The Association of Maternal $HBB$ Pathogenic Variant Status and Fetal Fraction in Non-invasive Prenatal Screening

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Disclosure

MP, MAH and DH
No conflict of interest

KEK and DM
Employed by Myriad Genetics, Inc, Salt Lake City, UT

Data obtained from Myriad Genetics
Participated in study design and data analysis
Not involved in final editorial decisions
Non-Invasive Prenatal Screening (NIPS) Fetal Fraction (FF)

Skrzypek et al, 2017
Liang et al, 2018
Factors Affecting FF

\[ \text{Fetal fraction} = \frac{\text{fetal cfDNA}}{\text{fetal cfDNA} + \text{maternal cfDNA}} \]

- **Fetal influences**
  - Gestational age
  - Multiple gestation
  - Fetal aneuploidy

- **Maternal Influence**
  - Body mass index
  - Maternal medical conditions

Taglauer et al, 2014
Women With *HBB* Hemoglobinopathies Have Lower Fetal Fraction

“5x increase no-call rate”
HBB gene carrier may have mild-severe clinical manifestations

Definite Associations

- Renal medullary cancer
- Hematuria
- Renal papillary necrosis
- Splenic infarction
- Exercise-related sudden death

Sickle Cell Trait
Objectives

To determine if:

1) *HBB* pathogenic variant carrier status is associated with altered Fetal Fraction in Maternal blood from NIPS samples

2) *HBB* pathogenic variant carrier status is associated with an altered rate of “No-Call” results
Study Design

Retrospective cohort study
Myriad NIPS and carrier screening lab database 2016-2019

**β-globin group**  
NIPS and β-globin (HBB) hemoglobinopathy carrier  
Structural (e.g. Hemoglobin S, C, E trait)  
Quantitative (beta-thalassemia minor and trait)

**α-globin group**  
NIPS and α-globin (HBA1/HBA2) hemoglobinopathy carrier  
alpha-thalassemia silent carrier and trait

**Comparison group**  
NIPS and non-carrier of β-globin/α-globin
Study Design

Exclusion criteria

β-globin and α-globin hemoglobinopathies

FF was adjusted using multivariate linear regression

Covariates: maternal age, gestational age, BMI → corrected FF

β-globin subgroup analyses

Hemoglobin S hemoglobinopathy carriers
Statistical Analysis

Kolmogorov-Smirnov test
Cohort Distributions

Estimate of no-call rate for hypothetical FF cutoffs
Study Cohorts

β-globin group

NIPS and \(HBB\)

- 19,929
  - HBB non-carrier: 19,686
  - HBB carrier: 243
  - Sickle cell trait: 213

α-globin group

NIPS and \(HBA1/HBA2\)

- 16,871
  - HBA1/HBA2 non-carrier: 15,854
  - HBA1/HBA2 carrier: 1,017
β-globin Group
Demographic Characteristics

**β-Globin**

<table>
<thead>
<tr>
<th></th>
<th>β-Globin Carriers</th>
<th>β-Globin Non-Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal Age</strong></td>
<td>32 [27-36]</td>
<td>33 [29-36]</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>26.8 [23.2-31.5]</td>
<td>25.2 [22.3-29.5]</td>
</tr>
</tbody>
</table>
Fetal Fraction Distribution

$\beta$-Globin

Adjusted Fetal Fraction

Probability density

- NON-CARRIER
- CARRIER

$p < 0.01$
Expected “No-Call” Rate

β-Globin
β-globin Subgroup Analyses: Hemoglobin-S carrier
## Demographic Characteristics

### Sickle Cell Trait

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sickle Cell Trait Median [IQR]</th>
<th>Non-Carrier Median [IQR]</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>28.3 [24.7-33.5]</td>
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</table>
Fetal Fraction Distribution

Hemoglobin S carrier

![Fetal Fraction Distribution Graph]

- Non-carrier
- Carrier
Expected “No-Call” Rate

Hemoglobin-S carrier
α-Globin Group
## Demographic Characteristics

### $\alpha$-Globin

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<thead>
<tr>
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<th>$\alpha$-Globin Carriers</th>
<th>$\alpha$-globin Non-Carriers</th>
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<tr>
<td>Maternal Age</td>
<td>32 [27-36]</td>
<td>33 [29-36]</td>
</tr>
<tr>
<td>BMI</td>
<td>26.5 [22.9-31.6]</td>
<td>25.1 [22.2-29.5]</td>
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</table>
Fetal Fraction Distribution

α-Globin

Probability density

Fetal fraction

p > 0.05
Conclusions

**β-globin carriers**
Lower FF and higher no-call rate

**Sickle cell traits**
Lower FF and higher no-call rate

**α-globin carriers**
No difference in FF and no-call rate
Implications

If confirmed on further studies, should be considered in pre- and post-test counseling

Impact of fetal hemoglobinopathy status is unknown

Impact of maternal carrier status on risk of aneuploidy among “no-calls” is unknown
Acknowledgement

Brian Mercer, MD

Justin Lappen, MD
Additional Slides
Study Design

• NIPS: Whole-genome sequencing methodology

• β-globin carrier screening: Sequencing with copy number analysis

• α-globin carrier screening: Analysis of homologous regions
ETHNICITY INFORMATION FOR HBB Group

**Carriers:**
- South Asian: 10.70%
- Ashkenazi Jewish: 0.82%
- African or African American: 22.63%
- Southeast Asian: 16.05%
- unknown: 13.17%
- Caucasian Other: 16.87%
- Northern European: 2.47%
- Southern European: 4.94%
- Hispanic: 4.94%
- East Asian: 4.12%

**Non-carriers:**
- Middle Eastern: 3.29%
- South Asian: 3.88%
- Middle Eastern: 1.62%
- Southeast Asian: 1.92%
- French Canadian or Cajun: 0.43%
- Native American: 0.26%
- Pacific Islander: 0.19%
- Finnish: 0.03%

- Caucasian Other: 35.88%
- Hispanic: 9.12%
- unknown: 13.60%
- African or African American: 7.29%
- Northern European: 15.68%
- Southern European: 2.03%
- Ashkenazi Jewish: 3.28%
- East Asian: 4.79%
ETHNICITY INFORMATION FOR HBA1/HBA2 Group:

CARRIERS:
Hispanic: 9.33%
Ashkenazi Jewish: 1.87%
African or African American: 39.98%
Middle Eastern: 3.93%
Southeast Asian: 2.95%
Caucasian Other: 14.34%
East Asian: 4.22%
South Asian: 7.37%
unknown: 11.59%
Northern European: 2.95%
French Canadian or Cajun: 0.20%
Southern European: 1.08%
Pacific Islander: 0.20%

NON_CARRIERS:
Caucasian Other: 36.81%
Hispanic: 9.34%
African or African American: 5.33%
Northern European: 16.10%
Southern European: 1.91%
Ashkenazi Jewish: 3.20%
East Asian: 5.13%
unknown: 14.00%
South Asian: 3.73%
Middle Eastern: 1.54%
Southeast Asian: 1.96%
French Canadian or Cajun: 0.44%
Native American: 0.30%
Pacific Islander: 0.18%
Finnish: 0.03%
<table>
<thead>
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<th>ETHNICITY INFORMATION FOR HbS PATIENTS:</th>
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<tbody>
<tr>
<td><strong>CARRIERS:</strong></td>
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<tr>
<td>Hispanic: 15.02%</td>
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<tr>
<td>Caucasian Other: 7.04%</td>
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<td>African or African American: 62.44%</td>
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<td>unknown: 11.27%</td>
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<td>Middle Eastern: 0.47%</td>
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Probability of Non-reportable Results in \textit{HBA1/HBA2} carriers
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<td>Fetal Fraction</td>
<td>7.9% [6.1%-10.1%]</td>
<td>8.6% [6.5%-11.2%]</td>
</tr>
</tbody>
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## HBA Statistics

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<tr>
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<th>HBA carriers</th>
<th>HBA non-carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>31.5 ± 5.9</td>
<td>32.3 ± 5.3</td>
</tr>
<tr>
<td>Gestational age</td>
<td>13.6 ± 3.9</td>
<td>12.8 ± 3.2</td>
</tr>
<tr>
<td>BMI</td>
<td>27.8 ± 6.3</td>
<td>26.5 ± 5.7</td>
</tr>
<tr>
<td>Fetal Fraction</td>
<td>9.1% ± 3.8%</td>
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</tr>
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</table>
## Non-HBS Statistics

<table>
<thead>
<tr>
<th></th>
<th>HBB carriers of non-HbS variant</th>
<th>HBB non-carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>$32.6 \pm 6.1$</td>
<td>$32.3 \pm 5.3$</td>
</tr>
<tr>
<td>Gestational age</td>
<td>$13.5 \pm 3.4$</td>
<td>$12.8 \pm 3.1$</td>
</tr>
<tr>
<td>BMI</td>
<td>$26.6 \pm 5.7$</td>
<td>$26.5 \pm 5.7$</td>
</tr>
<tr>
<td>Fetal Fraction</td>
<td>$8.6% \pm 3.7%$</td>
<td>$9.1% \pm 3.8%$</td>
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# HBS Statistics

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<tr>
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<th>HbS carriers (excluding affected)</th>
<th>HbS non-carriers</th>
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<td>BMI</td>
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<td>Fetal Fraction</td>
<td>7.7% [5.9%-9.8%]</td>
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Difference in fetal fraction in women with hbb hemoglobinopathies VS HEMOGLOBIN AA

Note: Lines are smoothed by locally weighted regression and confidence intervals that do not overlap indicate statistically significantly different mean fetal fraction values.
Methodology

Carrier Screening. β-globin carrier screening: Sequencing with copy number analysis and α-globin carrier screening: Analysis of homologous regions