PrenatalSafe®

Noninvasive prenatal test (NIPT) for genome-wide fetal chromosomal abnormalities

Cell-free fetal DNA analysis from maternal plasma providing karyotype-level insight
Case study
Case Study 1

31 y.o. patient

12^ weeks gestation

Negative Result

Fetal abnormal ultrasound findings
(cerebellar hypoplasia; Ventriculomegaly)

20^ week gestation

Amniocentesis

Reassessment of the NIPT data with

PrenatalSafe®
Detection of a deletion 5p confirmed by array-CGH
Case Study 2

32 y.o. patient

↓

13^ weeks gestation

PrenatalSafe®

5

↓

Negative Result

↓

21^ weeks gestation

Multiple fetal abnormal ultrasound findings

↓

Reassessment of the NIPT data with

PrenatalSafe®

↓

Amniocentesis
Identification of 2 deletions (18p / 18q) confirmed by array-CGH

Amniotic Fluid

Del18p11.32-p11.31
3.8 Mb

Del18q21.32-q23
21.3 Mb

Array-CGH

PrenatalSafe® Karyo

PrenatalSafe® KARYO

cfDNA
Case Study 3

32 y.o patient
Carrier of a reciprocal translocation 46,XX,t(7;9)(p15;q22)

11^ weeks gestation

Abnormal Result
detected fetal karyotype with an unbalanced translocation

Villocentesis

Results confirmed
Detected a fetal karyotype with an unbalanced translocation confirmed by array-CGH

Dup7p22.3p21.2 15 Mb

Dup9p24.3-q31.1 107.5 Mb
35 y.o patient

Amniocentesis (traditional karyotyping)

Negative Result (Fetal karyotype 46, XY)

Fetal abnormal ultrasound findings (Suspected DiGeorge syndrome)

Identified deletion 22q11.2 (compatible with DiGeorge syndrome)

Amniocentesis

13^ weeks gestation

20^ weeks gestation
Identification of 22q11.2 deletion confirmed by Array-CGH

Amniotic Fluid

Array-CGH

PrenatalSafe® Karyo

cfDNA

del22q11.21 DiGeorge Syndrome

4 Mb
Case Study 5

33 y.o. patient
(She was not aware to be carrier of a chromosomal translocation)

12^ weeks gestation

Abnormal Result
detected fetal karyotype with an unbalanced translocation

Villocentesis

Results confirmed
Detected a fetal karyotype with an unbalanced translocation confirmed by array-CGH.

- **Del13q33.1q34**: 11.4 Mb
- **Dup20q13.33**: 1.9 Mb

**Chr. 13**

**PrenatalSafe® Karyo (cfDNA)**

**Array-CGH (CVS)**

**Chr. 20**

**PrenatalSafe® Karyo (cfDNA)**

**Array-CGH (CVS)**
Case Study 6

40 y.o patient

↓

11^ weeks gestation

↓

Abnormal Result

detected fetal karyotype with duplication 18p

↓

Villocentesis

↓

Results confirmed
Detection of a duplication 18p confirmed by array-CGH
Case Study 7

38 y.o patient

↓

10^{\text{th}} \text{ weeks gestation}

↓

Abnormal Result

detected fetal karyotype with duplication 11p

↓

Amniocentesis

↓

Results confirmed
Detection of a duplication 11p confirmed by array-CGH

Dup11p15.4p15.1
16 Mb
Amniotic Fluid

11:00b

Chr. 11

Dup11p15.4p15.1
16 Mb
cfDNA

PrenatalSafe® Karyo

Array-CGH
Case Study 8

35 y.o patient

12^ weeks gestation

PrenatalSafe®

Abnormal Result
detected fetal karyotype with deletion Xp

Amniocentesis

16^ weeks gestation

Results confirmed
Detection of a deletion Xp confirmed by array-CGH

delXp22.33p11.1
58.5 Mb
Amniotic Fluid

Array-CGH

PrenatalSafe® Karyo

delXp22.33p11.1
58.5 Mb
cfDNA
40 y.o. patient

13^ weeks gestation

NIPT
(5 chromosomes screening)

Test performed by a different lab

Positive Result
High risk for Trisomy 21

Trisomy 21 not detected
Maternal CNV detected causing the false positive result
Lower the risk of false positive results determined by maternal CNVs

Copy-Number Variation and False Positive Prenatal Aneuploidy Screening Results

Matthew W. Snyder, M.S., LaVone E. Simmons, M.D., Jacob O. Kitzman, Ph.D., Bradley P. Coe, Ph.D., Jessica M. Henson, B.S., Riza M. Daza, B.S., Evan E. Eichler, Ph.D., Jay Shendure, M.D., Ph.D., and Hilary S. Gammill, M.D.
**Table 1. Algorithm Improvements and Reductions in False Positive Results.**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Original Result (as Reported in CARE Study)*</th>
<th>New Result (Current Algorithm)</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>Trisomy 13</td>
<td>Trisomy 13, female infant</td>
<td>Complete chromosome 13 gain confirmed on reanalysis</td>
</tr>
<tr>
<td>2†</td>
<td>Trisomy 13</td>
<td>Euploid</td>
<td>Maternal copy-number variant of approximately 5 Mb on chromosome 13q</td>
</tr>
<tr>
<td>3†</td>
<td>Trisomy 13</td>
<td>Euploid</td>
<td>Maternal copy-number variant of approximately 8 Mb on chromosome 13q</td>
</tr>
<tr>
<td>4†</td>
<td>Trisomy 18</td>
<td>Trisomy 18, male infant</td>
<td>Complete chromosome 18 gain confirmed on reanalysis</td>
</tr>
<tr>
<td>5¶</td>
<td>Trisomy 18</td>
<td>Euploid</td>
<td>Maternal copy-number variant of approximately 2 to 3 Mb on chromosome 18p</td>
</tr>
<tr>
<td>6¶</td>
<td>Trisomy 21</td>
<td>Euploid</td>
<td>Reduced coverage variability given additional normalization steps in current algorithm</td>
</tr>
<tr>
<td>7†</td>
<td>Trisomy 21</td>
<td>Euploid</td>
<td>Reduced coverage variability given additional normalization steps in current algorithm</td>
</tr>
<tr>
<td>8†</td>
<td>Trisomy 21</td>
<td>Trisomy 21, male infant</td>
<td>Complete chromosome 21 gain confirmed on reanalysis</td>
</tr>
<tr>
<td>9¶</td>
<td>Trisomy 21</td>
<td>Trisomy 21, male infant</td>
<td>Complete chromosome 21 gain confirmed on reanalysis</td>
</tr>
<tr>
<td>10¶</td>
<td>Trisomy 21</td>
<td>Trisomy 21, female infant</td>
<td>Complete chromosome 21 gain confirmed on reanalysis</td>
</tr>
<tr>
<td>11¶</td>
<td>Trisomy 21 and 18</td>
<td>Euploid</td>
<td>Reduced coverage variability given additional normalization steps in current algorithm</td>
</tr>
</tbody>
</table>
Lower the risk of false positive results determined by maternal CNVs

Improving the Accuracy of Prenatal Screening with DNA Copy-Number Analysis

Table 1. Identification of Maternal Microduplications as a Source of False Positive Results on Noninvasive Prenatal Screening.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Positive Trisomy</th>
<th>Maternal Microduplication</th>
<th>Confirmed on Microarray Analysis</th>
<th>Improvement in Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>313</td>
<td>12</td>
<td>9</td>
<td>+4 (from 94% to 98%)</td>
</tr>
<tr>
<td>18</td>
<td>106</td>
<td>21</td>
<td>3</td>
<td>+20 (from 72% to 92%)</td>
</tr>
<tr>
<td>13</td>
<td>93</td>
<td>28</td>
<td>2</td>
<td>+30 (from 39% to 69%)</td>
</tr>
</tbody>
</table>
Case Study 10

42 y.o patient

14\text{^ {weeks gestation}}

Abnormal Result

detected fetal karyotype with Trisomy 9

Amniocentesis

16\text{^ {weeks gestation}}

Results confirmed

Trisomy 9 mosaic (20\%)
Detection of Trisomy 9 mosaic confirmed by traditional karyotyping.

46,XX[80]/47,XX,+9[20]

PrenatalSafe® Karyo

Traditional Karyotyping
Case Study 11

40 y.o patient

10^ weeks gestation

Abnormal Result

detected fetal karyotype with Trisomy 22

Amniocentesis

16^ weeks gestation

Results confirmed
Trisomy 22 mosaic (16%)
Detection of Trisomy 22 mosaic confirmed by traditional karyotyping

46,XY[42]/47,XY,+22[8]
31 y.o patient

12 weeks gestation

Abnormal Result

detected fetal karyotype with Trisomy 7

Amniocentesis

16 weeks gestation

Results confirmed

Trisomy 7 mosaic (4%)
Detection of Trisomy 7 mosaic confirmed by traditional karyotyping

46,XX[48]/47,XX,+7[2]

Traditional Karyotyping
Prospective study
Background

- Conventional cfDNA-based NIPT approaches focus on detection of common trisomies and sex-chromosome aneuploidies.

- This leaves a gap of approximately 17% of clinically relevant chromosomal/subchromosomal abnormalities that would go undetected.

- Previous studies have shown the potential of extending conventional NIPT to detect fetal microdeletion syndromes from maternal plasma (Peters et al., 2011; Srinivasan et al., 2013).

- Other studies showed how genome-wide cfDNA testing can contribute in lowering the incidence of false positive results generated by maternal copy number variants (Snyder et al., 2015; Chudova et al., 2016).

- At present, there are limited data available on the potential of genome-wide screening to detect rare autosomal trisomies and structural chromosome anomalies in a general population of pregnant women.
Aim of the study

- From December 2015 through May 2016, genome-wide cell-free fetal DNA (cfDNA) testing was offered to pregnant women undergoing conventional cfDNA-based non-invasive prenatal testing (NIPT) for common fetal aneuploidy.

- We aimed to compare the performance of the two tests in a general obstetrical population.
## Demographic and pregnancy characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of eligible patients</td>
<td>12.114</td>
</tr>
<tr>
<td>Maternal age-yr</td>
<td></td>
</tr>
<tr>
<td>- Mean ±SD</td>
<td>35.3±4.1</td>
</tr>
<tr>
<td>- Min-max</td>
<td>20-58</td>
</tr>
<tr>
<td>Gestational age at sample collection -wk</td>
<td></td>
</tr>
<tr>
<td>- Mean ±SD</td>
<td>12.3±2.1</td>
</tr>
<tr>
<td>- Min-max</td>
<td>10-29</td>
</tr>
<tr>
<td>Indications for NIPT</td>
<td></td>
</tr>
<tr>
<td>- Parental Anxiety</td>
<td>3804 (31.4%)</td>
</tr>
<tr>
<td>- Advanced maternal age(^a)</td>
<td>4446 (36.7%)</td>
</tr>
<tr>
<td>- Positive prenatal screen</td>
<td>1199 (9.9%)</td>
</tr>
<tr>
<td>- Fetal ultrasound abnormality</td>
<td>472 (3.9%)</td>
</tr>
<tr>
<td>- Prior pregnancy with fetal aneuploidy</td>
<td>157 (1.3%)</td>
</tr>
<tr>
<td>- More than one indication</td>
<td>2035 (16.8%)</td>
</tr>
</tbody>
</table>
## Results of samples tested

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients analyzed</td>
<td>12.114</td>
</tr>
<tr>
<td>Samples with a call - no. (%)</td>
<td>12.078 (99.7)</td>
</tr>
<tr>
<td>Total cancellations - no. (%)</td>
<td>182 (1.5)</td>
</tr>
<tr>
<td>- Samples with low FF - no. (%)</td>
<td>145 (1.2)</td>
</tr>
<tr>
<td>- Samples with assay failure - no. (%)</td>
<td>36 (0.3)</td>
</tr>
<tr>
<td>Samples with a conclusive result - no. (%)</td>
<td>11.932 (98.5)</td>
</tr>
<tr>
<td>Chromosomally abnormal results</td>
<td></td>
</tr>
<tr>
<td>- Genome-wide cfDNA screening - no. (%)</td>
<td>196 (1.6)</td>
</tr>
<tr>
<td>- Conventional cfDNA screening - no. (%)</td>
<td>166 (1.4)</td>
</tr>
<tr>
<td>Pregnancies confirmed as chromosomally abnormal - no. (%)</td>
<td></td>
</tr>
<tr>
<td>- Genome-wide cfDNA screening - no. (%)</td>
<td>169 (1.4)</td>
</tr>
<tr>
<td>- Conventional cfDNA screening - no. (%)</td>
<td>151 (1.3)</td>
</tr>
</tbody>
</table>
Clinically relevant chromosomal abnormalities classes detected by genome-wide cfDNA analysis
Types of chromosome anomalies detected by conventional and genome-wide cfDNA testing

- Common aneuploidies
- Chr. abnormalities not detected by standard cfDNA testing
Clinically relevant chromosomal abnormalities, not detected by conventional cfDNA screening, potentially resulting in the birth of babies with chromosomal anomalies, have been considered as false negative.

A P-value of less than 0.05 was considered to indicate statistical significance (**).

<table>
<thead>
<tr>
<th></th>
<th>Conventional cfDNA screening</th>
<th>Genome-wide cfDNA screening</th>
<th>P-value&lt;sup&gt;§&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pregnancies assessed</td>
<td>11.932</td>
<td>11.932</td>
<td></td>
</tr>
<tr>
<td>Clinical relevant chromosomal abnormalities detected - no. (%)</td>
<td>166</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>Pregnancies confirmed as chromosomally abnormal - no. (%)</td>
<td>151</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>False Positive</td>
<td>15</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>False Negative</td>
<td>12*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>True Positive</td>
<td>151</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>True Negative</td>
<td>11.754</td>
<td>11.736</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>92.64%</td>
<td>100.00%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.87%</td>
<td>99.77%</td>
<td>0.064</td>
</tr>
<tr>
<td>Positive Predictive Value (PPV)</td>
<td>90.96%</td>
<td>86.22%</td>
<td>0.161</td>
</tr>
<tr>
<td>Negative Predictive Value (NPV)</td>
<td>99.90%</td>
<td>100.00%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>^</sup>Clinically relevant chromosomal abnormalities, not detected by conventional cfDNA screening, potentially resulting in the birth of babies with chromosomal anomalies, have been considered as false negative.

<sup>§</sup>A P-value of less than 0.05 was considered to indicate statistical significance (**).
# Performance of the Genome-wide cfDNA screening

<table>
<thead>
<tr>
<th></th>
<th>Trisomy 21 (n=11,932)</th>
<th>Trisomy 18 (n=11,932)</th>
<th>Trisomy 13 (n=11,932)</th>
<th>SCA (n=11,932)</th>
<th>Rare Trisomies (n=11,932)</th>
<th>CNV (n=11,932)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>True Positive</strong></td>
<td>88</td>
<td>15</td>
<td>12</td>
<td>36</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td><strong>False Positive</strong></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td><strong>True Negative</strong></td>
<td>11.843</td>
<td>11.916</td>
<td>11.919</td>
<td>11.884</td>
<td>11.915</td>
<td>11.919</td>
</tr>
<tr>
<td><strong>False Negative</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Sensitivity (95% CI)</strong></td>
<td>100.00% (95.89% - 100.00%)</td>
<td>100.00% (78.20% - 100.00%)</td>
<td>100.00% (73.54% - 100.00%)</td>
<td>100.00% (90.26% - 100.00%)</td>
<td>100.00% (69.15% - 100.00%)</td>
<td>100.00% (63.06% - 100.00%)</td>
</tr>
<tr>
<td><strong>Specificity (95% CI)</strong></td>
<td>99.99% (99.95% - 100.00%)</td>
<td>99.99% (99.95% - 100.00%)</td>
<td>99.99% (99.95% - 100.00%)</td>
<td>99.90% (99.82% - 99.95%)</td>
<td>99.94% (99.88% - 99.98%)</td>
<td>99.96% (99.90% - 99.99%)</td>
</tr>
<tr>
<td><strong>PPV (95% CI)</strong></td>
<td>98.88% (92.54% - 99.84%)</td>
<td>93.75% (67.88% - 99.07%)</td>
<td>92.31% (62.83% - 98.84%)</td>
<td>75.00% (63.02% - 84.08%)</td>
<td>58.82% (40.52% - 74.97%)</td>
<td>61.54% (39.98% - 79.35%)</td>
</tr>
<tr>
<td><strong>NPV (95% CI)</strong></td>
<td>100.00% (99.95% - 100.00%)</td>
<td>100.00% (99.95% - 100.00%)</td>
<td>100.00% (99.95% to 100.00%)</td>
<td>100.00% (99.95% - 100.00%)</td>
<td>100.00% (99.95% - 100.00%)</td>
<td>100.00% (99.95% - 100.00%)</td>
</tr>
</tbody>
</table>
The clinical utility of expanding NIPT to cover the entire genome is controversial, especially in low-risk pregnancies. In fact, it pertains a risk of overdiagnosis with a higher number of false positives because of chromosomal rearrangements which are confined to the placenta, potentially leading to an increase in unnecessary invasive testing.

The results of this study demonstrate that a high specificity may be maintained while extending the screen to all chromosomal abnormalities.
Thank you for your attention

http://www.laboratoriogenoma.eu/

Rome

Milan