A Tale of Two Hbs: DNA Sequencing and Hemoglobin Electrophoresis

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All authors were employed by Myriad Genetics, Inc. at the time of this study

INTRODUCTION

- Hemoglobinopathies, including α- and β-thalassemia, are the most common monogenic disorders worldwide.[1]
- Carrier Screening can identify individuals who are at risk of having affected offspring, but to be clinically effective, it must have both high sensitivity and specificity.[2]
- Current ACOG guidelines recommend traditional methods for hemoglobinopathy screening, i.e., a combination of complete blood counts and hemoglobin electrophoresis.[3]
- However, the most common pathogenic hemoglobinopathy variant worldwide, -α3.7, is not detected by these traditional screening methods.[4,5,6]
- Recent work by our group has demonstrated the superior sensitivity of next generation sequencing (NGS) over traditional screening methods for hemoglobinopathy variant detection (>99% detection rate), including the detection of -α3.7.[7]
- In this poster, we present support for the superior specificity of NGS and illustrate the undue burden caused by misidentification of hemoglobin variants through traditional screening methods.

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RESULTS

Figure 1. Variant Misidentification and Resolution

A) An internal case at Myriad Genetics was reported to be heterozygous for Hb S by traditional methods on two occasions, but negative for Hb S on the Foresight panel. Review of all variants detected by Foresight including those with no known disease association revealed the subject was heterozygous for Hb Hasharon, which is known to mimic Hb S on Hb electrophoresis[8,9,10] and has no known disease association. NGS was able to accurately report the subject's hemoglobin status and resolve the variant misidentification by electrophoresis. B) Only likely pathogenic or pathogenic variants are reported on the Foresight panel. If this subject had only been screened on the Foresight panel, they would not have been misinformed about their hemoglobin status.

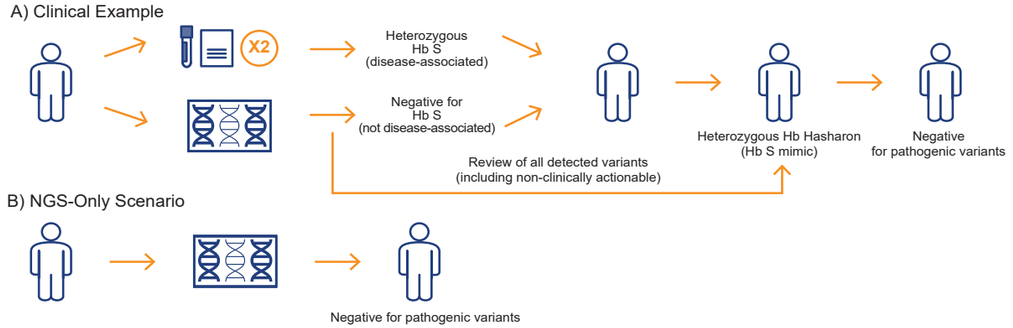

Figure 2. Hb S and Hb Hasharon: Comparison of Reproductive Risk and Implications

A) Hb S heterozygotes are at risk for symptoms and complications.[11] Their reproductive risk depends upon the partner's hemoglobin status, so partner testing is recommended. If the partner harbors a pathogenic β-thalassemia allele (β^0), the reproductive risk is 25% for an affected child (Hb S-β^0 thalassemia) and 25% for a child who is heterozygous for Hb S. Biological relatives of Hb S heterozygotes should also be tested given the personal and reproductive risks associated with the heterozygous state. B) Hb Hasharon has no known disease association. Hb Hasharon heterozygotes are not at risk of symptoms or complications. As there is no reproductive risk associated with Hb Hasharon, screening of partners or biological relatives is not necessary. β^N: wild-type *HBB*, β^S: Hb S, (*HBB*: p.E7V), β^0: β-thalassemia, β^H: Hb Hasharon (*HBA1/2*: p.D48H)

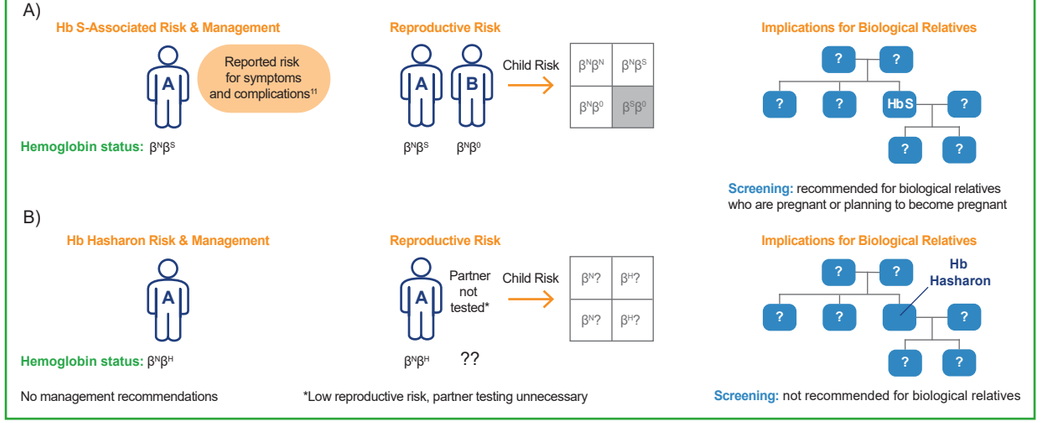

Table 1. Internal Observations of Hb S Mimics at Myriad Genetics

We compiled all *HBA1/2* and HBB variants reported at HbVar to have the same mobility as Hb S seen among 173,118 samples. We removed variants that met ACMG/AMP guidelines for classification as pathogenic or likely pathogenic,[27] and those lacking literature support for Hb S mimicry. Hb Hope was added without HbVar support when we encountered it in an internal case and found ample literature support for Hb S mimicry. Our internal data indicate a collective frequency of about 1 in 2100 in the general population. These data illustrate the potential scale of hemoglobin misidentification by hemoglobin electrophoresis.

Gene	Common name	Myriad (N=173,118)	GnomAD		Literature
			All ethnicities	Highest frequency (ethnicity)	
HBA1/2	Hb G-Philadelphia	24	6 in 164,532	6 in 15,068 (AFR)	Nair 2018[10]
	Hb Hasharon	18	12 in 124,140	11 in 6,734 (ASJ)	Nair 2018[10], Gracia de Oliveira 2012[12]
	Hb Q-Thailand	3	Absent	Absent	Jindadamrongwech 2010[13], Aksoy 1986[14]
	Hb Russ	3	2 in 216,482	2 in 48,906 (NFE)	Nair 2018[10]
	Hb Q-India	2	6 in 133,004	6 in 22,746 (SAS)	Aksoy 1986[14]
	Hb Etobicoke	1	Absent	Absent	Silva 2013[15]
	Hb Ottawa	1	Absent	Absent	Miranda 2018[16], Fucharoen 2007[17], Silva 2013[18]
HBB	Hb Korle-Bu	12	4 in 268,262	3 in 23,598 (AFR)	Konotey-Ahulu[19], Nagel 1993[20], Gracia de Oliveira 2012[12]
	Hb D-Iran	3	15 in 236,740	15 in 30,524 (SAS)	Miranda 2018[16], Rohe 1973[21]
	Hb Hope	3	3 in 236,836	3 in 14,902 (AFR)	Ducrocq 1998[22]
	Hb Dhofar	2	Absent	Absent	Daar 2008[23]
	Hb G-Coushatta	2	2 in 236,836	2 in 30,526 (SAS)	Miranda 2018[16]
	Hb Osu-Christiansborg	2	Absent	Absent	Nair 2018[10]
	Hb P-Galveston	2	Absent	Absent	Nair 2018[10]
	Hb Beograd	1	Absent	Absent	Aksoy 1984[24]
	Hb D-Ibadan	1	Absent	Absent	Itano 1951[25]
	Hb Hamadan	1	Absent	Absent	Dinçol 1984[26]
All variants		81 (1 in 2,100)	50 (1 in 3,400)	48	–

CONCLUSIONS

- The validated sensitivity and specificity of NGS allows for the detection of >99% of *HBB* and *HBA1/2* hemoglobinopathy variants. [7]
- We present case and literature support for the misidentification of hemoglobin variants due to the low specificity of traditional screening methods.
- Low specificity of traditional methods places an undue burden on:
  - Patients, potential for undue personal and reproductive clinical management.
  - Partners and biological relatives of patients, triggering unnecessary follow-up testing.
- Using data from individuals screened by Myriad, we demonstrate how NGS-sequencing of *HBB* and *HBA1/2* can mitigate undue clinical burden caused by false positives reported by traditional screening.
- This work suggests that adoption of NGS as the primary or sole method for hemoglobinopathy screening is in the best interest of the patient, reducing unnecessary emotional, financial, and reproductive burden.