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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

Presented by Genevieve Gould, PhD

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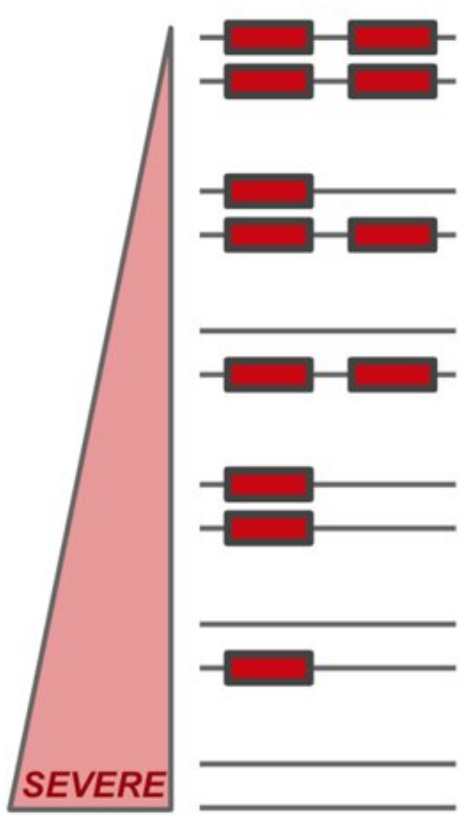
Disclosure Slide

Financial Disclosure for:
Genevieve Gould
Senior Manager, Computational Biology

Myriad Women's Health
Employee and share holder

Background

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



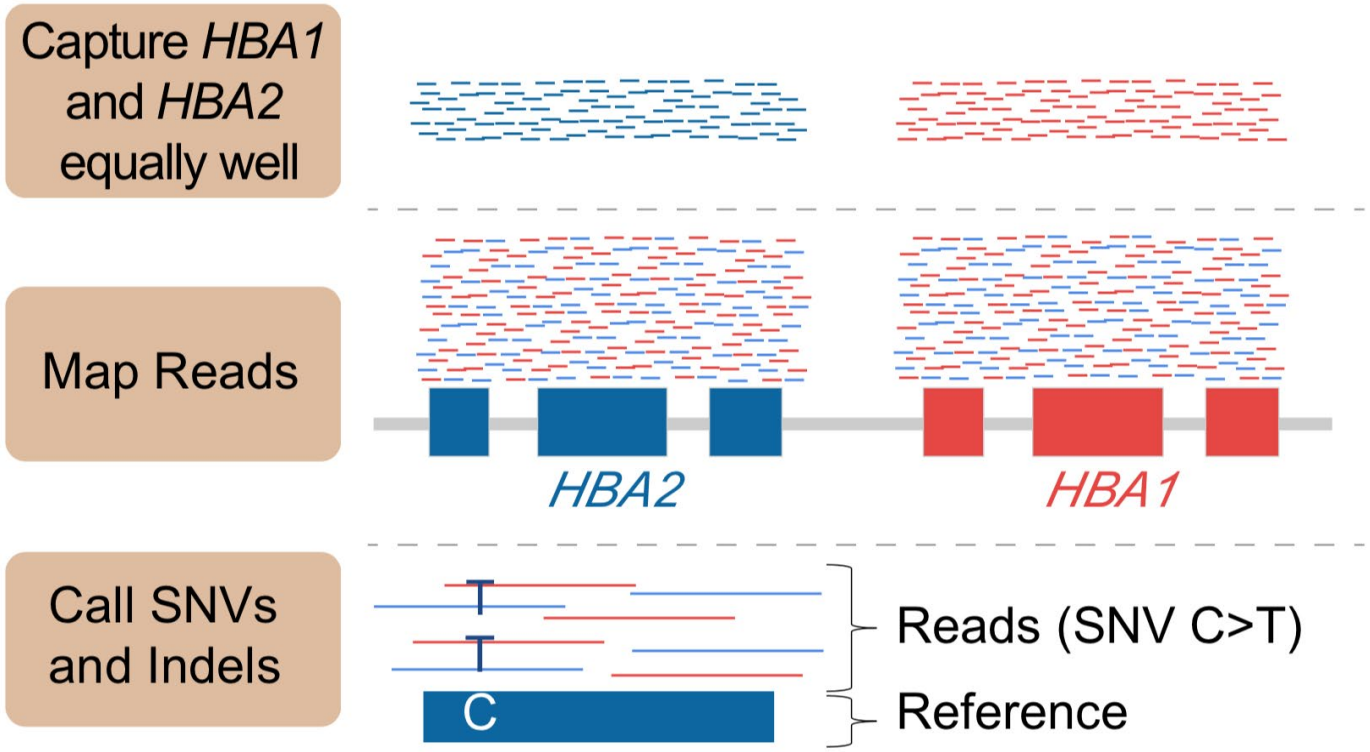
# of HBA copies	Phenotype
4	Normal
3	Carrier, no α-thalassemia
2 (cis) →	"α-thalassemia trait" → mild anemia
2 (trans) →	
1	"HbH disease" → moderate-severe anemia
0	"Hb Bart's hydrops foetalis" → lethal

Background

- 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.

Methods

Hybrid Capture



- Our previously established hybrid-capture assay was updated to detect novel SNVs and indel 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 FNs or FPs were identified.

Long Range PCR

Hybrid Capture: SNV	0	1	2	3	4	NC
0	11315	0	0	0	0	0
1	0	70	0	0	0	0
2	0	0	8	0	0	1
3	0	0	0	0	0	1
4	0	0	0	0	1	0
NC	0	0	0	0	0	0

Hybrid Capture: INDEL	0	1	2	3	4	NC
0	1026	0	0	0	0	0
1	0	10	0	0	0	1
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
NC	0	0	0	0	0	0

TN	FN
FP	TP

Conclusions

- These results demonstrate that the improved NGS can be used to detect novel SNVs and indels in the *HBA1* and *HBA2* 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.