Zygosity, Aneuploidy, and Gender Count Determination in Twin Gestations Using SNP-Based NIPT

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Introduction

- The prevalence of twin gestations is approximately 1/30 of all live births in the United States.[1]
- Twin gestations are at an increased risk of fetal loss and/or anomalies including structural and congenital abnormalities, and an increased risk for aneuploidy.[2]
- 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 chorionicity and zygosity.
  - For example, monozygotic (MZ), monochorionic twins have a 10% risk of fetal morbidity and mortality attributed to twin-to-twin transfusion syndrome (TTTS).[4,5]
- Given the prevalence of and complex antenatal management associated with twins, there is an unmet clinical need for accurate, early risk-determination of chromosomal abnormalities and other complications such as TTTS in twin gestations.
- Previously, we have demonstrated analytical[6] and clinical validation[7] of whole-chromosomal aneuploidy screening in singletons 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 clinics/prenatal centers.
  - Inclusion criteria were a) GA ≥9 weeks and b) clinical truth for zygosity status, fetal chromosome copy number, and/or gender count (acceptable sources were genetic test reports, verbal clinical follow-up with clinician/patient; visual assessment by parent [gender and aneuploidy only; for aneuploidy, only in twins ≥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[8]; analyses were performed using a proprietary algorithm.
  - Aneuploidy was not tested on X and Y chromosomes for DZ twins.
- 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 dizygotic (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 copy number, and gender accuracy were calculated using the Clopper-Pearson 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 (30:70)[9]; as described previously, aneuploidy no-call estimations included only samples with GA ≥10 weeks.[10]
  - Samples that received a no call were excluded from corresponding analyses.
- Confidence intervals were computed at the 95% confidence level.

Results

- A total of 126 patient samples from twin pregnancies were included in the study.
- The mean maternal age of the cohort was 32.8 ± 5.37 years; the mean GA was 15.5 ± 4.72 weeks (**Table 1**).

Table 1: Cohort Characteristics

	Samples (n=126)
Maternal Age (Years)	
Mean ± SD	32.8 ± 5.37
Median (Range)	33.0 (18.0 – 46.0)
Gestational Age (Weeks)	
Mean ± SD	15.5 ± 4.72
Median (Range)	13.64 (9.0 – 34.14)

- For MZ twins, the mean total FF was 13.0 ± 4.45; in DZ twins, the mean FF of the two fetuses were 6.37 ± 3.1 (Twin 1) and 7.96 ± 3.49 (Twin 2; **Table 2**).

Table 2: Fetal Fraction Estimates

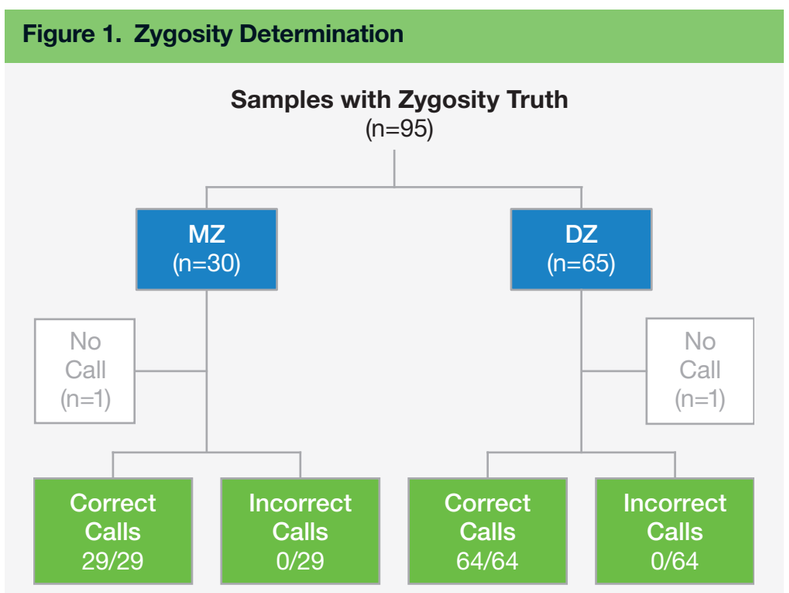
	MZ (n=44)	DZ; Twin 1/Twin 2 (n=82)
Fetal Fraction (%)		
Mean ± SD	13.0 ± 4.45	6.39 ± 3.1 / 7.96 ± 3.49
Median (Range)	12.9 (3.91 – 23.8)	6.12 (1.9 – 21.0) / 7.16 (2.6 – 21.3)

MZ, monozygotic; DZ, dizygotic.

Zygosity Determination

- Zygosity was evaluated in 95 samples (**Figure 1**).

Figure 1. Zygosity Determination



MZ, monozygotic; DZ, dizygotic.

- Overall zygosity accuracy was 100% (93/93; 96.1–100; **Table 3**).

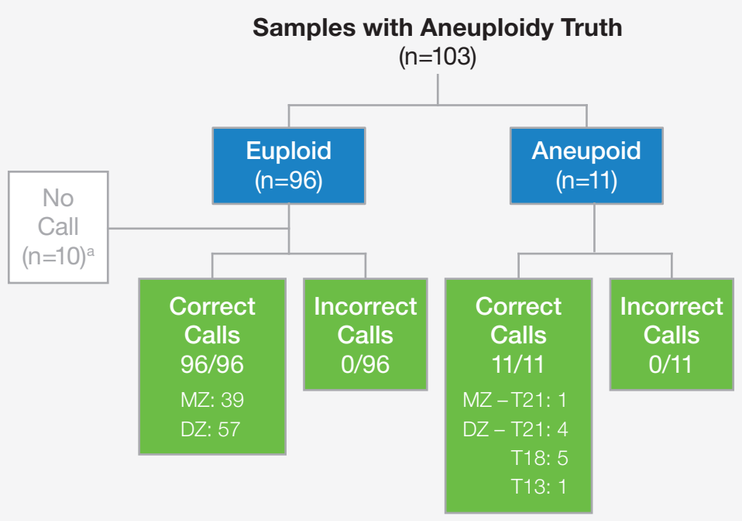
Table 3: Zygosity Test Performance

Performance	Performance, % (n; 95% CI)
Overall Zygosity Determination	100 (93/93; 96.1–100)
Monozygosity Detection	
Sensitivity	100 (29/29; 88.1–100)
Specificity	100 (64/64; 94.4–100)

Aneuploidy Determination

- Fetal chromosomal aneuploidy was evaluated in 103 samples (**Figure 2**).

Figure 2: Aneuploidy Determination



MZ, monozygotic; DZ, dizygotic.
[a]GA ≥10 weeks.

- Overall aneuploidy specificity was 100% (96/96; 95% CI 94.8–100; **Table 4**).

Table 4: Aneuploidy Test Performance

Performance	Performance, % (n; 95% CI)
Overall Specificity	100 (96/96; 94.8 – 100)
Sensitivity Breakdown	
MZ	100 (1/1)
DZ	100 (10/10; 69.2 – 100)
Specificity Breakdown	
MZ	100 (39/39; 91.0 – 100)
DZ	100 (57/57; 93.7 – 100)

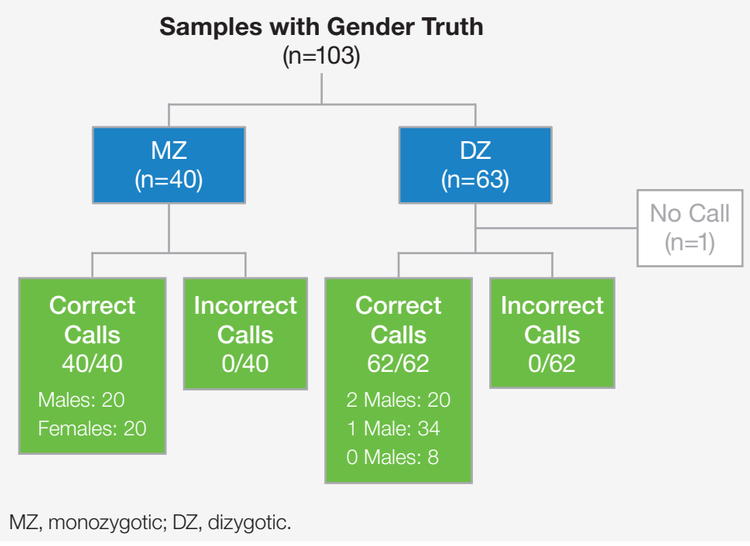
MZ, monozygotic; DZ, dizygotic.

- The estimated no-call rate (based on MZ:DZ ratio of 30:70)[9] was 10.6% (10/87 [MZ, 0/21; DZ, 10/66]; 95% CI 5.3–19.7).

Gender Determination

- Gender was evaluated in 103 samples **(Figure 3)**.

Figure 3: Gender Determination



MZ, monozygotic; DZ, dizygotic.

- **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

	Gender Truth		
Test Result	**2 Males**	**1 Male**	**0 Males**
2 Males	40	-	-
1 Male	-	34	-
0 Males	-	-	28

- One DZ case received a no call; the gender test no-call rate was 1.1% (1/103 [MZ, 0/40; DZ, 1/63]; 95% CI 0.03–6.6).

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

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