## Fetal Medicine - NIPT

<table>
<thead>
<tr>
<th>Code</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2-01</td>
<td>Simultaneous detection of fetal chromosome aneuploidy and monogenic diseases by a novel noninvasive prenatal testing method: Targeted and genome-wide simultaneous sequencing (TAGs-seq).</td>
</tr>
<tr>
<td>F2-02</td>
<td>Validation of the SNP–Based NIPT in twin gestations for zygosity-, individual-fetal-gender-, and aneuploidy-determination.</td>
</tr>
<tr>
<td>F2-03</td>
<td>Isolate FGR; is it an indication for CMA?</td>
</tr>
<tr>
<td>F2-04</td>
<td>Clinical evaluation of NIPT for women at advanced maternal age: a multicenter retrospective study.</td>
</tr>
<tr>
<td>F2-05</td>
<td>Not all chromosome aberrations can be detected by NIPT in women at advanced maternal age: a multicenter retrospective study.</td>
</tr>
<tr>
<td>F2-06</td>
<td>Haplotype-based noninvasive prenatal testing for duchenne muscular dystrophy: A pilot study in South China.</td>
</tr>
<tr>
<td>F2-07</td>
<td>The relationship between noninvasive prenatal testing failure and adverse prenatal outcomes.</td>
</tr>
</tbody>
</table>
Validation of the SNP-Based NIPT in Twin Gestations for Zygosity-, Individual-Fetal-Gender-, and Aneuploidy-Determination


Introduction

• The prevalence of twin gestations is approximately 1/30 of all live births in the United States.

• Twin gestations are at an increased risk of fetal loss and/or anomalies including structural and congenital abnormalities, and an increased risk for aneuploidy.

• Since non-invasive prenatal testing (NIPT) for fetal aneuploidy using cell-free DNA (cfDNA) in maternal plasma became clinically available in 2011, physicians have had a highly accurate, non-invasive method to detect the common trisomies in both singleton and twin pregnancies; this has led to a significant reduction of invasive prenatal diagnostic testing.

• Further, by detecting Y chromosome DNA, NIPT has enabled determination of whether a twin gestation has at least one male fetus, though it is not able to confirm the exact number of male fetuses.

• Despite these advancements in prenatal screening, antenatal management of twin gestations necessitates the accurate determination of both chorioity and zygosity.

• For example, monochorionic (MC), monochorionic twins have a 10% risk of fetal morbidity and mortality attributed to twin-to-twin transfusion syndrome (TTTS).

• Given the prevalence of and complex antenatal management associated with twins, there is an urgent clinical need for accurate, early determination of chromosomal abnormalities and other complications such as TTTS in twin gestations.

• Previously, we have demonstrated analytical and clinical validation of whole-chromosome aneuploidy screening in singlets using a single-nucleotide polymorphism (SNP)-based NIPT.

• This study evaluated the performance of SNP-based NIPT in twin gestations (from 9 weeks GA) with known clinical truth for the presence of fetal aneuploidy (chromosomes 21, 18, 13, X, and Y), determination of zygosity, and identification of individual fetal gender.

Methods

Study Cohort

• Maternal blood samples (20 mL) from pregnant women with twin gestations were collected from participating clinicians/patient centers.

• Inclusion criteria were GA ≥ 29 weeks and a clinical truth for zygosity status, fetal sex chromosome copy number, and/or gender count acceptable sources were genetic test reports, verbal clinical follow-up, clinician/patient visual assessment by parent/gender and aneuploidy; only for one twin (3 months of age), and/or CVS, amniocentesis, or baby buccal samples analyzed via SNP-based NIPT.

• Truth for zygosity, aneuploidy, and gender was not known for each sample.

• All women provided informed consent; samples were de-identified prior to testing.

Single Nucleotide Polymorphism-Based Analyses

• All samples were processed at a Clinical Laboratory Improvement Act (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory (Natera, Inc., San Carlos, CA) using a previously described validated methodology; analyses were performed using a proprietary algorithm.

• Aneuploidy was not tested on X and Y chromosomes for XYY cases.

• Fetal fraction (FF) estimates were generated for both fetuses. A combined FF was reported for MZ pregnancies and two distinct FF estimates were made for DZ pregnancies.

• Outcomes reliant on FF were calculated using the lower FF of the two fetuses (DZ twins).

Statistical Analyses

• Confidence intervals of sensitivity and specificity for zygosity, fetal sex copy number, and gender accuracy were calculated using the Clapper-Plonley exact binomial confidence intervals for a single proportion.

• Confidence intervals of no-call rates, overall aneuploidy specificity, and overall gender test accuracy were calculated using the Method of Variance Estimates Recovery (MOVER) for weighted averages based on the population prevalence of MZ and DZ twin gestations (0.07%). As described previously, aneuploidy no-calls estimated includes only samples with GA ≥ 10 weeks.

• Samples that received a no call were excluded from corresponding analyses.

• Confidence intervals were computed at the 95% confidence level.

Results

• A total of 128 patient samples from twin pregnancies were included in the study.

• The mean gestational age of the cohort was 32.8 ± 5.57 weeks; the mean GA was 15.2 ± 4.72 weeks (Table 1).

• For MZ twins, the mean FF was 15.2 ± 4.45. In DZ twins, the mean FF of the two fetuses was 8.07 ± 3.1 (Twins 1) and 7.96 ± 3.49 (Twins 2) (Table 2).

• Overall aneuploidy sensitivity and specificity were 100% (Table 4).

Zygosity Determination

• Zygosity was evaluated in 95 samples (Figure 1).

Figure 1: Zygosity Determination

- Samples with Zygosity Truth (n = 95)
  - MZ (n = 54)
  - DZ (n = 41)

- Correct Calls (GG): 54/54
- Incorrect Calls (GT): 3/41
- Incorrect Calls (TT): 1/41

- Overall zygosity accuracy was 100% (95% CI: 5.7-9.9). (Table 3)

Table 3: Zygosity Test Performance

<table>
<thead>
<tr>
<th>Performance</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Overall Zygosity Determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ (n = 54)</td>
<td>100% (92/92)</td>
<td>100% (42/42)</td>
<td>100% (92/92) (95% CI: 95-99.9)</td>
</tr>
<tr>
<td>DZ (n = 41)</td>
<td>100% (39/39)</td>
<td>100% (32/32)</td>
<td>100% (71/71) (95% CI: 95-99.9)</td>
</tr>
</tbody>
</table>

Gender Determination

• Gender was evaluated in 103 samples (Figure 3).

Figure 2: Aneuploidy Determination

- Samples with Aneuploidy Truth (n = 117)
  - MZ (n = 60)
  - DZ (n = 57)

- Correct Calls (GG): 60/60
- Incorrect Calls (GT): 2/57
- Incorrect Calls (TT): 15/57

- The estimated no-call rate (based on MZ: DZ ratio of 20:70) was 10.6% (95% CI: 6.9-14.3).

Table 5 shows the combined MZ and DZ gender counts; overall gender test accuracy was 100% (102/102; 95% CI: 95.2-100).

Table 5: Comparison of Gender Truth and Test Results

<table>
<thead>
<tr>
<th>Gender Truth</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Males</td>
<td>2 Males</td>
</tr>
<tr>
<td>1 Male</td>
<td>1 Male</td>
</tr>
<tr>
<td>1 Female</td>
<td>1 Female</td>
</tr>
<tr>
<td>0 Males</td>
<td>0 Males</td>
</tr>
</tbody>
</table>

- One DZ case received a no call; the gender test no-call rate was 1.1% (95% CI: 0.1-6.1).

Conclusions

• This study demonstrates that SNP-based NIPT can accurately detect aneuploidy in twin gestations and is the first study to accurately detect a) zygosity and b) gender of each fetus in twin gestations.

• The ability to determine zygosity could improve risk determination for conditions such as TTTS.

• Further studies are needed to confirm clinical performance.

References

11. The following authors contributed to the development of the NIPT test: L. Hsu, T. Cordero, A. Ananth, C. V. and C. T. Lee. All authors have contributed equally.

Presented at the 3rd ESHRE-PAS Joint Symposium in Clinical Genetics and Birth Defects, May 30-June 1, 2018 | Hong Kong.
Clinical evaluation of NIPT for women at advanced maternal age: a multicenter retrospective study.

B Yu, H Li, YP Chen, ZM Cai & T Wang.
Prenatal Diagnosis Center, Changzhou Women and Children Health Hospital affiliated to Nanjing Medical University, China.

Objective: To explore the clinical effect of non-invasive prenatal testing (NIPT) for the women at advanced maternal age (AMA) and discuss the relationship between women age and NIPT effect.

Methods: 14035 women at AMA who accepted NIPT from two prenatal diagnosis centers were recruited for this study. NIPT were checked by Illumina Next CN 500. All of the AMA women received prenatal genetic counseling, selected prenatal diagnosis and different clinical treatment according to the results of NIPT.

Results: ①114 cases (0.81%) got the NIPT positive results of T21/T18/T13. 104 cases accepted prenatal diagnosis and 87 cases were proved as true positive. The sensitivity, specificity, PPV and NPV were 100%, 99.88%, 92.55% and 100% respectively. ② 74 women (0.53%) showed NIPT positive results of SCAs. After informed consent, 46 women (62.2%) accepted fetus karyotype analysis. 19 cases were identified as true positive results, while 27 cases were false positive results. The PPV for SCAs in AMA women was 41.3%. ③ By univariate analysis, we found the risk of T21/T18/T13 for the women over than 40 would significantly increased (OR=2.90, p=0.0069).All of the parameters of the pregnant women over than 40 reached 100%. It was worth noting that PPV had greatly improved.

Conclusions: NIPT is a good choice for AMA pregnant women. It can not only achieve satisfactory clinical effect, but also greatly reduce invasive prenatal diagnosis. We will get better effect of NIPT by further manage AMA women stratified by their age.
Not all chromosome aberrations can be detected by NIPT in women at advanced maternal age: a multicenter retrospective study.  
YP Chen, Y Shi, B Zhang, Q Zhou, B Yu, & T Wang.  
Prenatal Diagnosis Center, Changzhou Women and Children Health Hospital affiliated to Nanjing Medical University, China.

Objective: To discuss the detectability of NIPT for pregnant women at advanced maternal age (AMA), and mainly focused on how many fetal abnormalities will be missed by NIPT.

Methods: A total of 4194 women at AMA who accepted cytogenetic prenatal diagnosis were recruited in this study. All the AMA women received amniocentesis at 18~23 weeks after clinical consultation. Combined with our detection level of NIPT and literature reports, we evaluated the detectability of NIPT.

Results: After cell karyotype analysis, a total of 233 (5.56%) fetuses were confirmed to have chromosomal abnormalities, including 91.0% were abnormal chromosome number and 9.0% were abnormal chromosome structure. According to the detectability of NIPT we calculated, 87.6% abnormal results could also be detected by NIPT. However, NIPT would miss 12.4% abnormal results which could be originally found by the karyotype analysis of amniotic fluid cells. The major types of missed fetal abnormalities include structural rearrangement, mosaic and triploidy. Meanwhile, there were no relationship between the detectability of NIPT and the age of AMA pregnant women.

Conclusions: About 12.4% of fetal chromosomal abnormalities will be missed if NIPT completely replaces invasive prenatal diagnosis in AMA women. The undetected abnormalities do not rise with the increase of pregnant women age.
Haplotype-based noninvasive prenatal testing for duchenne muscular dystrophy: A pilot study in South China.

M Chen, HC Yan, C Chen, QZ Zheng, YX Zeng, DJ Chen.
Department of Fetal Medicine and Prenatal Diagnosis, The Third Affiliated Hospital of Guangzhou Medical University, China.

Objective
To explore the accuracy and feasibility of a haplotype-based noninvasive prenatal testing (NIPT) for Duchenne Muscular Dystrophy (DMD).

Methods
Singleton pregnancies at 12-25 weeks of gestation from seventeen families, each with a proband affected by DMD were recruited in the antenatal clinic.

The causative mutations in probands and their mothers were previously identified by multiplex ligation-dependent probe amplification (MLPA). Captured sequencing was performed on genomic DNA from parents and proband using customized hybridization probes targeted at highly heterozygous 2358 SNPs located within the 1M region flanking DMD gene and its coding region to acquire parental haplotypes and the linkage to pathogenic mutations. Maternal plasma DNA obtained at 12-25 weeks of gestation also underwent targeted sequencing to deduce fetal haplotypes assisted by parental haplotypes. The fetal genotypes in DMD gene were further validated by invasive procedures of prenatal diagnosis.

Results
The haplotype-based NIPT was successfully performed in all families. Four female and six male fetuses were identified to be normal. Four female fetuses were carriers and three male fetuses were DMD patients due to exons 49-52 deletion, exons 8-37 deletion and c.628G>T, respectively. All these results were consistent with those of invasive procedures.

Conclusion
Haplotype-based noninvasive prenatal testing for DMD using targeted sequencing is promising and has potential for clinical implementation.
The relationship between noninvasive prenatal testing failure and adverse prenatal outcomes.

JX Li, PB Yuan, XJ Wang, WY Fu, QM Ou & Y Wei.
Department of Obstetrics and Gynaecology, Peking University Third Hospital, China.

Objective: This study aimed to follow up the prenatal diagnosis results and preinatal outcomes of pregnant women who fail to obtain a Noninvasive Prenatal Testing (NIPT) result, and assess risk of an adverse perinatal outcome for women with a no-call result on NIPT.

Methods: A retrospective cohort study whereby women with no calls were compared with women who had successful calls on NIPT. All pregnant women undergoing NIPT at the collatory from October 2016 to April 2017. Inclusion criteria were NIPT success at the first time as a control group named Successful Calls (SC), and NIPT failure women who have no calls results at least two times within a week or two as the dose group. According to the failure reason, two dose groups were divided by No Calls for Low Fatal Fraction (NCL) and No Calls for An Indeterminate Range (NCI). Exclusion criteria were women with chronic diseases before pregnancy, husband or wife had chromosomal abnormalities. Primary outcome was a composite of any of the following: miscarriage, fetal demise, neonatal death, preterm birth, pregnancy-associated hypertensive disorder, placental abruption, low birth weight, and fetal aneuploid. All data were performed by statistical analysis.

Results: 158 (74.2%) women had a SC result, and 55 (25.8%) women had a NC result. 47 (22%) women is NCL and 8 (3.8%) women is NCI. Women with a NCL result were more likely to have a higher body mass index (BMI) (mean BMI = 26.4 kg/m² vs BMI = 25.1 kg/m² vs BMI = 23.7 kg/m²; p ≤ 0.001) than women with an AC result and NCI result. The composite outcome was significantly more common in the NCL group (21/47 44.7% vs 27/158 17.1%; p = 0.001). After adjusting for BMI, NCL remained independently associated with adverse perinatal outcome with adjusted odds ratio = 4.613 (95% confidence interval 2.105-10.111; p < 0.001).