 years (range: 2 months-68 years). The overall survival rate was 86% (95% CI, 81-92) at 20 years and 77% (95% CI, 69-85) at 30 years of age (**Fig 3B**). Among living patients (243/260), 32% (78/243) were transfusion dependent, 22% (53/243) steroid dependent,

16% (40/243) in remission after HSCT, 14% (33/243) in remission after steroid treatment, and 11% (26/243) in spontaneous remission.

The overall cumulative incidence of remission (excluding post HSCT remission) is 9.4% at 5 years (95% CI, 6.4-13.9) and 17.2% (95% CI, 12.4-23.8) at 20 years from diagnosis. Specifically, the cumulative incidence of post-steroid remission was 8.5% at 5 years (95% CI, 5.5-12.6) and 16.4% (95 CI, 11.7-22.9) at 20 years from diagnosis and the cumulative incidence of spontaneous remission was 6.9% at 5 years (95% CI, 4.3 -10.9) and 10.4% (95 CI, 6.8-15.8) at 20 years from diagnosis.

The characteristics of patients who experienced spontaneous remission are reported in **Table II**. 80% of these patients were treated with sporadic transfusions; only 5 patients were transfusion dependent. The mean age at remission was different in these two groups (2.6 versus 17 years,  $p<0.001$ ). Among these patients 80% (12/15) of evaluated patients showed elevated eADA values and a pathological rRNA profile was found in all nine patients analysed. Only one patient showed a normalization of eADA and a normal rRNA profile (Pt 19, **Table II**).

#### Genetic spectrum of DBA mutations

Overall, 96% (271/283) of patients were characterized at a molecular level. Causative variants were found in 68% (184/271) patients. The *RPS* genes were involved in 62.5% (115/184) and *RPL* genes in 37% (68/115). Data on inheritance was available for 108 patients. The mutation was inherited in 26% of patients (28/108) and sporadic in 74% (80/108).

We most frequently found *RPS19* as a causative gene (44%, 81/184), followed by *RPL5* (18.5%, 34/184), *RPL11* (14%, 26/184), *RPS26* (12.5%, 23/184), *RPL35A* (4.5%, 8/184), *RPS17* (4.5%, 8/184), and *RPS24* (2%, 3/184). A *GATA1* mutation was found in only one single patient (Parrella, *et al* 2014).

Mutations mostly included single base changes identified by Sanger sequencing (81.5%, 150/184) leading to a missense (30%, 45/150), a splice-site change (18%, 27/150) a nonsense (12%, 18/150) or small insertions/deletions (40%, 60/150). Partial or complete gene deletions and partial gene duplications detected by MLPA represented an important mutation load (18.5%, 34/184).

Missense mutations were found more frequently in *RPS19* (42%, 34/81). Mutations detected in *RPL5* and *RPL11* were mostly small insertions/deletions (59%, 20/34 and 58%, 15/26, respectively). Gene deletions were particularly frequent for both *RPS17* (87.5%, 7/8) and *RPL35A* (87.5%, 7/8) (**Table III**). Interestingly, in one patient we found a single exon



duplication in *RPL35A* of 2,444 bp (c.165-759\_310-62dup; p.Met104GlnfsTer40, ENST00000265239.6; NM\_001316311.1).

#### Analysis of ribosomal RNA

Ribosomal RNA analysis was performed on 102 patients. The 54% (55/102) showed an increased 28S/18S ratio: 39 of these had a mutation in an *RPS* gene. A decreased 28S/18S ratio associated to an increase 32S/28S ratio was found in the 36% of patients; almost all (34/37) showed a mutation in *RPL5* or *RPL11*. A slightly reduced 28S/18S ratio without a corresponding increase in 32S/28S ratio was detected in the remaining 10 patients: four carried a *RPL35A* alteration.

#### Genotype/phenotype correlation

Patients with a mutation in an *RPL* gene showed a significant increased risk of both isolated and multiple malformations in comparison to patients with a mutation in an *RPS* gene ( $p < 0.0001$ ). Specifically, craniofacial and upper limb malformations were more associated to mutations in *RPL* genes ( $p < 0.0001$ ). This association seems to be mainly attributable to *RPL5* and *RPL11* for malformations in general ( $p < 0.0001$  and  $p = 0.02$ ) and upper limb malformations (both  $p = 0.001$ ). Patients with *RPL5* mutations have a significant risk of craniofacial malformation ( $p < 0.0001$ ). Response to steroids was not associated with a specific *RP* gene (**Fig 4**).

Patients with mutations in *RPS* genes showed a significant higher cumulative incidence of post-steroid remission (17.2%, 95% CI 10.2-28.8,  $p = 0.003$ ); specifically, no *RPL* gene mutated patient experienced post-steroid remission (**Fig 3C**). No association between spontaneous remission and *RPS/RPL* mutated gene was found.

#### **Discussion**

Our 20-year study allowed connecting 50 AIEOP centres and recruiting 283 cases, spreading the use of a uniform shared protocol for clinical and biological data collection. The Registry allowed for the screening of almost all the patients at a molecular level (96%, 271/283). Similarly, to other registries, the detection of the causative mutation was around 70% (Da Costa, *et al* 2018).

Considering the spectrum of DBA genes, we found *RPS* more frequently mutated than *RPL* genes (62.5%, 115/184), with *RPS19* covering 44% (81/184) of cases. Partial or complete deletions/duplications were detected in a significant percentage of the patients (18.5%), thanks to a specific MLPA assay (Quarello, *et al* 2012). Missense mutations were found more frequently in *RPS19* (Aspesi, *et al* 2018). *RPS17* and *RPL35A* were the genes most

frequently subjected to deletions. Notably, we found a single exon duplication in *RPL35A* gene, which to our knowledge, has never previously been described in a DBA patient.

Recently, the diagnostic pipeline was improved by the use of an rRNA assay, that directs mutation screening towards *RPL* or *RPS* genes (Quarello, *et al* 2016). Over the last few years, the rRNA assay was performed on 102/283, and it is now proposed as an initial functional screening in the diagnostic flowchart for DBA diagnosis.

Other groups have already exploited Next Generation Sequencing (NGS) panels for the diagnosis of DBA in clinical practice (Da Costa, *et al* 2018). Recently a Whole Exome Sequencing screening was used to achieve a comprehensive molecular diagnosis in 472 DBA patients (Ulirsch, *et al* 2018). Even if NGS approaches are widely used, we noted that an accurate clinical selection followed by a strategy based on Sanger/MLPA of *RPS19*, *RPS26*, *RPL5* and *RPL11* could detect up to 60.5% of pathogenic variants in our cohort.

Moreover, we confirmed the diagnostic value of eADA in DBA (Fargo, *et al* 2013). In our cohort, we found an increase in eADA in 88% of patients tested without transfusions. Notably, we report that patients that carried a mutation in *RPL* genes had higher eADA values in comparison to patients mutated in *RPS* genes. What is more, none of the DBA patients with normal eADA (12%, 15/125) showed a mutation in *RPL* genes.

The role of eADA in the pathophysiology of DBA remains unclear. It could be conceivable to consider the increase of the eADA activity as a compensation mechanism to dispose of the abnormal accumulation of rRNA precursors. In this sense an alteration of *RPL* genes could induce an increase in a greater quantity of precursors or of larger precursors so as to require higher levels of eADA. It will be interesting to confirm this hypothesis with further investigations.

One significant strength of the Italian DBA Registry is the genotype-phenotype correlation allowed by the large cohort of patients. We confirm that cases with a mutation in *RPL* genes showed a significant increased risk in both isolated and multiple malformations in comparison to patients with a mutation in *RPS* genes. More specifically, craniofacial and upper limb malformations were associated with *RPL* genes (Boria, *et al* 2010, Da Costa, *et al* 2018, Quarello, *et al* 2010).

A further notable advantage of DBA is represented in remissions (Narla, *et al* 2011). In our cohort, we observed that none of the patients that experienced post steroids remission had a mutation in *RPL* genes, suggesting that patients mutated in *RPL* genes have a more severe disease with a greater difficulty in rescue the haematological phenotype.

A small number of patients may also spontaneously enter remission. Notably, we have observed that the 80% (21/26) of these patients were treated with only sporadic transfusions. 20% of the patients (5/26) who regularly transfused, experienced remission significantly later. These are important considerations which could be fundamental for risk stratification and HSCT proposal.

The mechanisms of remission remain elusive, although some cases of both revertant mosaicism and somatic mosaicism associated with remission have been reported (Farrar, *et al* 2011, Garelli, *et al* 2019, Jongmans, *et al* 2018).

In one patient we have recently described the mechanism of remission as a revertant uniparental disomy ablating a de novo *RPS19* mutation (Garelli, *et al* 2019). This patient showed an eADA and rRNA profile normalization when remission occurred. On the contrary, we found that all tested patients in haematological remission had elevated eADA values and a pathological rRNA profile.

Based on this data, we speculate that DBA patients can have two different types of remission: i) a stable remission due to a “natural gene therapy” phenomenon, as in revertant mosaicism. In this case, not only the haematological phenotype, but also the ribosome biogenesis defect is completely rescued; ii) an unstable remission, with some persistent haematological DBA stigmata (e.g. increased eADA) and/or ribosome biogenesis defect with the probable risk of disease recurrence.

The incidence of malignancies is significantly increased in DBA with a median age of presentation of 35 years (range 11-70 years) (Alter, *et al* 2010, Vlachos, *et al* 2012). Overall, the cumulative incidence of evaluable cancer is 13.7% by 45 years of age with a striking increase of specific cancer types including myelodysplastic syndrome, acute myeloid leukaemia, colon carcinoma and osteogenic sarcoma (Lipton, *et al* 2001, Vlachos, *et al* 2012, Vlachos, *et al* 2018).

In our cohort we observed a lower median age at presentation (19 yrs.) with a similar cumulative incidence of 15.8% at 50 yrs. As already reported, we observed more solid tumours than hematologic malignancies with a high incidence of osteogenic sarcoma (37.5%, 3/8). No specific genotype correlations were possible due to the small numbers. The present data confirmed for us that DBA patients should receive appropriate counselling and surveillance for neoplastic events much earlier than the general population.

A further point we are working on is the collaboration with adult specialists to offer care in a lifelong setting. This aspect is particularly important considering that 39.5% of our patients are over 18 years old. We feel that a structured model of transition from paediatric

to adult care starting from 18 years of age is the only way to guarantee the regular follow-up of haematological parameters, the surveillance and treatment of iron overload in transfusion dependent patients, a screening for endocrine dysfunctions, and the early recognition of malignant conditions.

In conclusion, we have analysed clinical and molecular data from a different point of view finding that DBA patients with mutations in *RPL* or *RPS* genes showed significant differences with an impact on clinical management and the incentive to open new research fields. Thus, we confirm the importance of the existence of an active national registry for this challenging disease with the obvious wish that all DBA registries would merge into a single world-wide registry. This is a mandatory step towards an evidence-based medicine approach.

## FIGURE AND TABLES

**Figure 1. A) DBA registry structure.** The DBA Registry is coordinated by the Torino AIEOP Centre and involves a network of 50 AIEOP centres in Italy. At diagnosis, each patient is registered onto Mod.1.01, after having parents or a guardian's informed consent, and after being identified by a Unique Patient Number (UPN). All patients' personal data are encrypted except for responsible clinicians. Through the UPN, the patient can then be assigned to the DBA Registry. All information is loaded by the responsible clinician at each AIEOP Centre. Genetic analyses are performed following a standard workflow in a central lab and result analyses are inserted into the DBA database. The referral centre requires an annual update of the follow-up section.

### **B) Diagnostic molecular flowchart**

The screening for mutations in known DBA genes is anticipated by the rRNA analysis. Briefly, the 28S/18S and the 32S/28S ratio was analysed by Bioanalyzer on total RNA obtained from peripheral blood lymphocytes. If 28S/18S ratio was higher than 1.9 the patient's DNA was Sanger sequenced for *RPS19* and *RPS26*. Those patients that showed both a 28S/18S ratio lower than 1.5 and the presence of a 32S precursor were analysed for *RPL5* and *RPL11*. All mutation-negative patients, at this stage, were further analysed by a MLPA assay that allows screening for deletions/duplications for the following genes: *RPS17*, *RPS19*, *RPS26*, *RPL5*, *RPL11*, *RPL35A*. Finally, patients that are negative for these assays were subjected either to *RPS10* and *RPS17* Sanger sequencing if they had an increased (>1.9) 28S/18S ratio or to *RPL35A* sequencing if they showed a slightly normal 28S/18S ratio (1.5-1.6).

### **Figure 2. Analysis of Erythrocyte Adenosine Deaminase (eADA) in *RPS* and *RPL* mutated DBA cases.**

The eADA levels were increased in DBA cases with a mutated *RPL* vs. *RPS* genes. Symbols represent individual subjects within the patient groups. Bold horizontal axis lines represent the eADA upper limit of normal value (1.2 iu/g of haemoglobin).

eADA: erythrocyte Adenosine Deaminase, no RBCT: no Red Blood Cell Transfusion before eADA determination; RBCT: Red Blood Cell Transfusion performed less than 30 days from eADA determination. \*  $p=0.01$ ; \*\*\*\* $p<0.0001$  (Welch's *t*-test).

**Figure 3. Outcome of DBA patients**

**A. Cumulative incidence of malignancies in DBA patients**

**B. Overall survival of DBA patients**

**C. Cumulative incidence of post-steroids remission in DBA patients.**

**Figure 4. Genotype/phenotype correlation in DBA cases**

Associations between clinical variables and RP gene mutations are assessed with odds ratio (OR) and 95% confidence interval (CI) from logistic regression. ORs are drawn on a logarithmic scale. Legend: MALF=malformations, NS=not significant.

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## Authors contributions

PQ, ABr, ID, and UR participated in the conception and design of the study. PQ wrote the paper. PQ, EGa, AC, RC, ABr, EGi, and DF participated in the data collection, data analysis and drafting of the report. EGi, DF, and PQ performed the statistical analysis.

PQ, PC, MZ, ML, FP, MCP, MEC, PF, ABa, SC, GR, FF, UR enrolled patients in the study and had patients under their care. All authors revised and approved the final manuscript.

This study was realized on the behalf of the AIEOP working group on Diamond Blackfan Anaemia (Appendix 1).

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Figure 1

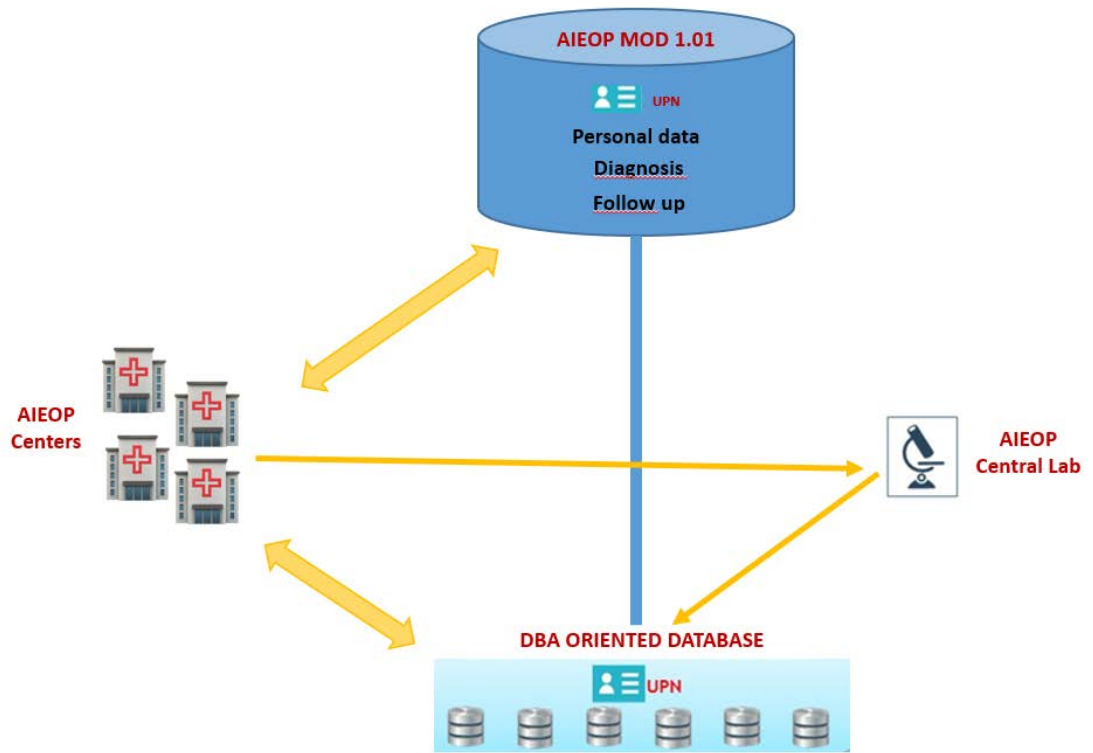


Figure 2

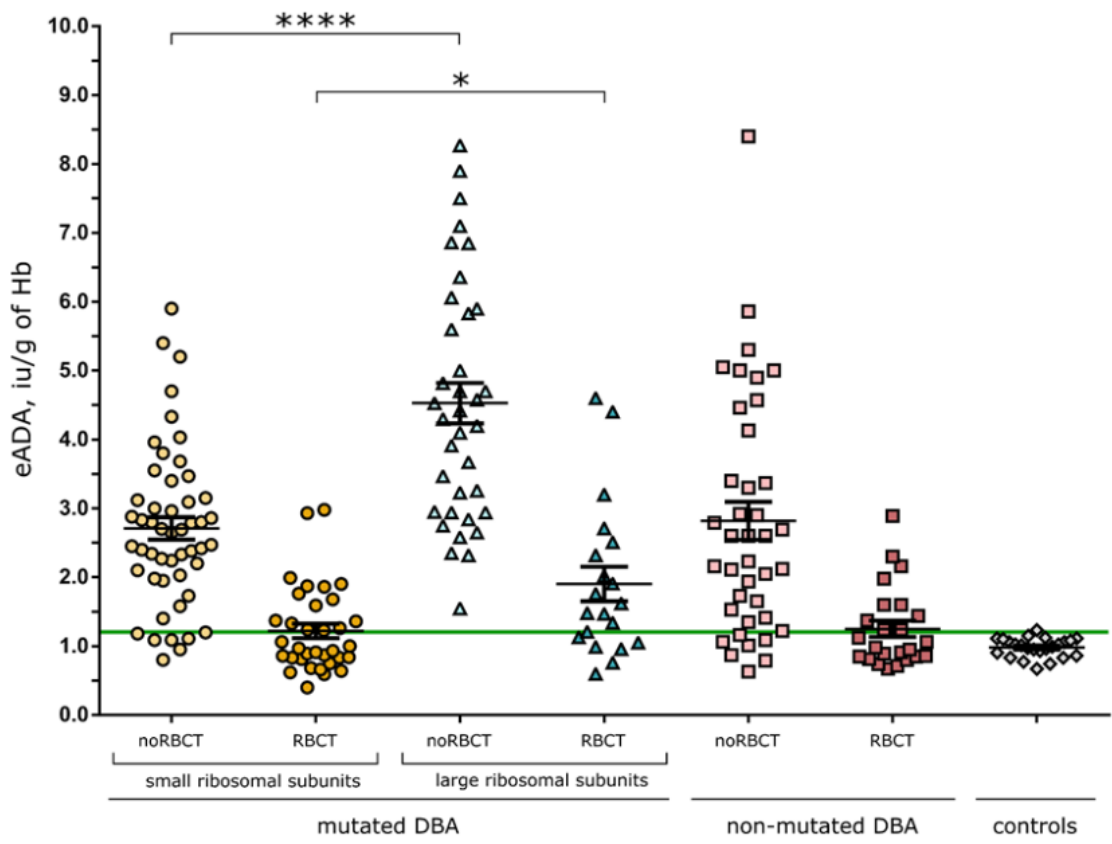


Figure 3

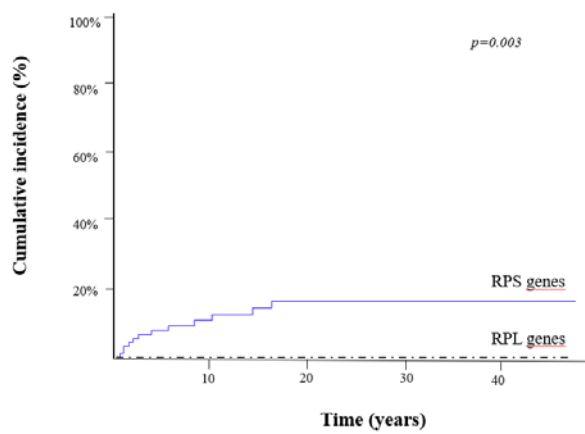
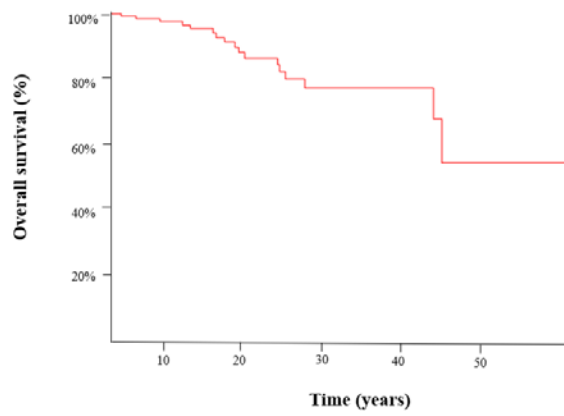
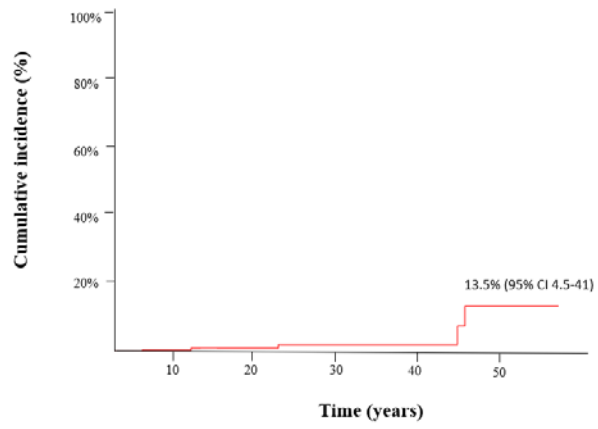


Figure 4

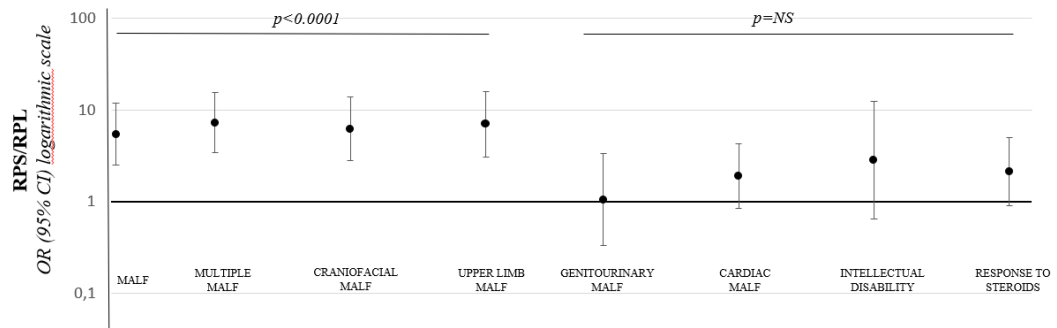




Table I. Clinical features of DBA patients with malignancies.

Patient No.	Gender	DBA status at diagnosis of malignancy	Age at diagnosis of malignancy (years)	Gene	Type of malignancy	Outcome	Follow up
1	F	TD	47	RPS19	Gastric carcinoma	Dead	1 yr
2	M	Remission after HSCT	19	RPS19	Osteogenic sarcoma	Alive	4 yrs
3	F	Remission after HSCT	18	RPS19	Osteogenic sarcoma	Alive	2 m
4	M	TD	4	GATA-1	Myelodysplastic syndrome	Alive	12 yrs
5	M	Remission after HSCT	9	No mutation	Cardiac Purkinje cell tumor	Alive	8 yrs
6	M	TD	22	RPS17	Thyroid cancer	Alive	14 yrs
7	F	SD	48	RPL5	Non-Hodgkin lymphoma	Alive	4 m
8	F	TD	11	RPS19	Osteogenic sarcoma	LFU	n.d.

Notes: F: female, M: male, TD: transfusion dependence, SD: steroid dependent, HSCT: Haematopoietic Stem Cell Transplant, LFU: lost at follow up; n.d.: not determined.



Table II. Clinical features of patients in spontaneous remission

Patient No.	Gender	Malformations	Age at diagnosis (months)	Age at remission (months)	Time from diagnosis to remission (months)	Response to steroid treatment	Status before remission	Follow up after remission (months)	eADA	Mutated gene
1	F	Cleft lip and palate, craniofacial dysmorphisms, hypoplastic thenar eminence	24	31	7	Nad.	ST	130	5.9 <sup>^</sup>	RPL5
2	F	No	9	358	349	Partial response, discontinued for inefficacy	TD	36	2.89 <sup>^</sup>	-
3	F	Clinodactyly, ventricular septal defect	1	35	34	Nad.	SpT	15	5.6 <sup>^</sup>	RPL11
4	M	Upper and lower extremities malformations, mental retardation, rectal atresia	39	58	18	NR	SpT	158	3.37 <sup>^</sup>	-
5	F	No	2	8	6	Nad.	SpT	105	1.24 <sup>^</sup>	-
6	M	No	5	199	194	NR	TD	53	3.12*	RPS19
7	F	Cleft lip and palate	1	27	25	Nad.	SpT	30	6.86*	RPL5
8	M	No	2	8	6	Nad.	ST	67	5.9*	RPS24
9	F	No	0	11	11	Nad.	ST	73	2.32*	RPL5
10	F	Patent foramen ovale	2	12	10	Nad.	ST	153	1.09*	-
11	F	No	8	65	58	Nad.	ST	566	Unknown	-
12	M	No	3	9	6	Nad.	ST	77	3.47*	RPS17
13	F	No	0	7	7	Nad.	ST	988	0.95*	RPS19
14	F	No	12	242	230	Nad.	TD	376	Unknown	RPS19
15	F	Patent foramen ovale, hypoplastic thenar eminence	2	293	291	Nad.	ST	254	6.06*	RPL11
16	F	Triphalangeal thumb	0	6	6	Nad.	ST	191	5.83*	RPL5
17	F	Triphalangeal thumb, craniofacial dysmorphisms	4	19	14	Nad.	SpT	179	4.7*	RPL5
18	M	No	0	7	7	Nad.	SpT	157	205*	-
19	M	Hypoplasia thenar eminence	2	121	119	NR	TD	749	3 <sup>^</sup> /0.56*	RPS19
20	M	Craniofacial dysmorphisms, cleft lip and palate, triphalangeal thumb, atrial septal defect, mental retardation	6	6	0	Nad.	ST	449	Unknown	RPL5
21	F	No	9	134	125	Response, stopped for side effects	TD	65	2.96*	RPS26
22	F	Craniofacial dysmorphisms	2	12	10	Nad.	SpT	93	4.57 <sup>^</sup>	-
23	M	No	2	12	10	Nad.	ST	275	Unknown	-
24	M	No	0	12	12	Nad.	ST	1508	7.5*	RPL11
25	M	Craniofacial dysmorphisms	4	10	6	Nad.	ST	225	2.94*	RPL35A
26	F	Craniofacial dysmorphisms	2	26	24	Nad.	SpT	81	1.68 <sup>^</sup>	RPS19

Notes: F: female, M: male, Nad.: not administered, NR: no response, ST: single transfusion, TD: transfusion dependent, SpT: sporadic transfusion, \*eADA: eADA determination after spontaneous remission, <sup>^</sup>eADA: eADA determination before spontaneous remission, -: mutation not found

Table III. DBA gene mutations

<u>Type of mutation</u>	<b>Number of cases</b>							
	<i>RPS17</i>	<i>RPS19</i>	<i>RPS24</i>	<i>RPS26</i>	<i>RPL5</i>	<i>RPL11</i>	<i>RPL35A</i>	<i>GATA1</i>
Complete/partial deletions and partial duplication	7	10	0	3	2	5	7	0
Small <u>insertions/deletions</u>	0	18	1	6	20	15	0	0
<u>Missense</u>	0	34	0	7	3	0	0	1
Nonsense	0	8	2	2	5	1	0	0
<u>Splice site</u>	1	11	0	5	4	5	1	0
<b>Total</b>	8	81	3	23	34	26	8	1

## **SUPPLEMENTARY MATERIAL**

### AIEOP DBA registry structure

CINECA IT infrastructure is certified for data quality procedures (ISO 27001:2013 certification, <https://www.cineca.it/en/content/certifications>) and through HTTP and SSL protocols encryption standards. The DBA-platform meets all the requirements set by the European General Data Protection Regulation. Through this approach, the entire data flow concerning clinical and molecular DBA data connects to existing patient demographic information and is based on a patient-centric approach (Fig1). Message alerts of the registration of a new patient guarantees a real-time traceable flow of information from all the local AIEOP centres to the referral one (Haematology Unit in Torino). The DBA Registry is organized into four different sections: the first includes data at diagnosis, the second covers information regarding the treatment and response to treatment, the third section consists of molecular results and the fourth concerns clinical evolution and follow-up. The number of mandatory parameters was limited to favour the filling-in of the form by the responsible physicians.

### Statistical analysis

The Welch's t-test was used for comparing eADA values between patients with a mutation in RPL or RPS genes. The significance of differences between the CI curves was estimated by doing a Gray's test. Statistical analysis was performed using NCSS (Hintze, 2001, NCSS PASS, Number Cruncher Statistical System, Kaysville, UT). The genotype-phenotype correlation was obtained by using logistic regression and odds ratio (OR) and 95% confidence interval (CI) was calculated. Data was analysed with the SAS ver. 8.01 or the Graphpad ver.5.0 software. P-Values less than 0.05 were considered statistically significant

### Ethical committee

This study was conducted according to the guidelines established by the Medical Ethics Committee of the "Città della Salute e della Scienza" University Hospital, Torino, Italy, that granted a waiver for the ethical review requirement. The guidelines were approved by the Medical Ethics Committee of each paediatric haematology unit involved in the Registry.