Noninvasive prenatal testing (NIPT) in multiple gestations: A report of laboratory experience.

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Introduction

Invasive prenatal diagnosis by CVS or amniocentesis has been widely used for decades despite the small risk of fetal and maternal complications. Not all women who are candidates for testing opt for invasive testing and those with multiple gestations, facing more than one invasive procedure, pursue it even less frequently. Saldivar, et al. have presented their overall experience in >60,000 patients undergoing NIPT for fetal aneuploidy[1]. This companion submission describes the subset of patients having multiple gestations, within a now larger laboratory experience of >90,000 patients.

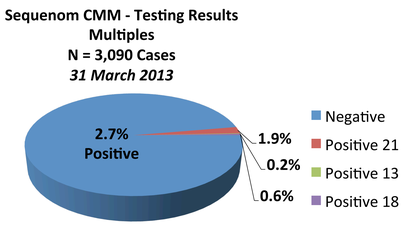
Methods

Noninvasive prenatal testing (NIPT) using massively parallel sequencing (MPS) to analyze cell-free fetal DNA in maternal plasma, detects a relative increase or decrease in a particular chromosome's representation. The Sequenom Center for Molecular Medicine has processed more than 90,000 MaterniT21™ PLUS laboratory developed test clinical samples following the methodology reported in the online supplement by Palomaki, et al.[2,3] All of the samples were referred for testing from women on the basis of an *a priori* increased risk for fetal aneuploidy. Results were tabulated and further detailed according to the indication for testing. All clinical samples tested in the Sequenom Center for Molecular Medicine laboratory were subject to multiple levels of quality control checks to ensure the highest quality prior to resulting by the laboratory director. Herein, we describe our clinical experience in multiple gestations, compared to the entire cohort of patient samples.

Results

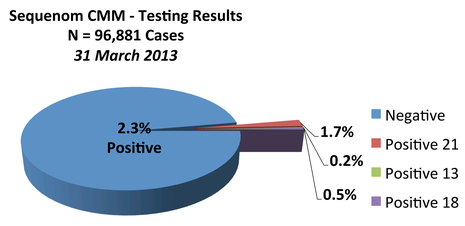
Of the 96,681 patients at increased risk for fetal aneuploidy tested by Sequenom Center for Molecular Medicine by the MaterniT21 PLUS test, there were 3,090 (3.2%) patients with multiple gestations. 2.7% of the twins had positive MaterniT21 PLUS test results for an increased representation of the target chromosomes: chromosome 21 (1.9%); chromosome 18 (0.6%); and chromosome 13 (0.2%), illustrated in Figure 1. The indications for testing in multiples included age-related risk (70.5%), ultrasound abnormalities (25.2%), positive serum screening results (11.5%), and history of aneuploidy (5.0%), illustrated in Figure 2. These findings are similar to the cohort of all clinical samples, 96.8% of which were from singleton pregnancies (see Figures 3 and 4) with the exception of the expected decline in serum screening indication in multiples given the less frequent use in these pregnancies. As expected, both fetal DNA counts (copies/mL) as well as fetal fraction (%) differed significantly when comparing multiple gestations to the entire cohort (Table 1). These data are depicted graphically in Figure 5.

Figure 1: Predicted Clinical Detection Rates in Multiple Gestations



The figure above depicts the predicted rates for the detection of fetal aneuploidy in 3,090 multiple gestation cases received by Sequenom CMM through 31 March 2013.

Figure 3: Predicted Clinical Detection Overall



The figure above depicts the predicted rates for the detection of fetal aneuploidy in 96,681 cases received by Sequenom CMM through 31 March 2013.

Figure 5: Graphic depiction of Fetal Fraction in Multiples vs. Singleton Pregnancies

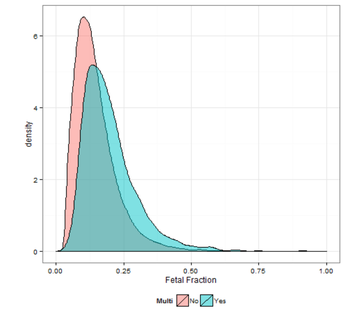
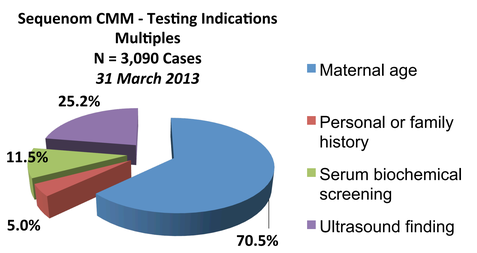
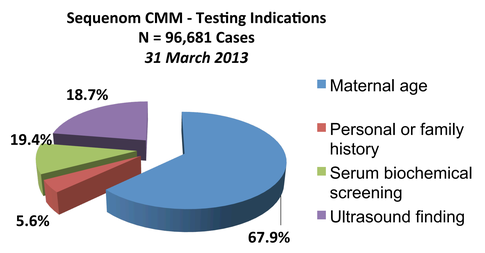


Figure 2: Clinical Indications for Testing in Multiple Gestations



The figure above depicts the indications for patient testing in the 3,090 cases in multiple gestations received by Sequenom CMM through 31 March 2013.

Figure 4: Clinical Indications for Testing Overall



The figure above depicts the indications for patient testing in the 96,681clinical cases received by Sequenom CMM through 31 March 2013.

Figure 6: Sequenom CMM Laboratory Cases, Through March 2013

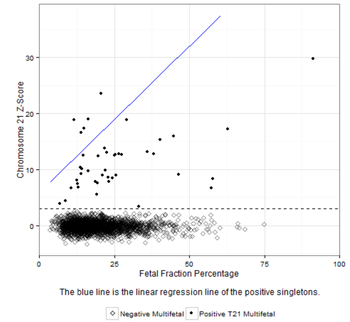


Table 1: Fetal DNA Counts and Fetal Fraction in Multiple Gestations

	Patient Samples	Median Fetal Fraction	Median Fetal DNA Counts
Multi-fetal Gestations	3,090	0.18	235
Singleton Gestations	93,591	0.13	131
Total Samples	**96,681**	**0.13**	**132**

	Difference in Median Values	H_a: $\mu_{multiple} > \mu_{singletons}$
Fetal Fraction	0.05	p-value <0.0001
Fetal Counts	104	p-value <0.0001

Table 2: Performance Based on *Ad Hoc* Feedback

Sequenom CMM Clinical Laboratory Performance Data

Laboratory Reported Cases (January-December 2012)

Chromosome	Number of cases reported as Negative	Number of cases reported as Positive	Number of False Negatives communicated to SCMM*	Number of False Positives communicated to SCMM*
21	58,273	1,087	7	7
18	57,315	272	5	10
13	57,315	124	4	10

** The above table summarizes the clinical outcome feedback we have received from ordering clinicians through February 28, 2013.*

(Data in multiples: Too few cases)

Conclusions

NIPT offers physicians and their pregnant patients at increased risk for fetal aneuploidies reliable information about risks for fetal aneuploidy in pregnancy. This report highlights our experience in pregnancies with multiple gestations. Apart from a larger percent of cases with an ultrasound finding indication in multiples, which might also explain the very slight increase in positivity rates, there appears to be no appreciable difference in the predicted results between singleton and multiple pregnancies. As expected, the fetal fraction and counts of fetal DNA are greater in multiple gestations than in singleton pregnancies, but not double, likely reflecting the overall increase in placental mass noted in multiples (but also not double). Finally, reported *ad hoc* feedback from ordering physicians as presented by Saldivar, et al.[1] (Table 2) included multiple gestations (encompassing cases with concordant and discordantly affected fetuses, which cannot be predicted by testing), and results are consistent with the findings in the original clinical validation studies published by Palomaki, et al.[2,3]

References

1. Saldivar, et al. Noninvasive prenatal testing (NIPT) using the MaterniT21 PLUS test: The clinical experience. American College of Medical Genetics Annual Meeting. Poster #533. Phoenix, AZ March 2013.
2. Palomaki GE, et al. DNA Sequencing of Maternal Plasma to Detect Down Syndrome: An International Clinical Validation. *Genet Med.* 2011; Nov 13; (11):913-920.
3. Palomaki GE, et al. DNA Sequencing of Maternal Plasma Reliably Identifies Trisomy 18 and Trisomy 13, as well as Down Syndrome: An International Collaborative Study.*Genet Med.* 2012; Mar 14; (3):296-305.