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The Increased Prevalence of Congenital Heart Disease (CHD) in Children with Diamond Blackfan Anemia (DBA) Suggests Unrecognized DBA as a Cause of CHD in the General Population: A Report of the Diamond Blackfan Anemia Registry

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Congenital heart disease (CHD) is one of the most commonly occurring congenital anomalies in the general population. In patients with Diamond Blackfan anemia (DBA), a rare inherited bone marrow failure syndrome, CHD represents ~30% of all congenital anomalies[1]. Affected individuals within multiplex families may have hematologic manifestations with or without CHD or have CHD alone. To support a link between these two conditions, we hypothesized that since CHD is common in the DBAR cohort, there are patients with occult DBA in the general CHD population. This study could reveal new knowledge regarding the etiology of non-syndromic CHD.

DBA, characterized by hypoproliferative, pro-apoptotic erythropoiesis and red cell failure, birth defects, growth failure, and cancer predisposition, presents with hypoplastic anemia; median age 2 months. Inactivating mutations in large or small subunit-associated ribosomal protein (RP) genes are found in 65-70% of cases. RP-associated DBA has autosomal dominant inheritance with variable penetrance or presents as sporadic new dominant mutations. A few cases are not RP-associated[1, 2]. The Diamond Blackfan Anemia Registry (DBAR) of North America, established in 1991, captures patients in a nonbiased fashion[1]. In the DBAR (N=744), 111 patients have CHD, representing a significantly greater prevalence of CHD in DBA patients (N=111/744, prevalence 1491.9/10,000; 14.9%) compared to the general population[3] (N=3,240/398,140; prevalence 81.4/10,000, <1%; p<0.0001[Chi-squared]). The relative distribution of CHD in DBA is similar to the general population (Figure).

To determine the prevalence of occult DBA presenting as CHD we evaluated 102 unselected patients with CHD followed by Pediatric Cardiology (after IRB-approved informed consent). Inclusion criteria were: age > 6 months (75% of DBA patients present prior to 6 months of age), no known syndrome associated with CHD, and no history of red cell transfusion in the past 120 days. Erythrocyte adenosine deaminase activity [eADA; sensitive (84%), highly specific (95%) and stable marker for DBA throughout life][4] and complete blood count (CBC) were determined on each patient. Five patients, ages 6 months to 4 years, with a vascular ring, Tetralogy of Fallot, transposition of great vessels, and 2 with complete atrioventricular canal (AVC) with double outlet right ventricle (DORV), respectively, were found to have elevated eADA activity (range, 1.01 – 1.35 EU/gm Hb; normal, 0.33-0.96) characteristic of DBA[4]. None of the patients had macrocytosis, a classical finding; one patient had mild anemia. Genetic analysis revealed one patient to be heterozygous for a mutation in *RPS24*, c.91G>A (p.Gly31Arg), a variant of unknown significance. The glycine residue at position 31 adjacent to invariant positions of *RPS24* is relatively conserved across

species. This variant was described likely pathogenic by prediction tools. Ribosomal RNA processing by Northern blot analysis was characteristic of the *RPS24* mutation (Figure).

The patient was born at term with complex CHD; DORV, complete AVC, and pulmonary stenosis with heterotaxy syndrome (asplenia, levocardia, juxtaposition of the aorta and inferior vena cava, right-sided stomach and intestinal malrotation). CBC at 15 months of age revealed hemoglobin 17.5 gm/dL, hematocrit 51.3%, MCV 87.3 fL and reticulocyte count 1.8%; at age 3.5 years he remains hematologically normal. Genetic testing revealed the same *RPS24* mutation in the father who has an essentially normal CBC and no CHD.

The analysis was extended to patients within the Italian DBA Registry where one patient had an AVC defect and a mutation in *RPS24*, c.64C>T (p.Gln22Ter). This variant was inherited by this patient's son who also had an AVC defect, elevated eADA activity and abnormal rRNA processing (Figure) but no overt hematological abnormalities.

In summary CHD is significantly more prevalent among those with DBA than in the general population, and subclinical blood abnormalities characteristic of variably penetrant DBA may be detectable among those patients with CHD, supporting that they are linked. The distribution of CHD in patients with DBA appears to be similar to the general population. We identify two individuals from unrelated families with mutations in *RPS24* for whom CHD is the only clinical manifestation of DBA. Both have mutations suggestive of loss of function with incomplete penetrance in both the hematological and CHD phenotypes.

Elevated eADA activity in unselected patients with CHD was noted in 5/102 patients with one confirmed to have "occult" DBA caused by a loss-of-function *RPS24* mutation. Further analysis of other CHD cohorts will be necessary to estimate the prevalence of DBA in the general CHD population as the variable penetrance of the DBA phenotype can be associated with the occurrence of congenital anomalies without the classic hematologic findings.

Changes in the dosage of genes that transcriptionally regulate cardiogenesis have been proposed to lead to CHD[5]. The similarity in distribution of CHD in both the DBAR and general population suggests that disrupted translation caused by RP haploinsufficiency in DBA, similarly reducing gene dosage, may be an unrecognized cause of CHD. Studies to detect loss-of-function germline mutations in RP genes in patients with CHD are likely to reveal additional patients similar to those described herein.

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References

1. Vlachos A, Blanc L, Lipton JM. Diamond Blackfan anemia: a model for the translational approach to understanding human disease. Expert Rev Hematol. 2014; 7:359–72. [PubMed: 24665981]
2. Lipton JM. Inherited thrombocytopenia and Occam's razor. Blood. 2017; 130:839–840. [PubMed: 28818975]
3. Reller MD, Strickland MJ, Riehle-Colarusso T, Mahle WT, Correa A. Prevalence of congenital heart defects in metropolitan Atlanta, 1998-2005. J Pediatr. 2008; 153:807–13. [PubMed: 18657826]
4. Fargo JH, Kratz CP, Giri N, Savage SA, Wong C, Backer K, et al. Erythrocyte adenosine deaminase: diagnostic value for Diamond-Blackfan anaemia. Br J Haematol. 2013; 160:547–54. [PubMed: 23252420]
5. Fahed AC, Gelb BD, Seidman JG, Seidman CE. Genetics of congenital heart disease: the glass half empty. Circ Res. 2013; 112:707–20. [PubMed: 23410880]

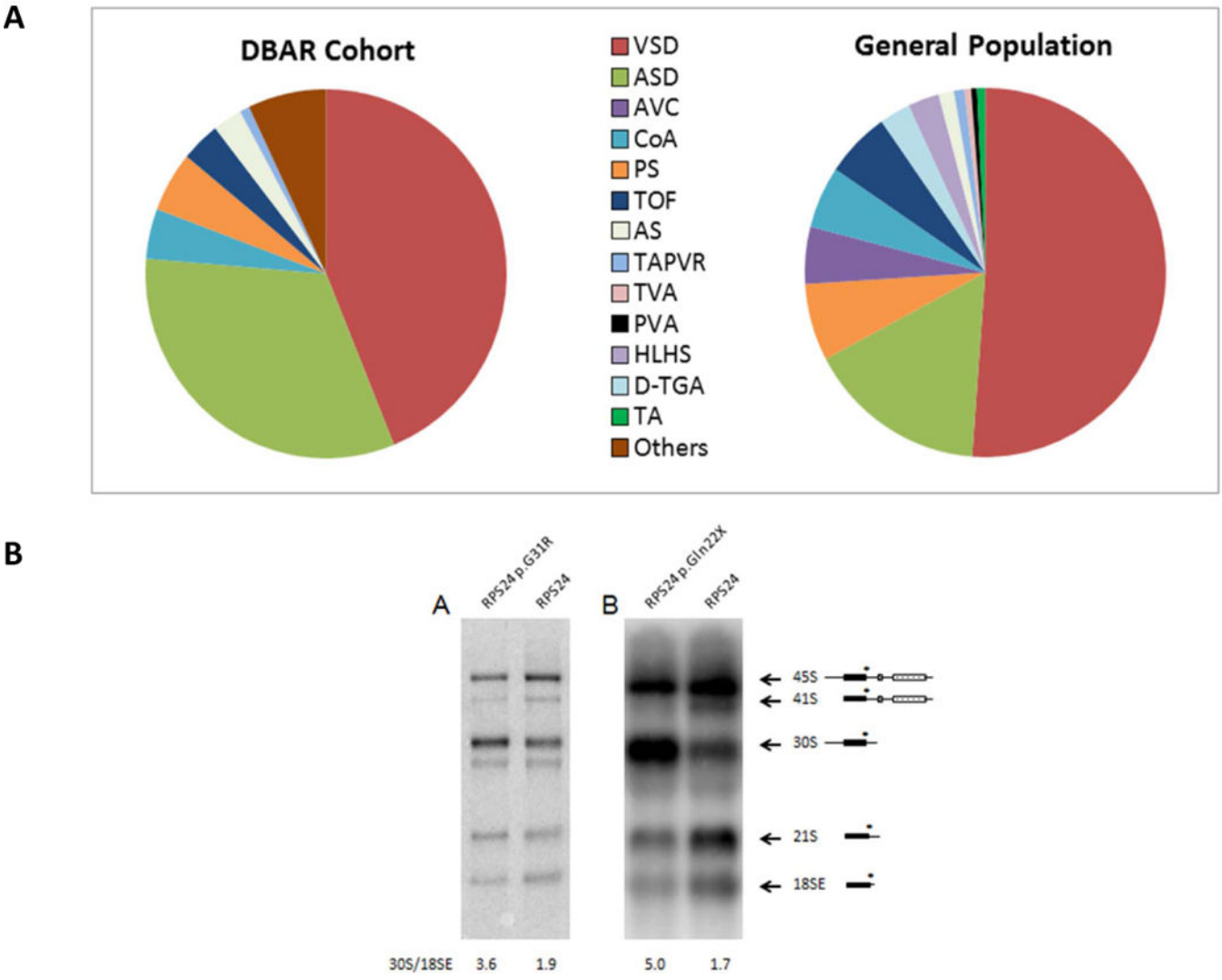

Figure. Congenital heart disease in Diamond Blackfan anemia (DBA)

(A) The CHD anomalies in the DBAR included: ventricular septal defect (VSD; 44%), atrial septal defect (ASD; 32.5%), coarctation of the aorta (CoA; 4.4%), pulmonic valve stenosis (PS; 5.3%), Tetralogy of Fallot (TOF; 3.5%), aortic valve stenosis (AS; 2.6%), total anomalous pulmonary venous return (TAPVR; 0.9%) and others (7%). Patent ductus arteriosus and patent foramen ovale were not included. The defects were similar in relative frequency to those found in the general CHD population[6], with VSD, ASD, CoA, PS, and TOF being the five most common CHD diagnoses in the DBAR but with the exception that there were no cases of tricuspid atresia (TVA), pulmonary atresia (PVA), hypoplastic left heart syndrome (HLHS), dextro-transposition of the great arteries (D-TGA), truncus arteriosus (TA) or atrioventricular canal (AVC). Due to the limited size, it is difficult to ascertain if any of the diagnoses not found are truly underrepresented in the DBAR. However restricting the analysis to age ≥ 6 months likely precludes representative enrollment of patients with severe fatal lesions. The absence of AVC defects in the DBAR cohort can be explained by the fact that most AVC defects in the general population are

usually seen in relation to syndromes, in particular Down syndrome. **(B)** Northern blot analysis of pre-rRNA processing was performed on total RNA isolated from primary peripheral blood mononuclear cells (left panel) on the DBAR patient and immortalized lymphoblastoid cells lines (right panel) from the Italian patient, and healthy controls, respectively. Protein translations of mutational genotypes for index patients are shown above appropriate lanes. The membranes were interrogated with a probe against the 5′ end of ITS1 represented as the asterisk above the schematic views of pre-rRNA species shown to the right. The values shown below each lane represent the ratio of phosphorimage units derived for 30S and 18SE species in each lane demonstrate increased 30S pre-rRNA and diminished 18SE, consistent with a pre-rRNA processing defect reported in patients with *RPS24* mutations.