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RESEARCH ARTICLE

Genotype-phenotype associations in individuals with Diamond Blackfan anaemia

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Abstract

Introduction: Diamond Blackfan anaemia (DBA) is a rare disorder characterized by failure of red blood cell production, congenital abnormalities and cancer predisposition, primarily caused by pathogenic germline variants in genes encoding ribosomal proteins.

Methods: We conducted a genotype-phenotype and outcome study of 121 patients with DBA spanning the 20-year history of the National Cancer Institute's Inherited Bone Marrow Failure Syndromes study. Patient phenotypes were compared by large versus small ribosomal protein genes, across genes with >5 cases (*RPS19*, *RPS29*, *RPS26* and *RPL35A*) and by type of pathogenic variants (hypomorphic versus null, large deletions versus others).

Results: A pathogenic germline variant was identified in 71% of patients ($n = 86/121$) from 54 families. After adjusting for multiple testing, we found that patients with *RPS29* variants were least likely to need treatment for anaemia while those with large ribosomal protein subunit variants had a higher proportion of intellectual disability and gastrointestinal abnormalities compared with small ribosomal protein subunit variants ($p < 3.5 \times 10^{-4}$). There were no statistically significant differences in overall survival or cancer incidence among patients with large or small ribosomal subunit genes.

Conclusion: This detailed genotype-phenotype study of DBA improves our understanding of the role of germline genetics in the clinical manifestations that may help guide the management of people with DBA.

KEYWORDS
anaemia, Diamond Blackfan anaemia, genotype, phenotype, ribosomal protein

1 | INTRODUCTION

Diamond Blackfan anaemia (DBA) is a rare inherited bone marrow failure syndrome (IBMFS) characterized by a failure of red blood cell (RBC) production, congenital abnormalities, and cancer predisposition [1, 2]. Classic DBA consists of profound anaemia diagnosed below 1 year of age characterized by reticulocytopenia, macrocytosis, and paucity of erythroid precursors in the bone marrow [2, 3]. However,

not all patients present with typical DBA features, and some may be diagnosed later in childhood or as young adults with bone marrow failure. Congenital malformations are common but variable in severity including midline craniofacial defects, and cardiac, thumb and/or renal abnormalities; though approximately half of patients do not have a reported congenital malformation [1, 4–6]. Individuals with DBA are at a 4.8-fold increased risk of cancer compared with the general population [7], including myelodysplastic syndrome (MDS), acute myeloid leukaemia, colorectal adenocarcinoma and osteosarcoma [7–9].

Corticosteroid treatment for anaemia is associated with response in about 80% of patients with DBA, resulting in transfusion independence or decreased need for packed red blood cell (pRBC) transfusions. Gradual tapering of corticosteroids is successful in about 20% of patients with DBA and prolonged remission of anaemia is possible [4, 10]. However, about 40% of patients become steroid-resistant and require lifelong chronic pRBC transfusions with aggressive iron chelation, or haematopoietic cell transplant (HCT) [4].

DBA is predominately caused by heterozygous pathogenic or likely pathogenic (P/LP) variants in at least 25 different ribosomal proteins affecting the large (*RPL*) or small (*RPS*) subunits. P/LP variants in two X-linked genes, *TSR2* and *GATA1*, encoding a ribosome chaperone and haematopoietic transcription factor, respectively have also been reported [1, 11]. There are limited, relatively small, and inconsistent genotype-phenotype association studies based on specific DBA gene(s) or genes grouped by *RPL* and *RPS* subunits [1, 5, 6]. Here, we explored the genotype-phenotype and outcome associations in patients with DBA across the 20-year history of the IBMFS study at the National Cancer Institute.

2 | MATERIALS AND METHODS

2.1 | Study populations

This retrospective and prospective longitudinal observational cohort study was approved by the National Institutes of Health (NIH) Institutional Review Board (ClinicalTrials.gov Identifier: NCT00027274). Patients with DBA and their family members were enrolled in the National Cancer Institute (NCI) IBMFS cohort [12] between July 2002 and October 2021 and met well-established DBA diagnostic criteria based on genetic, clinical, and laboratory findings [2]. Briefly, this includes 1) "classical" DBA diagnosed by the presence of macrocytic anaemia below 1 year of age along with paucity of RBC precursors in the bone marrow; 2)"non-classical" DBA presenting with anaemia later in life; or 3) individuals without anaemia but meeting additional criteria including being a family member with the same gene variant as the proband identified [2]. The field cohort consists of all patients, or their proxies, who signed informed consent for participating in the research study and medical release forms for obtaining medical records. The clinic cohort consists of a subset of patients who underwent further detailed evaluation at the NIH Clinical Center, including physical examination, imaging, echocardiography, and subspeciality evaluations in addition to laboratory studies.

Detailed questionnaires were completed by all participants, including personal, medical, and family history. Medical records were reviewed by the study team members. All data were extracted and finalized as of February 15, 2023. A positive phenotypic finding was counted as present, and a clinical finding marked absent or not reported was considered absent. Standard criteria for defining cytopenias were used [13]. Definitions of haematological, physical abnormalities and other phenotypes studied are shown in Table S1. This includes remission, defined as physiologically stable and acceptable haemoglobin not requiring corticosteroids, transfusions, or other therapy. The classification of a patient as having MDS was based on medical record review.

2.2 | Genetic testing

Germline genetic variants were deemed P/LP based on clinical laboratory reports provided by genetic testing companies. For cases without a known P/LP variant and available DNA samples, we performed targeted genetic testing, whole exome sequencing, and/or array comparative genomic hybridization at the NCI's Cancer Genomics Research Laboratory to identify disease-causing variants as described (Figure 1A) [14, 15]. The details of the sequencing pipeline have been previously published and are based on the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) variant curation guidelines [16] and focus on a rare variant (minor allele frequency <1%) predicted to be P/LP [15, 17, 18].

2.3 | Statistical analysis

The Fisher's exact test was used for association analyses. We compared phenotypes by gene for those with P/LP variants in more than five cases (*RPS19*, *RPS29*, *RPS26*, and *RPL35A*) versus all other ribosomal genes (Figure 1A). We also compared cases with P/LP gene variants in *RPL* versus *RPS*, large deletion versus all other mutation types, and null versus hypomorphic mutation types. Null was defined as nonsense, frameshift insertion or deletion, and splice site variant. Hypomorphic was classified as in-frame insertion or deletion and missense. Seven cases were excluded from the congenital abnormality analysis due to insufficient data on physical phenotype (see grouping of congenital abnormality outlined in Table S1). A Bonferroni correction for 142 tests was applied, and p-values $<3.5 \times 10^{-4}$ were considered significant [19].

We used the Kaplan-Meier estimator to calculate probabilities of overall survival and a non-parametric estimator of the cumulative incidence function (Coviello and Boggess) to calculate the cause-specific cumulative incidence of cancers [20]. Age was the time scale. Follow-up started at birth and ended at the age of the outcome of interest

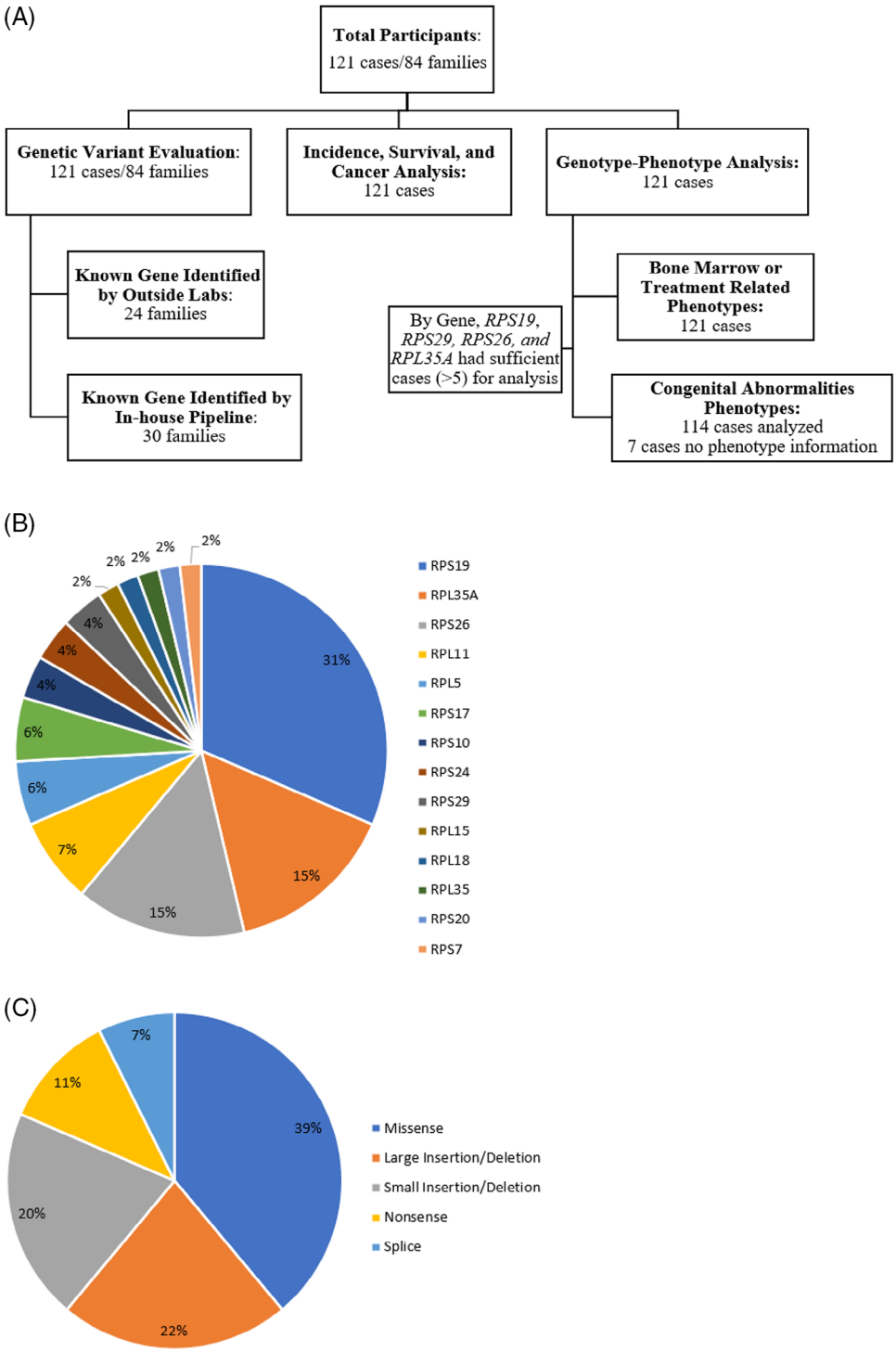

FIGURE 1 Summary of the study population and causative genes identified: (A) Study population; (B) Causative Gene by family; (C) Mutation type by family. (A) Distribution of patients with Diamond Blackfan anaemia by genetic variant evaluation and patients available for analyses. Seven cases were excluded from congenital abnormality analyses due to insufficient clinical data. (B, C) Germline pathogenic variants were only counted once per family. Per cent is based upon a total of 52 unique unrelated families.

(death or cancer). Participants were censored at the age of last contact or at the study endpoint (15 February 2023). For overall survival estimates, participants were also censored at the age of HCT, as this event changes the risk of death. Death from non-cancer causes was considered a competing event for cancer cumulative risk analysis [20]. We used STATA 17 software (StatCorp.) for the described analyses; $p < 0.05$ was considered statistically significant for overall survival and cumulative incidence.

3 | RESULTS

3.1 | Demographics

This study included 121 patients with DBA from 84 different families. The cohort was 46% male and predominately self-reported as non-Hispanic white (86%) or Hispanic (7%). The median age at DBA diagnosis was 0.25 years (range 0–65.1 years), at study enrollment was 14 years (range 0.43–85.9 years) and at last follow-up was 23.2 years (range 0.89–85.91 years). Forty-seven of the 121 patients (39%) were evaluated at the NIH Clinical Center as part of the clinic cohort.

3.2 | Genetic characterization

The germline genetic cause of DBA was identified in 64% of the families (54/84) and 71% of the study participants (86/121; Figure 1A). Of these 54 families, 67% (36/54) had a P/LP variant in an *RPS* gene and 33% (18/54) had a P/LP variant in an *RPL* gene. The most frequently affected genes were *RPS19* (n = 26/86 participants, 30%) followed by *RPS29* (n = 16/86, 19%), *RPS26* (n = 10/86, 12%) and *RPL35A* (n = 8/86, 9%). By family, the most frequently affected genes were *RPS19* (n = 17/54, 31%), followed by *RPS26* (n = 8/54, 15%), *RPL35A* (n = 8/54, 15%) and *RPL11* (n = 4/54, 7%; Figure 1B). The most common P/LP variant type was missense at 39% (21/54 families) followed by a large insertion/deletion at 22% (12/54 families) and small insertion/deletion at 20% (11/54 families) (Figure 1C and Table S2). Only four identical variants were detected in more than one unrelated family, including an *RPS26* variant (NM_001029.5: c.1A > G) that was present in four unrelated families (Table S2).

3.3 | Clinical phenotypes

Most patients had anaemia (90.9%; 110/121 individuals) at diagnosis and 86 of 121 (71.1%) required treatment at the last follow-up. Seventeen patients had mild anaemia not needing treatment and 18 patients had spontaneous remission (Table 1). Of those requiring treatment for anaemia, 33 were pRBC transfusion dependent, 35 were corticosteroid-responsive, 18 were treated with HCT, and 18 patients underwent spontaneous remission while receiving corticosteroids. A small proportion of cases (6%, 7/121) also had thrombocytopenia and 24% (29/121) had neutropenia. Erythrocyte adenosine deaminase (eADA) values were elevated in 80% (n = 60) of the 75 patients with data prior to pRBC transfusion. The proportion of cases with elevated eADA was highest in *RPS19* at 88% and lowest in *RPS29* at 60% (Figure S1).

Physical phenotype details were available for 114 patients, 60% (68/114) of whom had at least one congenital abnormality (Table 1). The most common abnormality was craniofacial in 31% of cases (35/114) and included 13 individuals with a cleft lip and/or palate. Thumb abnormalities were noted in 25% (28/114) and intellectual disabilities in 23% (26/114) of cases. This includes 17 patients diagnosed by their paediatricians with delays in speech, motor, or language milestones, four diagnosed with autism spectrum disorder, and three with difficulty in school requiring an Individualized Education Program (IEP).

3.4 | Genotype–phenotype relationships

When comparing DBA cases with and without an identified P/LP variant, a lower proportion of those with a P/LP variant required treatment for their anaemia (62.8%, 54/86 vs. 91.4%, 32/35, respectively; $p = 0.002$), a lower proportion were on corticosteroid treatment (19.8%, 17/86 vs. 51.4%, 18/35, p = 0.001), and a higher proportion achieved spontaneous remission of their anaemia (19.8%, 17/86 vs. 2.9%, 1/35, respectively, p = 0.022; Table 1). The prevalence of craniofacial abnormalities was higher in cases with an unknown gene at 47.1% (16/34) versus 23.8% (19/80) in cases with a known gene variant ($p = 0.025$; Table 1).

Phenotypes and treatment requirements varied based on the affected gene group (i.e. *RPL* vs. *RPS*) and mutation type (i.e. null vs. hypomorphic). Intellectual disability was more prevalent in cases carrying an *RPL* (55%, 11/20) variant compared with the *RPS* variant (12%, 7/60, p = 0.00020; Table 2 and Figure S2A). Of note, six of the 11 cases with *RPL* variants with intellectual disability had *RPL35A* variants. In addition, a higher proportion of cases with intellectual disabilities had large deletions (58%, 7/12) compared with all other variants (16%, 11/68, p = 0.004; Figure S2A). Individuals with an *RPL* P/LP variant had a higher proportion of gastrointestinal abnormalities (40%, 8/20) than patients with an *RPS* variant (3%, 2/60, p = 0.00014; Table 2 and Figure S2B). Gastrointestinal abnormalities in these cases with *RPL* and *RPS* variants included the following: one patient with ulcerative colitis, three with malabsorption, four with failure to thrive, one with colonic angiodysplasia, one with tracheoesophageal fistula, and two requiring gastrostomy tubes. These included three *RPL35A* cases and three *RPL5* cases. Patients with gastrointestinal abnormalities also had a higher proportion of null variants (24%, 8/34) compared with hypomorphic variants (4%, 2/46; $p = 0.015$), and a higher proportion had large deletions although this was not significant (25%, [3/12] vs. 10% [7/68]; $p = 0.168$; Figure S2B). For all patients with known P/LP variants, neutropenia was more likely to be associated with the presence of a null variant than with a hypomorphic variant (35%, [13/37] vs. 12%, [6/49]; $p = 0.017$) and the presence of a large deletion versus all other variants (67%, [8/12] vs. 15%, 11/74; $p = 0.0004$; Figure S2C).

By gene, fewer individuals with *RPS29* (19%, 3/16) were currently on treatment for anaemia compared with 73% (51/70) of patients with all other RP genes (p = 9.6E-05; Table 2, Figure S3A). Although not statistically significant, a higher proportion of patients with *RPS29* had mild anaemia that never required treatment and experienced spontaneous remission (Figure S3B,C). A significantly higher proportion of patients with *RPL35A* P/LP variants had neutropenia (100%, 8/8) compared with all other RP gene variants (14%, 11/78; p = 1.4E-06; Table 2, Figure S2C).

TABLE 1 Clinical phenotypes of patients with Diamond Blackfan anaemia (DBA) in the National Cancer Institute cohort.

	Total cases		Cases with known gene		Cases with unknown gene		
Bone marrow phenotypes[1]	***n* = 121**	**Per cent**	***n* = 86**	**Per cent**	***n* = 35**	**Per cent**	***p*-value**
Anemia	110	90.9%	76	88.4%	34	97.1%	0.174
Neutropenia	29	24.0%	19	22.1%	10	28.6%	0.485
Thrombocytopenia	7	5.8%	5	5.8%	2	5.7%	1
Treatment-related phenotypes	***n* = 121**	**Per cent**	***n* = 86**	**Per cent**	***n* = 35**	**Per cent**	***p*-value**
On treatment for anaemia	86	71.1%	54	62.8%	32	91.4%	0.002
On steroid treatment	35	28.9%	17	19.8%	18	51.4%	0.001
On chronic pRBC treatment	33	27.3%	22	25.6%	11	31.4%	0.509
HCT	18	14.9%	15	17.4%	3	8.6%	0.269
Spontaneous remission	18	14.9%	17	19.8%	1	2.9%	0.022
Anaemia not needing treatment	17	14.0%	15	17.4%	2	5.7%	0.147
Congenital abnormalities	***n* = 114**	**Per cent**	***n* = 80**	**Per cent**	***n* = 34**	**Per cent**	***p*-value**
Any congenital abnormality	68	59.6%	43	53.8%	25	73.5%	0.061
Craniofacial	35	30.7%	19	23.8%	16	47.1%	0.025
Cardiac	21	18.4%	13	16.3%	8	23.5%	0.430
Thumb	28	24.6%	20	25.0%	8	23.5%	1
Urogenital	15	13.2%	8	10.0%	7	20.6%	0.140
Intellectual disability	26	22.8%	18	22.5%	8	23.5%	1
Other	13	11.4%	6	7.5%	7	20.6%	0.057
Other abnormalities	***n* = 114**	**Per cent**	***n* = 80**	**Per cent**	***n* = 34**	**Per cent**	***p*-value**
Short stature[2]	51	44.7%	33	41.3%	18	52.9%	0.305
Chronic GI problems	17	14.9%	10	12.5%	7	20.6%	0.267

Abbreviations: GI, gastrointestinal; HCT, haematopoietic cell transplantation; RBC, red blood cell.

[1]Standard criteria for defining cytopenia were used (also outlined in Table S1): thrombocytopenia = platelet count <150, absolute neutrophil count (ANC) $<1.5 \times 10^9$/L, and anaemia = haemoglobin <2 standard deviations below the mean for the normal population based on age and sex.

[2] <10th percentile for height based on age and sex.

All cases meeting sufficient phenotypic information were included, seven cases were excluded for congenital abnormality analysis for insufficient exam records. A positive finding was counted as present, and a clinical finding marked absent or not stated was considered absent. *p*-Values were based upon Fisher's exact test comparing phenotypes of cases with and without a known pathogenic or likely pathogenic variant. Anaemia, thrombocytopenia, and neutropenia indicate any history of those cytopenias, while treatment status is the patient's current status. Congenital exam findings were grouped, and a list of specific abnormalities identified is outlined in Table S1.

TABLE 2 Association of phenotypic features of patients with Diamond Blackfan anaemia (DBA) by gene and ribosomal protein subtype.

Phenotype	Gene	Per cent of cases (counts)	Per cent of comparison cases (counts)	*p*-value
Requiring treatment	*RPS29*	19% (3/16)	73% (51/70)	9.6E-05
Neutropenia[1]	*RPL35A*	100% (8/8)	14% (11/78)	1.4E-06
Intellectual disability	*RPL*	55% (11/20)	12% (7/60)	0.00020
Chronic GI abnormality	*RPL*	40% (8/20)	3% (2/60)	0.00014

Abbreviation: GI, gastrointestinal.

[1]Standard criteria for defining neutropenia were used (also outlined in Table S1): absolute neutrophil count (ANC) $<1.5 \times 10^9$/L.

Only phenotypes with a *p*-value $<3.5 \times 10^{-4}$ after Bonferroni correction using Fisher's exact tests are included. Comparisons shown are for significant phenotypes by genes and by *RPL* versus *RPS* subtypes. Treatment for anaemia included steroids, chronic transfusion, and haematopoietic stem cell transplant (HCT). Congenital exam findings were grouped, and a list of specific abnormalities identified is outlined in Table S1.

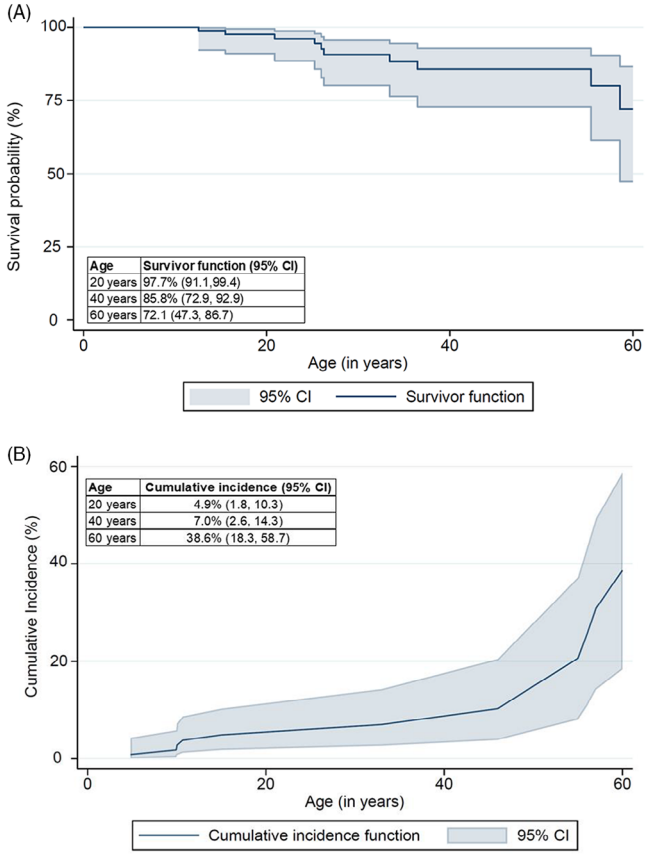

FIGURE 2 Summary of Diamond Blackfan anaemia (DBA) in the National Cancer Institute cohort by: (A) Overall survival among people with DBA; (B) Cumulative incidence of cancer estimates overall among people with DBA. (A) Kaplan-Meier was used to calculate probabilities of overall survival. Follow-up started at birth and ended at the age of death. Participants were also censored at the age of last contact, study endpoint (15 February 2023), or the age of haematopoietic cell transplant. (B) Non-parametric cumulative incidence function [20] was used to calculate the cumulative incidence of cancers. Death from non-cancer causes was considered a competing event for cancer cumulative risk analysis.

3.5 | Overall survival and malignancies

Vital status and cancer information were available on all 121 patients. The probability of overall survival was 97.7% at age 20 years (95% confidence interval [CI]: 91.1%, 99.4%), 85.8% at age 40 years (95% CI: 72.9%, 92.8%), and 72.1% at age 60 years (95% CI: 47.3%, 86.7%; Figure 2A). Nine of 64 patients (14.1%) with P/LP variants in the *RPS* gene died during follow-up compared with one of 22 patients (4.5%) with *RPL* gene variants, but this difference was not statistically significant ($p = 0.44$; data not shown).

About 14% of the cohort (17/121) developed a first malignancy (cancer or MDS) at a median age of 33 years (range 4.8–69.7 years, Table S3). One patient developed two cancers, lung cancer at age 46 and cervical cancer at age 52. The most common malignancy was colorectal cancer ($n = 4$). The specific types of lung and colon cancer were not reported, and neither was the human papillomavirus status of the cervical cancer. Three patients developed malignancies after HCT; one had post-transplant lymphoproliferative disease, another had rhabdomyosarcoma, and a third had osteosarcoma of the left radius. *RPS29* was the most common gene affected among patients who developed cancer ($n = 6$ with 5/6 patients coming from one family). P/LP variants in *RPS19* were the second most common, with four cancer cases from three different families. Five of the 14 patients who developed solid malignancies had either never needed treatment for anaemia or were in haematological remission prior to their cancer diagnoses. Of note, treatment for malignancy was associated with prolonged cytopenia in four patients who became pRBC transfusion-dependent for 2–10 years following treatment.

All three cases of MDS were diagnosed in patients in their twenties who were steroid-resistant and on chronic transfusions. Two were treated with HCT and one died from transplant-related complications. The third patient died from cardiac failure secondary to iron overload (Table S3).

The cumulative incidence of cancer was 4.9% by age 20 years (95% CI: 1.8%–10.3%), 7.0% by age 40 years (95% CI: 2.6%–14.3%), and 38.6% by age 60 years (95% CI: 18.3%–58.7%; Figure 2B). When comparing patients with a pathogenic variant in *RPL* versus *RPS* gene, 10 patients with *RPS* genes and three patients with *RPL* genes developed cancer, but there was no difference in the cumulative incidence of cancer between the two groups (hazard ratio [HR] = 1.35, 95% CI: 0.32, 5.7; $p = 0.68$).

4 | DISCUSSION

DBA is a complex IBMFS and cancer predisposition syndrome with variable clinical phenotypes and numerous germline genetic etiologies. In this study, 121 patients with DBA from 84 different families underwent comprehensive clinical phenotyping and genetic evaluation to determine whether certain genes or gene families were associated with phenotypic manifestations. The presence of germline genetic aetiology was associated with certain phenotypes, including age at diagnosis, clinical features, and treatment outcomes.

A P/LP variant in an *RPL* or *RPS* gene was identified in 71% of our patient cohort. This is consistent with other studies reporting 70 to 80% germline genetic aetiology of DBA [1, 5]. We did not have any cases with a non-*RPL*/*RPS* gene, such as *GATA1*. The RP gene distribution in our study was similar to previous publications, supporting that *RPS* was more common than *RPL* and *RPS19* was by far the most prevalent [1, 5]. Our pathogenic variant detection rate was on the lower end of the reported range, possibly due to enrollment bias of more gene unknown families, as our cohort included gene discovery in its aims. The clinical manifestations of our cohort included nearly 60% of cases with congenital malformations and 80% with elevated eADA, both consistent with prior reports [1, 5, 6]. The 22% (26/114 individuals) intellectual disability rate noted in our cohort is higher than the ~8% learning disabilities reported for children 3–17 years in the United States National Survey for Children's Health (21).

In this study, individuals with a clinical diagnosis of DBA (29%) but no identified genetic aetiology were more likely to require treatment for their anaemia, had lower rates of spontaneous remission, and higher rates of craniofacial abnormalities compared with the gene-identified DBA group. This raises the possibility of a non-RP gene or other genetic etiologies in some, manifesting as clinically severe DBA, or perhaps a selection bias of more severe undiagnosed cases.

Grouping patients by small or large ribosomal subunit genes found that *RPL* P/LP variants were more likely to be associated with intellectual disabilities and gastrointestinal abnormalities. Moreover, we identified a significant association between intellectual disability and *RPL*, with nearly all cases being *RPL35A*, and most of the *RPL35A* variants (seven out of eight) were large deletions. This association with *RPL35A* has been previously reported and is likely associated with large deletions, including the 3q29 deletion syndrome, which has overlapping symptoms and several other gene deletions in this region [17, 22]. We also identified *RPL* cases as associated with a higher proportion of chronic gastrointestinal problems, and most of the cases were either *RPL35A or RPL5*. Although not specific to gastrointestinal abnormalities, *RPL5* has been known to be associated with multiple malformations [1, 6, 23], and we have previously reported on the association of *RPL35A* large deletions and chronic gastrointestinal problems [17]. It is important to note that these findings of both intellectual disabilities and gastrointestinal abnormalities in patients with large deletions may be associated with other genes deleted in the region, and it's not clear how ribosome dysfunction contributes to these heterogeneous phenotypes.

To our knowledge, our study is the first to identify *RPS29* pathogenic variants with a milder anaemia phenotype compared with other RP gene cases. However, these findings are driven by 18 cases from two different families and need to be interpreted with caution due to small numbers and the potential for other unrecognized inherited or environmental aetiologies. We also identified a significant association between *RPL35A* and neutropenia. Most *RPL35A* cases with neutropenia had large deletions, suggesting that genes outside of the *RPL35A* region may be contributing to neutropenia, such as *RNF168* which underlines the immune deficiency Riddle Syndrome and was often co-deleted in our cases [24].

We explored whether the type of P/LP variant, regardless of the gene affected, was associated with a specific phenotype and whether individuals harbouring null variants had a higher proportion of neutropenia, intellectual disabilities, and chronic gastrointestinal abnormality. However, most of the null variants were in *RPL35A* and reflect our previously described findings of *RPL35A* with neutropenia and chronic gastrointestinal abnormalities. No other variant type grouping and phenotype had p-values <0.05.

This study confirmed the increased risk of cancers in DBA, including colorectal cancers and osteosarcoma. We also observed cancers not typically associated with DBA, such as testicular seminoma. Many of our patients were relatively young at the age of cancer diagnosis (median age 33 years) and many were asymptomatic from an anaemia perspective prior to their cancer diagnosis, such as two patients who had colon cancer (NCI-50-6 and NCI-578-1). This underscores the importance of cancer surveillance even in young adults and in those with relatively few DBA symptoms. Colorectal cancer screening beginning at the age of 20 years is now recommended for individuals with DBA by the Diamond Blackfan Anaemia Registry [9].

This longitudinal cohort study of DBA systematically evaluated the association of phenotype with genotype in this complex disorder. Although our study is limited by statistical power, and we cannot make definitive treatment recommendations from these data, the presence of clinically significant differences by gene, gene grouping, and underlying pathogenic variants, may help guide management in DBA. Future studies are needed to validate the observations from this study.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study. D. Matthew Gianferante and Sarah Cole extracted clinical charts. D. Matthew Gianferante, Kyra J. W. Mendez, Neelam Giri and Shahinaz M. Gadalla analyzed the data. D. Matthew Gianferante, Kyra J. W. Mendez, Neelam Giri and Sharon A. Savage wrote the paper. All authors revised and approved the final version of the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The genetic data for this project are publicly available on the database of Genotypes and Phenotypes (dbGaP), dbGaP Study Accession: phs001481.v1.p1.

ETHICS STATEMENT

This retrospective and prospective longitudinal observational cohort study was approved by the National Institutes of Health (NIH) Institutional Review Board (ClinicalTrials.gov Identifier: NCT00027274).

PATIENT CONSENT STATEMENT

Informed consent was obtained from all participants included in the study in accordance with Health and Human Services regulation 45 CFR 46.

CLINICAL TRIAL REGISTRATION

ClinicalTrials.gov Identifier: NCT00027274.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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