The Yale Blood Disease Reference Laboratory Program (BDRLP) has been established as a national reference laboratory under Yale's CLIA certified Molecular Diagnostic Pathology Laboratory. The function of the BDRLP is to provide a resource to physicians and their patients for the diagnosis of complex hereditary intrinsic red cell disorders, particularly those involving defects in the cell's plasma membrane.

The plasma membrane provides structural support for the anucleate erythrocyte, accounting for its antigenic, transport, and mechanical characteristics. Inherited red cell disorders with altered membrane and cell function can be broadly divided into two categories: 1) altered function due to mutations in various membrane, skeletal, or metabolic proteins, such conditions include hereditary spherocytosis (HS), hereditary elliptocytosis (HE), hereditary stomatocytosis, and hereditary hemolytic anemia; and 2) altered function due to secondary effects on the membrane resulting from mutations in globin genes; these conditions include sickle cell disease, Hb SC disease, Hb C disease, unstable hemoglobin, and thalassemias. As a result of natural selection driven by severe forms of malaria and other diseases affecting the red cell, 1 in 6 individuals in the world – more than 1 billion people – are affected by red cell abnormalities. Such diseases are thus the most common of all inherited disorders.

Typically, genomic DNA extracted from a patient's blood is subjected to target exon capture, followed by high-throughput next generation genomic sequencing (NGS) using the third generation Ion Torrent platform (NGS). Analysis of the resulting sequencing data against various genetic databases as well as comparison with detailed knowledge of erythrocyte biology and physiology is used to determine the etiology of a patient's defective red cells.

Bioinformatics Variant Annotation Pipeline

- Scanning by Next Generation Sequencing

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The Yale Blood Disease Reference Laboratory Programs: Rapid Mutation Scanning by Next Generation Sequencing

Pei Hui, MD, PhD, Vincent Shultz, PhD, Michael Krauthammer, PhD, Matthew Holford, BA, Suping Chen, MS, Teresa Silva, MS, Patrick Gallagher, MD and Jon Morrow, MD, PhD

Departments of Pathology and Pediatrics, Yale School of Medicine

Clinical Operation Period: 08-2011 to 05-2012
• 20 patients examined (clinically 18 HS and 2 HE)
• Ages 5 months to 61 years
• No variation of SPTA1, SPTB, ANK and SLC4A1 found in 9 patients (45%)
• 11 patients with causal mutations involving SPTA1, SPTB and ANK1 (55%)
  - Five novel alpha spectrin variants likely pathologic
  - Four novel beta spectrin variants, likely pathologic
  - One novel variant found in ANK1, likely pathologic
  - All clinically relevant mutations are heterozygous
  - No pathologic variants found in SLC4A1 (band 3)

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**KEY POINTS**
- Established and fully operational Clinical Reference Laboratory for the analysis of rare non-malignant blood cell disorders
- Targeted NGS to identify variations in red cell proteins
- Exploring informatics tools to speed/deliver interpretations
- Rapid turn-around with very high sensitivity
- High yield of informative variations in samples examined
- Currently BCDRL analyzes patient samples from the United States, Canada and European Countries.