THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Diamond-Blackfan Anemia

Clinical Features:
Diamond-Blackfan anemia (DBA) is an inherited red blood cell aplasia disorder associated with reduced or absent erythroid precursors in bone marrow, macrocytic anemia and reticulocytopenia [1]. Approximately 30% of cases have growth retardation and 50% have congenital anomalies, which may include thumb anomalies, congenital heart defects and midline facial defects such as cleft palate and hypertelorism [1]. Patients have an increased risk of malignancies, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and solid tumors such as osteogenic sarcoma [1]. The cumulative incidence of solid tumors or leukemia is 22% by age 46 [2]. DBA is a genetically heterogeneous condition, with the currently known genes accounting for 50-70% of cases [1]. All the DBA genes included on this panel are inherited in an autosomal dominant manner. An estimated 55-60% of cases are caused by *de novo* mutations; DBA has variable expressivity and penetrance is incomplete.

Our Diamond-Blackfan Anemia Panel includes sequence and deletion/duplication analysis of the 11 genes listed below.

Diamond-Blackfan Anemia Sequencing Panel					
GATA1	RPL5	RPL11	RPL15	RPL26	RPL35A
RPS7	RPS10	RPS19	RPS24	RPS26	

Gene	Clinical Features
GATA1	Patients with inherited thrombocytopenia in a concurrent hemolytic anemia should raise the suspicion of thrombocytopenia caused by *GATA1* mutations or sitosterolemia [3]. Recent exome sequencing has identified a novel splice site mutation in *GATA1* in two siblings with DBA [4]
RPL5	DBA type 6, caused by heterozygous mutations in *RPL5*, is typically associated with multiple physical anomalies, including craniofacial, thumb and cardiac anomalies [5].
RPL11	Heterozygous mutations in *RPL11* are associated with DBA type 7. In terms of observed congenital malformations, mutations in *RPL11* are predominantly associated with isolated thumb defects [5].
RPL15	Deletions of *RPL15* have been identified in patients with Diamond-Blackfan anemia recently [6, 7].
RPL26	Gazda HT *et al.* (2012) identified a frameshift mutation in p53 regulator *RPL26* that is associated with multiple physical abnormalities and a specific pre-ribosomal RNA processing defect in diamond-blackfan anemia[8].
RPL35A	Mutations in *RPL35A* have been identified in both familial and sporadic cases of DBA type 5. In one familial case, some individuals were found to have subclinical DBA with macrocytic anemia [9].
RPS7	*RPS7* has been associated with DBA type 8 [10]. At least one individual with no associated physical anomalies has been described [5].
RPS10	*RPS10* mutations are associated with DBA type 9, and are estimated to account for 2.6% of all DBA cases [11].
RPS19	Mutations in the *RPS19* gene account for an estimated 24% of all DBA cases overall [12].
RPS24	*RPS24* mutations are associated with DBA type 3, and account for an estimated 2% of DBA cases [13]. Both sporadic and familial mutations have been described [13].
RPS26	Mutations in *RPS26* are associated with DBA type 10, and account for an estimated 6.4% of DBA cases overall. Based on available data from a limited number of cases, physical malformations appear to be rare in patients with *RPS26* mutations [11].

Test methods:
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the

coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Diamond-Blackfan Anemia Panel

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube. NOTE: blood samples are not accepted if patient has a history of MDS or leukemia. Please send 2 T-25 flasks of cultured skin fibroblasts instead.
Cost:	$3,000
CPT codes:	81406, 81407
Turn-around time:	6 weeks

Note: We cannot bill insurance for the Diamond-Blackfan Anemia panel

Results:

Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire Diamond-Blackfan Anemia Sequencing Panel. All abnormal results are reported by telephone or email.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

1. Wilson, D.B., et al., *Inherited bone marrow failure syndromes in adolescents and young adults.* Ann Med, 2014: p. 1-11.
2. Vlachos, A., et al., *Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry.* Blood, 2012. **119**(16): p. 3815-9.
3. Pecci, A., *Diagnosis and treatment of inherited thrombocytopenias.* Clin Genet, 2016. **89**(2): p. 141-53.
4. Sankaran, V.G., et al., *Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia.* J Clin Invest, 2012. **122**(7): p. 2439-43.
5. Gazda, H.T., et al., *Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients.* Am J Hum Genet, 2008. **83**(6): p. 769-80.
6. Landowski, M., et al., *Novel deletion of RPL15 identified by array-comparative genomic hybridization in Diamond-Blackfan anemia.* Hum Genet, 2013. **132**(11): p. 1265-74.
7. Farrar, J.E., et al., *Exploiting pre-rRNA processing in Diamond Blackfan anemia gene discovery and diagnosis.* Am J Hematol, 2014. **89**(10): p. 985-91.
8. Gazda, H.T., et al., *Frameshift mutation in p53 regulator RPL26 is associated with multiple physical abnormalities and a specific pre-ribosomal RNA processing defect in diamond-blackfan anemia.* Hum Mutat, 2012. **33**(7): p. 1037-44.
9. Farrar, J.E., et al., *Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia.* Blood, 2008. **112**(5): p. 1582-92.
10. Gerrard, G., et al., *Target enrichment and high-throughput sequencing of 80 ribosomal protein genes to identify mutations associated with Diamond-Blackfan anaemia.* Br J Haematol, 2013. **162**(4): p. 530-6.
11. Doherty, L., et al., *Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia.* Am J Hum Genet, 2010. **86**(2): p. 222-8.
12. Willig, T.N., et al., *Mutations in ribosomal protein S19 gene and diamond blackfan anemia: wide variations in phenotypic expression.* Blood, 1999. **94**(12): p. 4294-306.
13. Gazda, H.T., et al., *Ribosomal protein S24 gene is mutated in Diamond-Blackfan anemia.* Am J Hum Genet, 2006. **79**(6): p. 1110-8.

Committed to CUSTOMIZED DIAGNOSTICS, TRANSLATIONAL RESEARCH & YOUR PATIENTS' NEEDS