



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

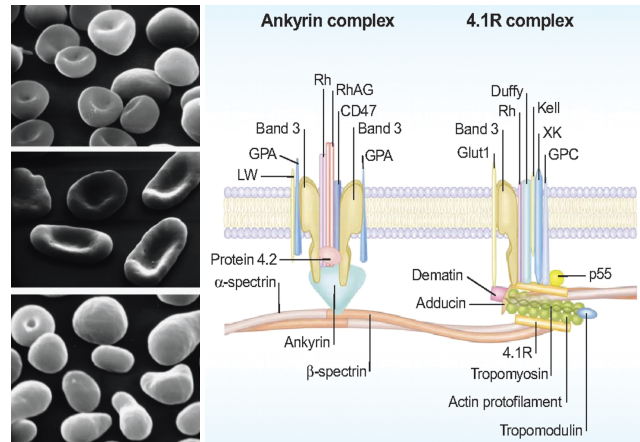


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INTRODUCTION

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 ovalocytosis, and hereditary stomatocytosis; 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 CC disease, unstable hemoglobins, 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.



Left panel: Hereditary spherocytosis (HS) top panel; nonhemolytic hereditary elliptocytosis (HE), middle panel (elliptocytes, poikilocytes, and fragmented red cells in hemolytic HE (bottom panel) (Reprinted with permission from Mohandas & Gallaher, 2008). **Right panel:** The membrane is a composite structure in which a plasma membrane envelope composed of amphiphilic lipid molecules is anchored to a 2-dimensional elastic network of skeletal proteins through tethering sites (transmembrane proteins) embedded in the lipid bilayer.

The Yale BDRLP laboratory is currently focused on the former category of disorders, i.e., those involving the red cell's membrane, cytoskeletal, and metabolic proteins. This information allows physicians to expertly handle their patients and to select the most appropriate therapies. Beyond the analysis of membrane proteins, the BDRLP will offer in the near future diagnostic studies for other inherited red cell disorders, including abnormalities of metabolism (e.g. glycolytic and anticoincitant enzyme defects), congenital dyserythropoietic anemias, and disorders associated with familial erythrocytosis.

METHODS

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.

Ion Torrent Sequencer

Next Gen sequencing for tumor profiling: advantages - extent and depth of coverage can identify mutations, gene fusions, amplification, CNV can analyze selected genes or whole "exome" can analyze expression (RT-PCR), methylation disadvantages - cost, turnaround time, development (working out bugs) too much information!

Suping Chen and Teresa Silva

Fast Direct Detection

DNA → Ions → Sequence

- Nucleotides flow sequentially over Ion semiconductor chip
- One sensor per well per sequencing reaction
- Direct detection of natural DNA extension
- Millions of sequencing reactions per chip
- Fast cycle time, real time detection

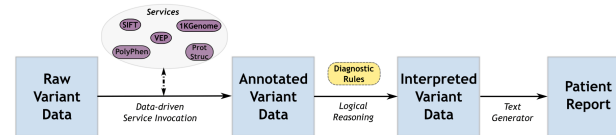
Sensing Layer, Sensor Plate, Bulk, Drain, Source, Silicon Substrate, To column receiver

Red Cell Disorders

Hereditary Spherocytosis
Hereditary Elliptocytosis
Hereditary Pyropoikilocytosis
Hereditary Stomatocytosis
CDA type I
CDA type II/HEMPAS
CDA unspecified
Familial Erythrocytosis

Gene Sequenced by NGS

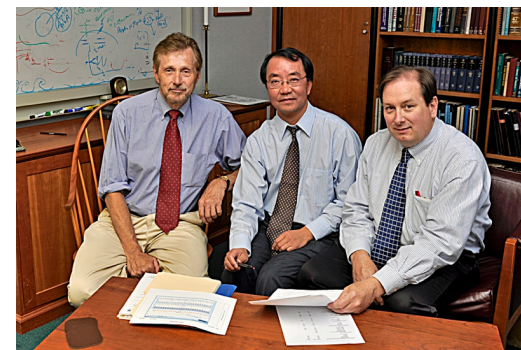
SPTA, SPTB, ANK1, SLC4A1 (band-3)
SPTA, SPTB
SPTA, SPTB
SLC4A1
CDAN1
SEC23
KLF1
Alpha and Beta globins



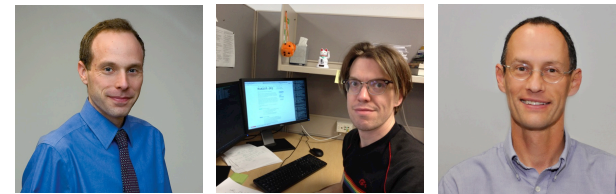
Bioinformatics Variant Annotation Pipeline

Raw patient variant data from the Ion Torrent sequencer is annotated using diverse data sources such as the 1000 Genome Project (common polymorphisms), and is in-silico inspected for deleterious variants using tools such as SIFT or PolyPhen. Annotated variants are then subjected to a set of in-house diagnostic rules, and the resulting interpretation are transformed into a physician-friendly text report.

TEAM MEMBERS



Jon Morrow, Pei Hui, and Patrick Gallagher



Michael Krauthammer

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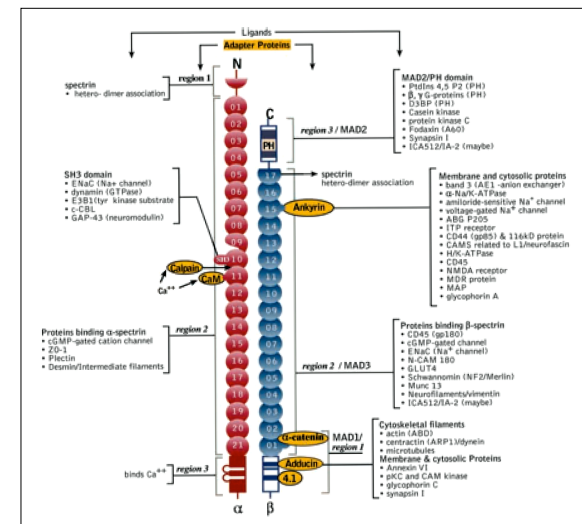
Vincent Schultz

CLINICAL OPERATION SUMMARY

Clinical Dx	Age	Sample	SPTA	SPTB	ANKYRIN	SLC4A1	Comments
7HS	47 yrs	Blood	No	Exon 13C - R827Q (Heterozygous)	No	No	Unreported mutation. It involves the repeat areas. Recommended testing for BIC encephalopathy and unstable hemoglobinopathy
HS	4 yrs	Blood	No	No	No	No	
7HS	8 months	Blood	Exon 4 - 154In(L155 (Heterozygous)	Exon 11-R508H (Heterozygous)	n/a	n/a	The insertion is seen also in patient's mother and is a common mutation in African. R508H is also seen in patient's father
7HS	6 months	Blood	Exon 4 - 154In(L155 (Heterozygous)	No	n/a	n/a	The insertion is seen also in patient's mother and is a common mutation in African patient.
7HS	44 yrs	Blood	No	Exon 10-E435Q (Heterozygous)	No	No	Novel, likely causal mutation
7HS	1 yr	Blood	Exon 24 - N1130S (Heterozygous)	No	n/a	n/a	A novel variant, located at conserved region, likely causal
7HE	6 yrs	Blood	ND	No	No	No	One common variant
Rule out HS	5 yrs	DNA	No	No	No	No	Based on clinics, mutation analysis of PK gene is recommended
7HE	11 months	Blood	Exon 6 - L260P (Heterozygous)	No	n/a	n/a	L260P of SPTA is a reported causal mutation of hereditary elliptocytosis
HS	5 yrs	Blood	Exon 29 - A1347T (Heterozygous)	Exon16-R1227S and Exon23-R1625X (Heterozygous)	No	No	The 2 novel SPTB mutations are causal. Novel SPTA A1347T is uncertain.
7HS	49 yrs	Blood	No	No	No	No	Refer patient to Dr. Gallagher for clinical consultation
5HS	53 yrs	blood	n/a	n/a	n/a	No	
5HS	53 yrs	blood	No	No	No	No	
5HS	47 yrs	blood	No	No	No	No	
5HS	5 yrs	blood	Exon 23-Y1088X (Heterozygous)	No	No	No	Causal mutation
5 months	blood	Exon 2 - R26H (Heterozygous)	No	n/a	n/a	n/a	Likely causal mutation
19 yrs	blood	No	No	Exon 11-S198X (Heterozygous)	No	No	Causal mutation
58 yrs	blood	No	No	No	No	No	
35 yrs	blood	No	No	No	No	No	
34 yrs	DNA	Exon 2 - R 28 H (Heterozygous); Exon 5 - L207P (Heterozygous)	No	No	n/a	n/a	Likely causal mutations

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 SCL4A1 (band 3)



Genotyping results: novel variants in spectrin identified suspected in disease phenotype

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
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- Exploring informatics tools to speed/aid 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.