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RESEARCH LETTER

Increased Prevalence of Congenital Heart Disease in Children With Diamond Blackfan Anemia Suggests Unrecognized Diamond Blackfan Anemia as a Cause of Congenital Heart Disease 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 2 conditions, we hypothesized that because CHD is common in the Diamond Blackfan Anemia Registry (DBAR) cohort, there are patients with occult DBA in the general CHD population. This study could reveal new knowledge regarding the pathogenesis of nonsyndromic CHD.

DBA, characterized by hypoproliferative, proapoptotic 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 RP (ribosomal protein) genes are found in 65% to 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 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 patients with DBA (n=111 of 744; prevalence, 1491.9/10 000; 14.9%) compared with the general population[3] (n=3240 of 398 140; prevalence, 81.4/10 000; <1%; $P<0.0001$ [χ^2]). 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 patients with DBA present before 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 (sensitive [84%], highly specific [95%], and stable marker for DBA throughout life)[4] and complete blood count were determined on each patient. Five patients, aged 6 months to 4 years, with a vascular ring, tetralogy of Fallot, and transposition of great vessels and 2 with complete atrioventricular canal with double outlet right ventricle, respectively, were found to have elevated erythrocyte adenosine deaminase 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; 1 patient had mild anemia. Genetic analysis revealed 1 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).

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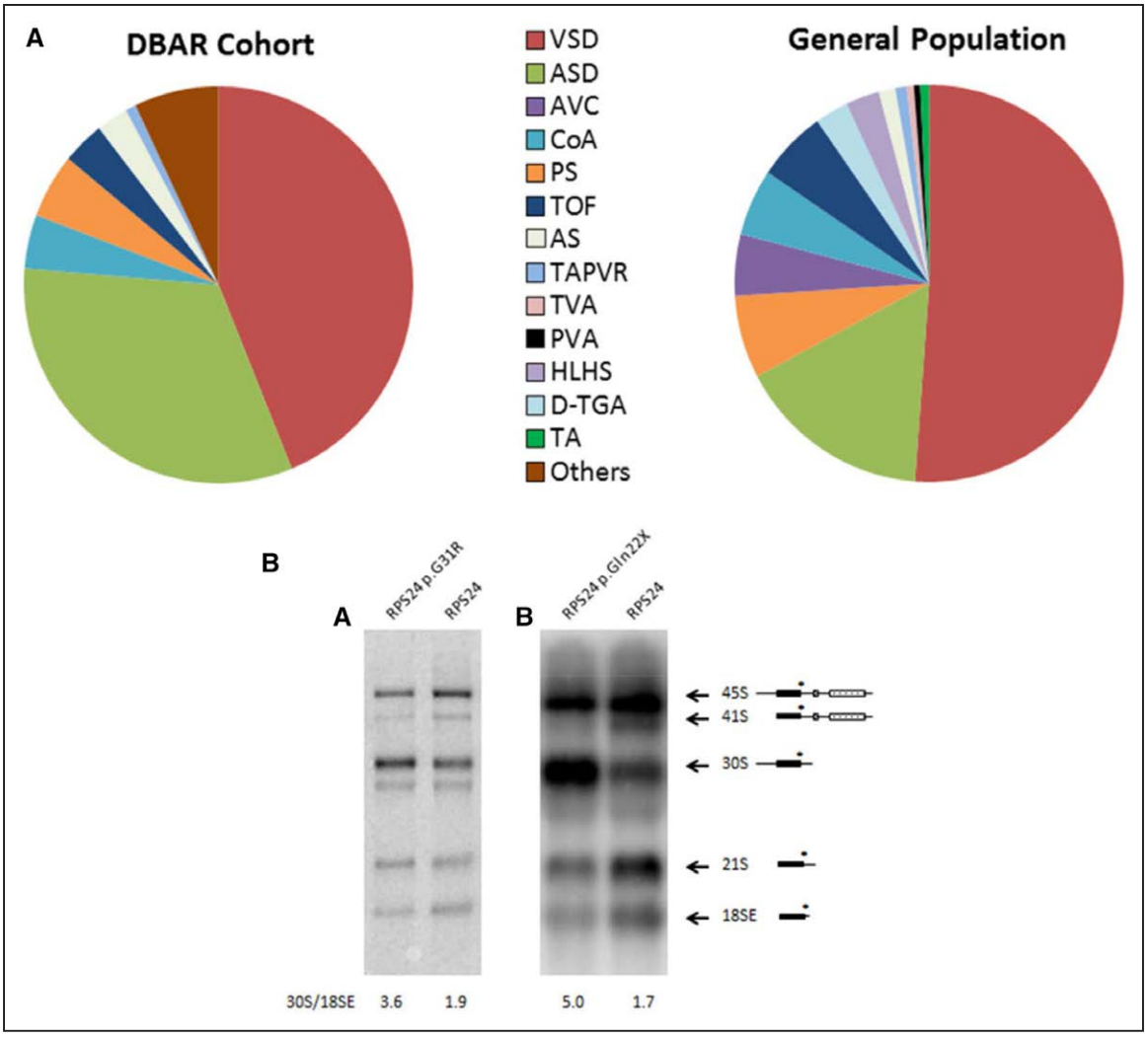

Figure. Congenital heart disease (CHD) in Diamond Blackfan anemia (DBA).
A, The CHD anomalies in the Diamond Blackfan Anemia Registry (DBAR) included ventricular septal defect (VSD; 44%), atrial septal defect (ASD; 32.5%), coarctation of the aorta (CoA; 4.4%), pulmonic valve stenosis (PS; 5.3%), tetralogy 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,[3] with VSD, ASD, CoA, PS, and TOF being the 5 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). Because of the limited size, it is difficult to ascertain whether any of the diagnoses not found are truly underrepresented in the DBAR. However, restricting the analysis to age ≥6 mo 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**) on the DBAR patient and immortalized lymphoblastoid cells lines (**right**) 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 internal spacer region 1 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.

The patient was born at term with complex CHD; double outlet right ventricle, complete atrioventricular canal, and pulmonary stenosis with heterotaxy syndrome (asplenia, levocardia, juxtaposition of the aorta and inferior vena cava, right-sided stomach, and intestinal malrotation). Complete blood count at

15 months of age revealed hemoglobin, 17.5 g/dL; hematocrit, 51.3%; mean corpuscular volume, 87.3 fL; and reticulocyte count, 1.8%, at 3.5 years of age, he remains hematologically normal. Genetic testing revealed the same *RPS24* mutation in the father who has an essentially normal complete blood count and no CHD.

The analysis was extended to patients within the Italian Italian DBA registry where 1 patient had an atrioventricular canal defect and a mutation in *RPS24*, c.64C>T (p.Gln22Ter). This variant was inherited by this patient's son who also had an atrioventricular canal defect, elevated erythrocyte adenosine deaminase activity, and abnormal rRNA processing (Figure) but no overt hematologic 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 seems to be similar to the general population. We identify 2 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 hematologic and CHD phenotypes.

Elevated erythrocyte adenosine deaminase activity in unselected patients with CHD was noted in 5 of 102 patients with 1 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 because 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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Disclosures

None.

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