

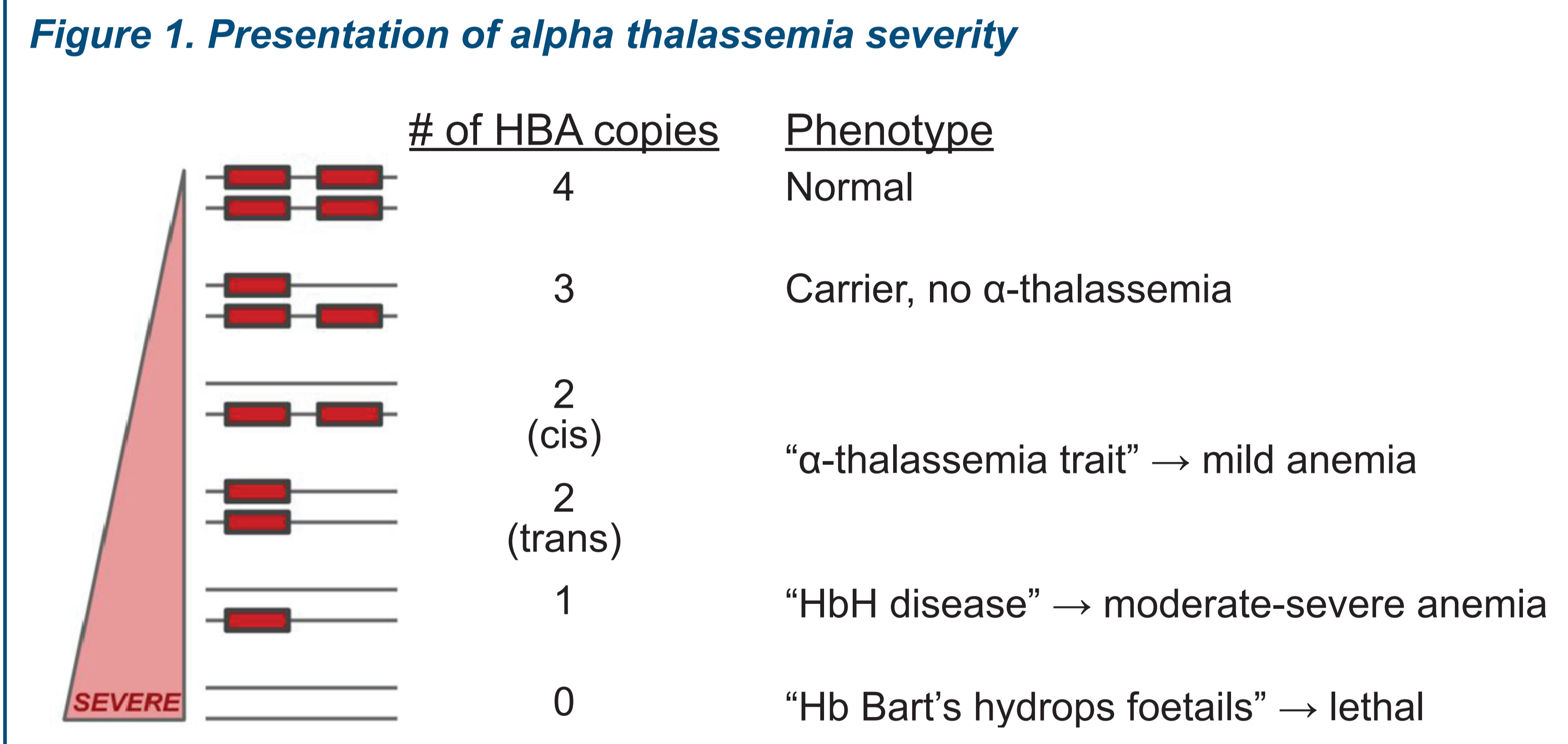
Detecting Novel Variants in Alpha Thalassemia Carriers

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

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

BACKGROUND

- Alpha thalassemia is caused by the loss of alpha globin chains encoded by *HBA1* and *HBA2* (Figure 1).
- Alpha thalassemia carrier screening is recommended for all women who are pregnant or planning a pregnancy.¹



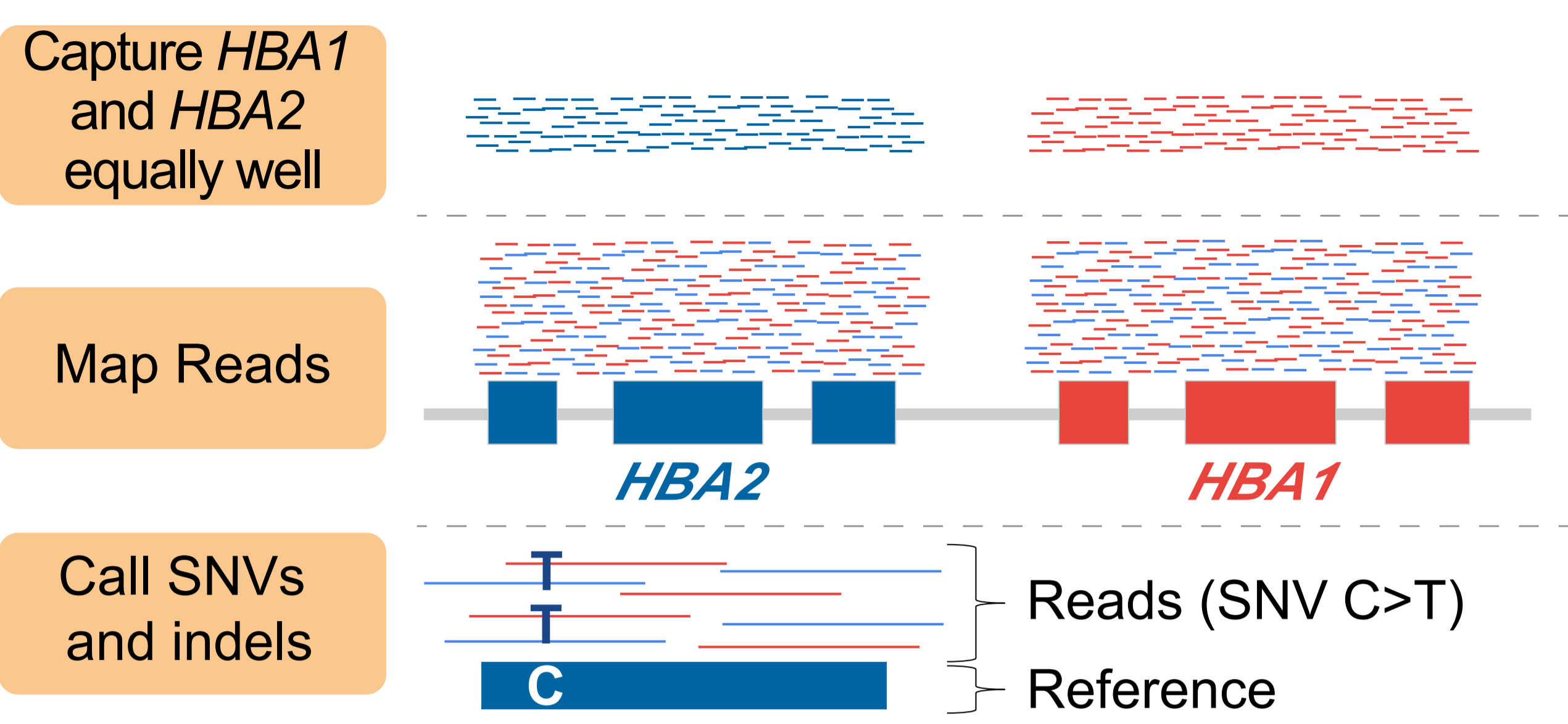
- Determining alpha thalassemia carrier status via NGS is technically challenging because of high homology between *HBA1* and *HBA2*.
- We previously developed a hybrid capture-based NGS assay that detects common copy number variants (CNVs) and the Constant Spring variant², resulting in a 90% detection rate for alpha thalassemia in high-risk ethnicities.³

Objective

- 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.

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HYBRID CAPTURE



- Our previously established hybrid-capture assay was updated to detect novel SNVs and indels via tetraploid calling.
- 259 patient samples were analyzed with the improved assay.
- Long range PCR (LR-PCR), utilizing unique regions in the genome, was also performed on all samples and served as an orthogonal truth dataset.

RESULTS

- 79 SNVs and 10 indels were identified in the set of 259 samples.
- The improved alpha thalassemia hybrid capture (HC) assay achieved 100% concordance with the LR-PCR data.
- No false negatives (FNs) or false positives (FPs) were identified.

Table 1. Long Range PCR

		Long Range PCR						Long Range PCR						TN	FN
		0	1	2	3	4	NC	0	1	2	3	4	NC	FP	TP
Hybrid Capture	SNV														
	0	11,315	0	0	0	0	0	1,026	0	0	0	0	0		
	1	0	70	0	0	0	0	0	10	0	0	0	0		1
	2	0	0	8	0	0	1	0	0	0	0	0	0		
	3	0	0	0	0	0	1	0	0	0	0	0	0		
	4	0	0	0	0	1	0	0	0	0	0	0	0		
	NC	0	0	0	0	0	0	0	0	0	0	0	0		
Hybrid Capture	INDEL														
	0														
	1														
	2														
	3														
	4														
	NC														

Not Captured (NC)

CONCLUSION

- These results demonstrate that the improved NGS assay can be used to detect novel SNVs and indels in the *HBA1* and *HBA2* genes.
- This 10x decrease in alpha thalassemia false negative rate is important in the detection of carriers and more importantly, at-risk carrier couples, to help inform pregnancy related decisions for individuals and couples who are pregnant or planning a pregnancy.

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.