Detecting Novel Variants in Alpha Thalassemia Carriers

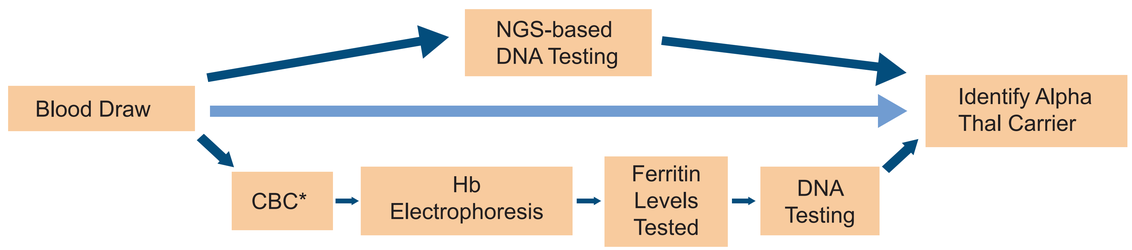
J. Sherry Wang, PhD; Laura Melroy, MS; Robert Chin, MS; Katherine Winters, BS; Dale Muzzey, PhD;
Katie Johansen Taber, PhD; Aishwarya Arjunan, MS, MPH, CGC; Rotem Ben-Shachar, PhD; Genevieve Gould, PhD

All authors are current or former employees of Myriad Genetics and/or Myriad Women's Health

BACKGROUND

- Alpha thalassemia is caused by the loss of alpha globin chains encoded by *HBA1* and *HBA2*. Disease severity varies from mild anemia to in utero fetal demise.
- Carrier screening by complete blood count analysis and hemoglobin electrophoresis is recommended for this prevalent genetic disease for all women who are pregnant or planning a pregnancy.[1]
- The recommended screening workflow is a complicated, multi-step process that results only in a diagnosis of exclusion. Screening via next-generation sequencing (NGS) is appealing due to its simple workflow and definitive results (Figure 1).
- Determining alpha thalassemia carrier status via NGS is technically challenging because of high homology between *HBA1* and *HBA2*.
- We previously developed a hybrid capture-based NGS assay that detects common copy number variants (CNVs) and the Constant Spring variant[2], resulting in a 90% detection rate for alpha thalassemia in high-risk ethnicities.[3]
- Here we present an improvement to the assay to identify novel variants (both single nucleotide variants (SNVs) and insertions/deletions (indels), resulting in a >99% detection rate in high-risk ethnicities.

Figure 1. Workflow for detecting alpha thalassemia carriers. Top: NGS-based workflow. Bottom: Traditional CBC/ hemoglobin electrophoresis workflow.

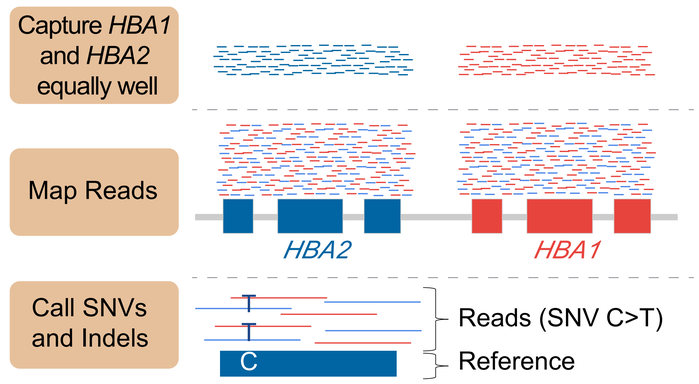


*Silent carriers are typically not detected

METHODS

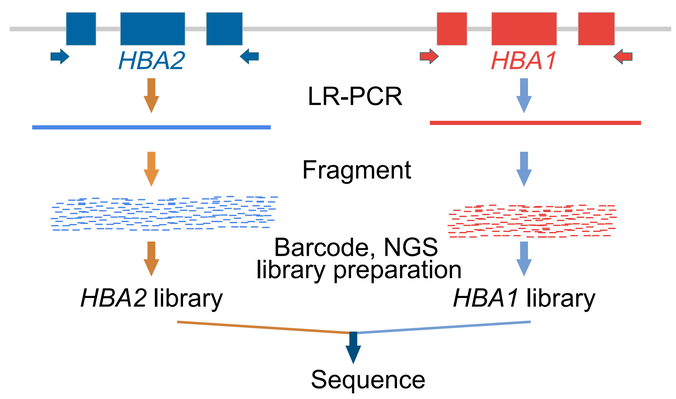
- We identified novel SNVs and indels using the hybrid capture assay described below.
- LR-PCR was also performed on all samples and served as an orthogonal truth dataset.

HYBRID CAPTURE



- Hybrid capture probes were designed to enrich for *HBA1* and *HBA2* with equal efficiency. Sequencing was performed on a HiSeq 2500.
- NGS reads were permissively aligned to both *HBA1* and *HBA2* and variant calling was performed using the genome analysis tool kit with the expectation of observing 4 alleles (tetraploid calling).[4]

LONG RANGE PCR (LR-PCR)



- *HBA1* and *HBA2* were amplified separately for each sample using gene-specific primers designed to unique regions of the genome.
- Amplicons were fragmented, library prepped with gene and sample specific barcodes, and sequenced on a HiSeq 2500.

RESULTS

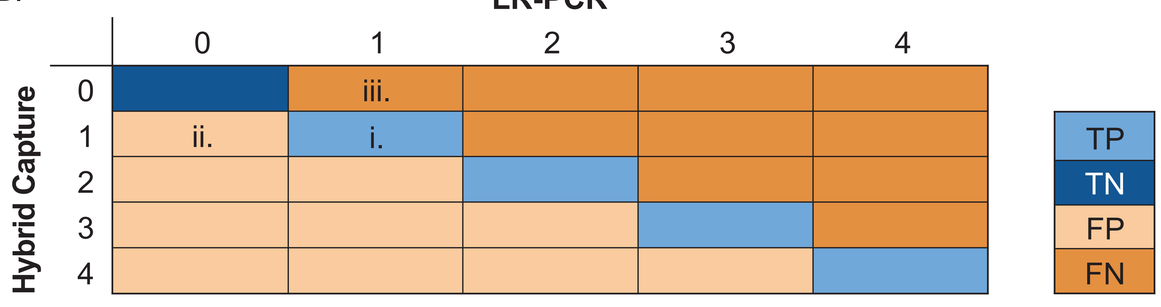
- The concordance analysis compares the LR-PCR and hybrid capture results (Table 1).
- Approximately half of the 502 patient samples were selected randomly. The rest were a combination of samples known to have *HBA1* and *HBA2* copy number variants and samples selected based on self-reported ethnicity.

Table 1. Example concordance analysis. (A) Example genotype data. (B) Example concordance table. Rows and columns show the number of alt counts identified by each assay.

A.

Concordance Type	LR-PCR			Hybrid Capture	
	HBA1	*HBA2*	Alt Counts	*HBA1+HBA2*	Alt Counts
i. True positive (TP)	A/A	A/T	1	A/A/A/T	1
ii. False positive (FP)	A/A	A/A	0	A/A/A/T	1
iii. False negative (FN)	A/A	A/T	1	A/A/A/A	0

B.



- 42 SNVs and 3 indels were identified in the set of 502 samples.
- The improved alpha thalassemia hybrid capture assay achieved 100% concordance with the LR-PCR data (Table 2).
- No FNs or FPs were identified.

Table 2. Concordance results (N=502). Rows and columns show the number of alt counts identified by each assay.

Hybrid Capture \ LR-PCR	0	1	2	3	4
0	10,497	0	0	0	0
1	0	39	0	0	0
2	0	0	6	0	0
3	0	0	0	0	0
4	0	0	0	0	0

TP
TN
FP
FN

CONCLUSIONS

- These results demonstrate that NGS can be used to detect novel SNVs and indels in the *HBA* genes.

REFERENCES: **1.** American College of Obstetricians and Gynecologists' Committee on Genetics. *Obstet. Gynecol.* 2017. 129, 41–55 **2.** Hogan et al. *Clinical Chemistry.* 2018. 64:7 1063–1073 **3.** Shang et al. *EBioMedicine.* 2017. 23, 150–157 **4.** DePristo et al. *Nature Genetics.* 2011. 43, 491–498.

Presented at ACMG on March 20, 2020