MAGEE-WOMENS HOSPITAL OF UPMC'S CLINICAL EXPERIENCE WITH NON-INVASIVE PRENATAL TESTING

by

Joya J. Petersen

BS, Molecular Biology, University of Pittsburgh, 2016

Submitted to the Graduate Faculty of the

Department of Human Genetics

the Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2018

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

This thesis was presented

by

Joya J. Petersen

It was defended on

April 3, 2018

and approved by

Thesis Director: Robin E. Grubs, MS, PhD, LCGC
Associate Professor and Director of Genetic Counseling Program
Department of Human Genetics
Graduate School of Public Health, University of Pittsburgh

Committee Members:

Luanne Fraer, MS, LCGC
Supervisor, Magee-Womens Hospital of UPMC Pregnancy Screening Lab
Adjunct Instructor, Department of Human Genetics
Graduate School of Public Health, University of Pittsburgh

Robert Krafty, PhD
Associate Professor, Department of Biostatistics
Graduate School of Public Health, University of Pittsburgh

Aleksandar Rajkovic, MD, PhD
Medical Directory, Center of Medical Genetics and Genomics
Magee-Womens Hospital of UPMC
Associate Professor, Department of Human Genetics
Graduate School of Public Health, University of Pittsburgh

Svetlana Yatsenko, MD, PhD
Director, Pittsburgh Cytogenetics Laboratory
Visiting Associate Professor, Department of Human Genetics
Graduate School of Public Health, University of Pittsburgh

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ABSTRACT

The clinical introduction of Non-invasive Prenatal Testing (NIPT) in the United States in 2011 significantly impacted the field of prenatal genetic screening. Through molecular analysis of cell-free fetal DNA extracted from maternal serum samples, NIPT provides pregnant women with their risk to have a child with the most common aneuploidies seen in live born children: trisomy 21, 18, 13, and sex chromosome abnormalities. Moreover, the detection rates for the conditions screened for by NIPT are higher than those offered by prior prenatal genetic screening tests and NIPT poses no additional risk to the fetus unlike prenatal genetic diagnostic testing (i.e., chorionic villi sampling and amniocentesis). Clinical utilization of NIPT to screen for fetal aneuploidy is rapidly growing; multiple professional organizations have released position statements recommending that NIPT be offered to all women as a genetic screening option.

With the rapid expansion of NIPT it is important to assess the clinical outcome of this emerging technology to inform genetic counseling practices and policy development. This study is a retrospective medical records review of data from women who had NIPT through Magee-Womens Hospital of UPMC's Center for Medical Genetics and Genomics, a high-risk referral center, from January 1, 2014 to December 31, 2016. A total of 2,589 women had non-invasive prenatal testing (NIPT) and 95 women (3.67%) from the original cohort who received a positive or failed result were included in the analysis. Our results showed that the positive predictive

value for trisomy 21 was 100% (95% CI:90.5-100), 66.7% (95% CI:9.4-99.2) for trisomy 18, and 50% (95% CI: 98.7) for trisomy 13. The NIPT fail rate was 1.62% and concordant with the test fail rates published by laboratories and prior studies. Continued analysis of the clinical outcomes and utilization of NIPT should be performed in order to provide more accurate genetic counseling, inform universal screening practices, and improve prenatal care of pregnancies with aneuploidy. This study is relevant to public health because it contributes to current knowledge of the clinical outcomes of NIPT to aid healthcare professionals in assessing patients' risks for fetal aneuploidy.

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PREFACE

I would like to express the deepest gratitude to my committee members: Mrs. Luanne Fraer, Dr. Robert Krafty, Dr. Aleksandar Rajkovic, and Dr. Svetlana Yatsenko. Without your support and expertise, this project would not have been possible. I also want to extend a resounding thank-you to my outstanding thesis advisor, Dr. Robin Grubs. As my thesis advisor and program director, your boundless patience, dedication, and guidance has inspired me beyond words. The same can be said for my assistant program director, Dr. Andrea Durst. Simply stated, Andrea you are the best!

Special thanks to my outstanding classmates, who have been immense sources of humor, encouragement, and compassion over the past two years. I cannot wait to see how we will transform the world of genetic counseling in the years to come! To the magnificent Dr. Devereux Saller, Jr and Elizabeth Rusiewicz, in addition to Magee-Womens Hospital's superlative genetic counseling assistants, prenatal genetic counselors, you all deserve an honorable mention.

With the fullest of hearts, I must thank my friends and family who have been a never-ending wealth of love and support throughout my thesis journey. Whether it is sarcastic memes, short phone calls, or late night texts, I've felt your love from hundreds of miles away. Because of your unfaltering faith in me, I've soared to higher heights than I could ever imagine.

1.0 INTRODUCTION

Fetal aneuploidy is the leading known cause of miscarriage and congenital birth defects in the United States[1,2]. It is estimated that 10-30% of pregnancy losses are due to fetal aneuploidy and 0.65% of live born infants have chromosomal abnormalities[3,4]. Pregnancies with chromosome abnormalities pose a significant risk to the mother for miscarriage and complications due to miscarriage such as infection and excessive bleeding. Aneuploid fetuses are at an increased risk for miscarriage/fetal demise and can exhibit multiple congenital anomalies, growth restriction, and other pre- and postnatal complications. The chance for individuals to have a child with a chromosome abnormality increases with maternal age[5]. Women who will be age 35 or older at the time of delivery, are classified as advanced maternal age and are considered to be at an increased risk to have a child with aneuploidy[5,6].

Prenatal genetic screening tests are designed to aid healthcare professionals in identifying pregnant women who are at an increased risk for fetal aneuploidy within the general population. Throughout the United States, pregnancy screening for fetal aneuploidy is offered to all pregnant women as the standard of care. Early identification of fetuses at an increased risk for chromosome abnormalities enables families to diagnose medical conditions affecting the fetus prior to birth and make medical management decisions regarding the developing fetuses. Medical decision making can include planning of neonatal medical interventions, coordination of palliative care services, and/or discussion of pregnancy termination options. Genetic counseling

can also be offered to families to provide information with regards to the clinical features and prognosis of genetic conditions, and other genetic testing options for the pregnancy, as well as to support families in autonomous decision making[5,7].

Prenatal genetic screening tests include ultrasound and analysis of maternal serum samples for specific hormone and protein markers. The sensitivities of these screening methods are in the range of 69-95% for the detection of trisomies 21, and 18. Diagnostic testing options of amniocentesis and chorionic villus sampling offer pregnant women higher sensitivities of approximately 99% but these procedures pose a risk of miscarriage of less than 1%[8]. In 2011, a new prenatal screening test known as Non-invasive Prenatal Testing (NIPT) or cell-free fetal DNA testing was made commercially available throughout the United States[9,10].

NIPT offers higher detection rates and screens for a broader array of conditions than prior prenatal screening options at no additional risk to the fetus[10]. The American College of Obstetricians and Gynecologists (ACOG) in conjunction with the Society for Maternal Fetal Medicine (SMFM) have released a committee statement supporting the use of NIPT to screen high-risk pregnancies for fetal aneuploidy[6,11]. NIPT utilizes next generation sequencing to analyze maternal serum samples for the presence of fetal DNA in order to determine if there are an increased or decreased amounts of chromosomes 13, 18, 21, X and Y. The sensitivity and specificity of NIPT to detect trisomies 21, 18, and 13 are approximately 97-99% as reported by commercial testing companies[12–17]. Due to its high accuracy and non-invasive methodology, NIPT is becoming increasingly popular as a screening option for high-risk pregnancies[18].

However, studies on the clinical outcomes and accuracy of NIPT have been fairly limited to those sponsored by commercial testing companies or clinical centers outside of the United States. Furthermore, only a few studies that have analyzed the clinical use of NIPT have included

follow-up of NIPT results to determine how frequent a NIPT result is congruent with the birth outcome and/or diagnostic testing result[19,20]. Follow-up of NIPT results can help to determine the positive predictive value, true positive rate, false positive rate, false negative rate, and true negative rate of NIPT. These types of results can aid genetic counselors and other healthcare providers in providing more accurate information about NIPT to their patients considering prenatal screening for chromosome conditions and inform healthcare professionals of further pregnancy management options that may be indicated by specific NIPT results.

2.0 LITERATURE REVIEW

2.1 ANEUPLOIDY

Aneuploidy is defined as the presence of abnormal amounts of chromosomes in a cell[3,21]. According to ACOG, aneuploidy embryos accounts for 10-30% of all pregnancies[1,22]. The most common aneuploidies observed in liveborn infants are trisomy 21, trisomy 18, trisomy 13, and sex chromosome aneuploidies that include XXY, XYY, XXX, and 45, X[3,23]. Newborns with aneuploidy can experience shortened lifespans, congenital birth defects, failure to thrive, and/or intellectual disability[21].

Due to the high proportion of early pregnancy losses associated with fetal aneuploidy, the incidence of newborns with chromosomal abnormalities is estimated to be less than 1% (1 in 150) livebirths[6]. Approximately 1 in 691 liveborn infants in the United States are diagnosed with trisomy 21, a condition also known as Down syndrome[23]. The incidence of trisomy 18 and 13 is estimated to be 1 in 3,762 livebirths and 1 in 7,906 livebirths, respectively[23]. The incidence of sex chromosome aneuploidies can range from about 1 in 400 to 1 in 4000 births depending on the exact condition.

The prevalence of aneuploid pregnancies increases with maternal age[6]. Women who are 35 years or older at the time of delivery, are defined as “advanced maternal age” and are considered to be at an increased risk for fetal aneuploidy[6]. A woman’s age-related risk for fetal aneuploidy is not influenced by their race or ethnicity however, family history of inherited chromosome rearrangements (for example, a balanced translocation), abnormal ultrasound findings, a prior pregnancy history and/or positive pregnancy screening results can increase an

individual's baseline risk[6]. Additional prenatal screening and diagnostic testing in addition to alternative medical management plans and services should be offered to individuals who are identified to be at an increased for fetal aneuploidy[5,6].

2.2 PRENATAL SCREENING AND DIAGNOSIS FOR GENETIC CONDITIONS

2.2.1 PRENATAL DIAGNOSTIC TESTING

There are two types of prenatal diagnostic testing currently available: chorionic villus sampling (CVS) and amniocentesis[24,25]. Both of these procedures are invasive requiring physicians to extract a sample from the pregnancy to perform genetic analysis[24,26,27]. Genetic analytic methods performed on prenatal tissue samples obtained from amniocentesis and chorionic villi include karyotype, fluorescence in-situ hybridization (FISH), and/or targeted molecular analysis. Karyotyping of cultured cells is estimated to be 97.5-99.8% accurate, while FISH and microarray analysis are estimated to be greater than 99% accurate[28].

The use of amniocentesis has been reported in the medical literature as early as the 1870s[25,26]. During the 19th century, amniocentesis was primarily utilized as a means of reducing the buildup of amniotic fluid in cases of polyhydramnios[26,29]. Physicians began to expand their use of amniocentesis in the 1930s in order to manage cases of erythroblastosis fetalis, a hematological condition that arises when there is an incompatibility between the Rh-status of the mother and fetus[25]. Following the advent of karyotyping, researchers were able to develop a method of culturing fetal cells obtained from amniocentesis to create a karyotype by the late

1960s[26,27,29]. Through this technological advancement, physicians were able to identify chromosomal abnormalities affecting pregnancies such as Down syndrome, and modify women's pregnancy management in relation to the amniocentesis results.

By the mid-1970s amniocentesis became the standard procedure for obtaining fetal karyotypes[27,29]. Amniocentesis can be performed for pregnancies that reach at least 15 weeks gestation[26,29]. In addition to karyotype analysis, amniotic fluid samples can be used to assess a pregnancy's risk for open neural tube and abdominal wall defects by measuring alpha-fetoprotein (AFP) and acetylcholinesterase (AChE) levels[30].

Chorionic villus sampling (CVS) is a prenatal diagnostic method that was developed in the 1980s to offer patients a diagnostic test that can be safely performed earlier than amniocentesis; CVS can be performed at 11-13 weeks of gestation[24]. There are two approaches for sample extraction including transcervical and transabdominal, the method used depends on the position of the placenta relative to the fetus[8,27,31]. The accuracy of karyotyping and microarray analysis for samples obtained by CVS is generally over 99%, however in cases of suspected placental mosaicism clinicians may recommend amniocentesis and/or additional testing to clarify results[24].

Both CVS and amniocentesis confer a risk for complications leading to a miscarriage or preterm labor due to the invasive nature of their sample collection procedures[27,29]. Multiple studies have shown that due to the earlier age of pregnancy during which CVS is performed, there is a higher risk of miscarriage associated with CVS when compared to amniocentesis[32]. However, there has been no difference observed in the pregnancy loss rate for transcervical vs. transabdominal CVS procedures[8,27]. While, randomized trials have found the risk for miscarriage

after CVS or amniocentesis range from 0.1- 1%, the risk of pregnancy loss for both procedures is often lower than in more experienced centers[8].

2.2.2 MATERNAL SERUM SCREENING

In order to identify pregnancies at an increased risk for fetal aneuploidy and/or congenital birth defects without increasing individuals' risk for complications leading to miscarriage or preterm delivery, pregnancy screening tests were developed[4,33]. The first type of pregnancy screening test developed was ultrasonography[34,35]. Ultrasound imaging provides clinicians with information regarding pregnancy viability, dating, placental location, fetal number and anatomical features[34]. Fetuses with chromosome abnormalities can have distinctive congenital anomalies which can be detected by ultrasound[34,36]. Ultrasound findings commonly observed in fetuses with Down syndrome include shortened long bones, absent nasal bones, echogenic intracardiac focus, increased nuchal thickness, and choroid plexus cysts[37]. However, all fetuses with chromosome abnormalities will not have congenital abnormalities that can be detected by ultrasound[35,38,39].

A detailed anatomical ultrasound can be performed at 18 to 22 weeks of pregnancy to assess the fetus' growth, position, movement, and anatomy[34,40]. This analysis confers a higher detection risk for common aneuploidies than that offered by standard ultrasounds[37,41]. Approximately, 73% of Down syndrome pregnancies can be detected by ultrasound (when performed at 18-20 weeks of pregnancy and by an experienced ultasonographer) and 90% of pregnancies with trisomy 18 or trisomy 13 can be detected at this time[36,37,41]. The false positive rate for trisomy 21 detected by ultrasound is about 4%[36,41]. Ultrasound imaging can also be used to detect open neural tube and abdominal wall defects.

Multiple marker screening was developed in the late 1980s to detect pregnancies that are at an increased risk for Down syndrome and open neural tube defects[42]. In the early 1990s, researchers discovered that multiple marker screening could also be used to screen for trisomy 18[42]. Multiple marker screening computes a risk for a pregnancy to have trisomy 21, trisomy 18, and open neural tube defects by measuring the presence of biochemical markers in the maternal serum: alpha-fetoprotein (AFP), human gonadotrophin (hCG), unconjugated estriol (uE3) and inhibin-A[37,43]. Multiple marker screening is typically performed at 16- 20 weeks of gestation[36]. The exact number of biochemical markers that are analyzed varies based on the laboratory used, typically three (triple screen) or four (quad screen) markers are measured, although a pentascreen that analyzes five biochemical markers is also available. The markers measured by quad screens are AFP, hCG, uE3 and inhibin- A, while triple screens measure AFP, hCG, and uE3[35]. In addition to the precise measurements of these biochemical markers present in the maternal serum sample, maternal age, race, weight, diabetic status, pregnancy history, and the gestational age are all tabulated to compute a unique risk estimate for the fetus to have Down syndrome, trisomy 18, and/or an open neural tube defect[35]. The detection rate of quad screening is approximately 70-75% for trisomy 21, 60% for trisomy 18, 80% for spina bifida, and 90% for pregnancies with anencephaly (**Table 1**)[35]. The false positive rates for multiple marker screening are approximately 5% for trisomy 21 and 8% for trisomy 18 (**Table 1**)[37].

To increase the detection rate for common aneuploidies, trisomy 21 and trisomy 18, first trimester screening (FTS) was developed. First trimester screening can be performed at 11 to 14 weeks of pregnancy and involves two components, maternal serum screening and ultrasound analysis[36]. Ultrasound imaging is used to obtain a measurement of the fetus' nuchal translucency. The maternal blood sample is analyzed for the presence of pregnancy associated plasma protein

A (PAPP-A) and total human chorionic gonadotropin (total hCG). An increased nuchal translucency measurement is associated with the presence of chromosomal abnormalities.

The detection rate of first trimester screening is 90% for trisomies 18, and 21. The false positive rate of first trimester screening is about 5%[44]. Nuchal translucency alone can also be measured as a means of detecting pregnancies with aneuploidy. Measurement of the nuchal translucency has a 70% detection rate for Down syndrome[44].

To continue to improve aneuploidy detection integrated screening was developed. Integrated screening combines elements of first trimester screening and second trimester screening to quantify a pregnancy's risk to be affected with Down syndrome. During the first trimester, the nuchal translucency, maternal serum PAPP-A and hCG are measured[35]. Then the AFP, uE3, hCG, and inhibin A levels in a maternal serum sample are measured during the second trimester. The detection rate for Down syndrome by integrated screening is 94% with a 5% false positive rate (**Table 1**)[35]. Patients are only informed of the combined integrated screening result.

Table 1. Comparison of Prenatal Screening Test Detection and False Positive Rates

Screening Tests for Common Trisomies (21, 18, 13)	Detection Rate			False Positive Rate		
	21	18	13	21	18	13
Anatomic Ultrasound[36,37,41]	73%	80%	90-100%	4%	n/a	n/a
Multiple Marker Screen[37]	70-75%	60%	n/a	5%	8%	n/a
First Trimester Screen[37]	90%	90%	90%	5%	5%	5%
Integrated Screen[35]	94%	n/a	n/a	5%	n/a	n/a

2.3 NON-INVASIVE PRENATAL TESTING (NIPT)

Non-invasive Prenatal testing (NIPT), also known as cell-free fetal DNA (cffDNA) can detect pregnancies with chromosomal abnormalities including but not limited to trisomies 21, 18, 13 and Monosomy X. NIPT can be performed in pregnancies that are at least 10 weeks of gestation. In order to detect aneuploidies a maternal blood sample is collected and the amount of cffDNA contained in the sample is measured; this measurement is referred to as the fetal fraction. The cffDNA is analyzed through the massively parallel sequencing or single-nucleotide polymorphism (SNP) approach to assess whether or not the pregnancy is at an increased risk for a specific aneuploidy. Massively parallel sequencing detects fetal aneuploidy based on the representation of specific chromosomes within the sample compared to unaffected, diploid pregnancies[45]. Natera Inc. is the only commercial laboratory in the United State that uses the SNP based approach for NIPT[12]. This method has been validated by multiple research studies[44,46].

Evidence suggestive of feto-maternal cell transfer has been reported as early as the 1960s[47]. In 1969, scientists karyotyped cultured lymphocytes from 30 pregnant women and discovered cells indicative of 46, XY present in 21 samples from the pregnant women[48]. Of those 21 women with 46, XY cells detected via karyotype, 19 of them delivered male fetuses and 2 had females[48]. Although additional studies confirmed these findings of cffDNA circulating in the maternal bloodstream throughout pregnancy, a molecular diagnostic technique to precisely identify the fetal DNA had yet to be developed. However, in 1996 researchers were able to develop a method to detect tumor DNA in the bloodstream of cancer patients[48]. Drawing upon this discovery, scientists began to investigate whether similar mechanisms could be applied to

the precise detection and isolation of cffDNA circulating in the maternal bloodstream during pregnancy[48,49].

In the following year, researchers were able to accurately detect specific chromosome through the analysis of isolated cffDNA from the maternal bloodstream[48]. Through this initial study, maternal blood samples were collected from 43 pregnant women and additional non-pregnant controls[48]. Signals from the Y-chromosome were detected in 24 out of the 30 total fetal DNA PCR-products of women pregnant with male fetuses[48]. Y-chromosome signals were not detected in the plasma or serum samples of any of the controls or PCR-products generated from the cffDNA extracted from women pregnant with female fetuses[48]. Moreover, this same group of researchers determined that 10μL of maternal serum and plasma is sufficient for the accurate detection of cffDNA[50].

Through the culmination of these breakthrough discoveries, numerous validation studies, and the innovation of Next-Generation sequencing technology NIPT became clinically available in the United States in 2011. Following the release of Committee Opinion 545 from ACOG in conjunction with SMFM there was a significant uptake in the clinical adoption of NIPT. In their position statement ACOG and SMFM supported the use of NIPT to screen high-risk pregnancies for fetal aneuploidy, and the utilization of NIPT began to rapidly increase[21]. “High-risk pregnancies” are defined as those in which one or more of the following risk factors were present: mother to be 35 years or older at the time of delivery, family history of a child with a known trisomy, parent(s) is a balanced Robertsonian translocation carrier, fetus with ultrasound findings associated with an increased risk for aneuploidies, or a positive pregnancy screening test indicating an increased risk for fetal aneuploidy[5,21]. NIPT was not recommended for use in low-risk pregnancies and cases of multiple gestations, as it was not validated in these populations at

the time of its clinical release in 2011[5,9,11,21,51]. The American College of Medical Genetics and Genomics (ACMG) and ACOG published updated position statements endorsing the clinical use of NIPT to detect fetal aneuploidy for all women regardless of age in 2016[5,6]. The sensitivities and specificities of the NIPT offered by commercial laboratories is summarized in **Table 2** and the positive predictive values reported by each lab are reported in **Table 3**.

Table 2. Comparison of Clinically Available NIPT Sensitivities and Specificities

	MaterniT21/InformaSeq (15,17)		**Verifi** (16)		**Harmony** (14)		**Panorama** (12)		**Qnatal** (13)	
Aneuploidy	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
21	99.1%	99.9%	99.2%	99.9%	>99.9%	>99.0%	>99%	100%	99.1%	99.9%
18	98.3%	99.9%	96.3%	99.87%	97.4%	>99.0%	>96%	100%	99.9%	99.6%
13	98.2%	99.9%	91.0%	99.87%	93.8%	>99.0%	>99%	100%	91.7%	99.7%
X	95.0%	99.0%	n/a	n/a	n/a	n/a	>92%	100%	n/a	n/a
XX	97.6%	99.2%	97.6%	99.2%	n/a	n/a	>99.9%	100%	n/a	n/a
XY	99.1%	98.9%	99.1%	98.9%	n/a	n/a	>99.9%	100%	n/a	n/a
SCAs	96.2%	99.7%	n/a	n/a	n/a	n/a	100%	100%	96.2%	99.7%
Triploidy	n/a	n/a	n/a	n/a	n/a	n/a	>99.9%	100%	n/a	n/a

*T21=trisomy 21, T18= Trisomy 18, T13= Trisomy 13; SCA= sex chromosome aneuploidy; sex= XX and XY

Table 3. Comparison of Commercially Available NIPT Positive Predictive Values and Test Failure Rates

	MaterniT21/ InformaSeq[15,17]	Verifi[16]	Harmony[14]	Panorama[12]	Qnatal[13]
PPV	92.0%	83.5%	n/a	85.05%	n/a
Test Failure Rate	n/a	3.8%	n/a	n/a	n/a

*PPV= positive predictive value; n/a= information not available; the positive predictive values presented in this table are overall values for trisomies 21, 18, 13

NIPT's high detection rates for trisomies 21, 13, and 18 in conjunction with its average false positive rates of 0.5% are unprecedented by any other pregnancy screening test (**Table 2 & 3**). As a result, NIPT has quickly been adopted into widespread clinical use as a prenatal screening tool for fetal aneuploidy. ACMG states that clinicians should continue to offer

maternal serum AFP testing to women who choose to pursue NIPT for the detection of open neural tube defects[5]. Moreover, NIPT should not be used as a substitution for anatomic ultrasound screening[5]. It is important to recognize that a negative NIPT result does not exclude the possibility that a pregnancy may be affected[5,21]. For positive NIPT results, the positive predictive value should be calculated based on the gestational age of the pregnancy at the time of NIPT blood-draw, maternal age, and the particular condition indicated in order to interpret the result(s)[5,15,21,52]. In addition, diagnostic testing should be offered to confirm the positive NIPT result.

Patients may also receive a failed or inconclusive NIPT result when they choose to undergo screening. The most commonly reported reasons for failed NIPT results are low fetal fraction (<4%), maternal obesity, and administrative/technological failures[53–55]. NIPT results that are failed or inconclusive due to unknown reasons are considered to be an increased risk for fetal aneuploidy[53,56]. However, few clinical laboratories publicly report their test failure rates (**Table 3**). According to ACOG and ACMG, women who receive failed NIPT results should be offered additional follow-up through: repeat maternal serum sample blood draw and cffDNA analysis, additional ultrasound and/or biochemical screening, and/or diagnostic testing[5,21].

In addition to the aneuploidy conditions that NIPT companies initially screened for, most have begun to offer screening for microdeletion and duplication syndromes (see **Table 4**). Information regarding the positive predictive values, sensitivity, and specificity for the detection of these microdeletion syndromes with respect to each clinical laboratory is limited or not provided[12–15,52]. Presently, Panorama is the only non-invasive prenatal testing company that has expanded its services to offer screening for fetal triploidy[12].

Table 4. Comparison of Expanded NIPT Screening Options

	Harmony [14]		InformaSeq [15]		Materni21 Plus [17]		Panorama [12]		Qnatal Advanced [13]		Verifi Plus [16]	
Extra screening options available	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
22q (DiGeorge syndrome)	●		●		●		●		●		●	
15q deletion (Prader-Willi/ Angelman syndromes)		●	●		●		●		●		●	
11q (Jacobsen syndrome)		●	●		●			●	●			●
8q (Langer-Giedion syndrome)		●	●		●			●	●			●
5p (Criu-du-chat syndrome)		●	●		●		●		●		●	
4p (Wolf-Hirschhorn syndrome)		●	●		●			●	●		●	
1p36 deletion syndrome		●	●		●		●		●		●	
Trisomy 16		●	●		●			●		●	●	
Trisomy 22		●	●		●			●		●	●	
Triploidy		●		●		●	●			●		●

2.4 CLINICAL STUDIES ON NIPT PERFORMANCE

Following the commercial launch of NIPT in the United States, multiple validation studies were performed. In a blinded research study conducted in 2012, Bianchi et al. massively parallel sequenced 534 maternal serum samples obtained from pregnant women enrolled in the MELISSA study and undergoing prenatal diagnostic testing at 60 clinical testing centers across the United States[57]. Through massive parallel sequencing, Bianchi et al. were able to detect trisomy 21 with 100% sensitivity, trisomy 18 with 97.2% sensitivity, and trisomy13 with 78.6% sensitivity[57]. "Unclassified results" were obtained for 2.8% of participants and 10 false negative results were obtained, with sex chromosome aneuploidies comprising the majority of false negative results[57]. Meta-analysis of clinical validation studies have found similar results[52,58]. The pooled sensitivity for trisomy 21 was 99.3% (95% CI 98.9% to 99.6%), 97.4% (95.8% to 98.4%) for trisomy 18, and 97.4% (86.1% to 99.6%) for trisomy 13[58]. Another meta-analysis calculated a positive predictive value of 88.6% (95% CI 83.0–93.1) for Monosomy X and 93.8% (95% CI 85.9–98.7) for all sex chromosome abnormalities excluding Monosomy X[59].

Several clinical studies of NIPT outcomes have reported decreased detection rates for sex chromosome abnormalities[19,20]. A study conducted by Petersen et al. performed cytogenetic analysis on 712 maternal serum samples that received an abnormal NIPT result[19]. The positive predictive value for Monosomy X was 27%, while the positive predictive value for Klinefelter syndrome (XXY) was approximately 85%[19].

Adverse pregnancy outcomes and fetal aneuploidy have been associated with failed and inconclusive NIPT results[53,54]. Some risk factors that predispose individuals to receive a failed NIPT result have been identified. Multiple studies have shown that maternal use of anticoagulants during pregnancy, maternal metastatic malignancies, and maternal weight

exceeding 270 pounds all increase an individual's risk to receive a failed NIPT result[54,57,60,61]. The average test failure rate reported by clinical sites and meta-analysis is approximately 1%, however test failure rates as high as 6% within a cohort have been reported[19,20,54,58,59]. More research is need to elucidate the biological mechanisms and risk factors that contribute to NIPT failure.

2.5 FUTURE DIRECTIONS FOR NIPT

Only five years after NIPT entered the clinical market, Sequenom became the first clinical testing company in the United States to launch a genome wide non-invasive prenatal test [62]. Entitled, MaterniT Genome, this screening test can be performed as "early as nine weeks gestation" through a maternal blood draw[62]. MaterniT Genome reports information regarding the fetus' risk for common trisomies (13,18,21), sex chromosome aneuploidies, seven microdeletions (**Table 4**), and "clinically relevant" microdeletions or duplications at least 7 Mb in size on every chromosome throughout the genome[62]. Globally, clinical testing companies have also began to investigate the use of NIPT to detect monogenic disorders[63,64].

However, ACOG strongly cautions against the use of NIPT for the detection of microdeletion disorders[6]. Although ACMG does not discourage the use of NIPT microdeletion panels they assert that extensive pretest counseling and/or genetic counseling should be provided to patients if they choose to have this screening[5]. No professional organizations have recommended the use of NIPT to screen for triploidy or genome-wide NIPT analysis[6,9,51,65,66].

With the rapid evolution and expansion of NIPT subsequent information on its clinical performance is needed in order for clinicians and genetic counselors to provide patients with

adequate information about the test. The false positive rates, positive predictive values, detection rates, and test failure rates are crucial measures to assess the accuracy and clinical validity of expanded NIPT options. Presently, few studies independent of commercial laboratory sponsorship have been published regarding the clinical use/experience of genetic testing centers with NIPT.

3.0 MANUSCRIPT

3.1 BACKGROUND

3.1.1 ANEUPLOIDY

Aneuploidy is defined as the presence of abnormal amounts of chromosomes in a cell[3]. According to the American College of Obstetricians and Gynecologists (ACOG), aneuploidy embryos accounts for 10-30% of all pregnancies[3,4]. The most common cause is nondisjunction[3,39,67]. Due to the high proportion of early pregnancy losses associated with fetuses with aneuploidy, the incidence of newborns with chromosomal abnormalities is estimated to be less than 1% (1 in 150) livebirths[6].

The most common aneuploidies observed in live born infants are trisomy 21, trisomy 18, trisomy 13, and sex chromosome aneuploidies that include XXY, XYY, XXX, and 45, X[6,23,65,67]. Approximately 1 in 691 live born infants in the United States are diagnosed with trisomy 21, a condition also known as Down syndrome[23]. The incidence of trisomy 18 and 13 is estimated to be 1 in 3,762 livebirths and 1 in 7,906 livebirths, respectively[23]. The incidence of sex chromosome aneuploidies ranges from 1 in 400 to 1 in 4000 based on the exact condition specified[23].

There several are risk factors known to increase an individual's chance to have a child with aneuploidies. One risk factor is advanced maternal age, as women age their chance to have a child with aneuploidy increases due to the fact that more errors in nondisjunction occur in oocytes as maternal age increases[3,67]. "Advanced maternal age" is defined as women who will be

35 years or older at the estimated time of delivery[4–6]. A woman's age-related risk is not influenced by her race or ethnicity[3,68]. Balanced translocation carriers are also at an increased risk for fetal aneuploidy[4,5,11,51]. Prenatal screening and diagnostic testing to detect fetal aneuploidy should be offered to individuals who are at an increased risk for fetal aneuploidy[6].

3.1.2 PRENATAL SCREENING AND DIAGNOSTIC TESTING

Prenatal screening and diagnostic testing in the United States began in the 1970s[1,69]. While diagnostic testing such as amniocentesis and chorionic villi sampling can identify fetal aneuploidy with high accuracy, pregnancy screening tests determine the risk for a fetus to be affected. If a woman is at an increased risk for a condition based on her screening test results, then she can choose to pursue diagnostic testing to verify the screening test results.

The first pregnancy screening test developed was ultrasonography. Fetuses with aneuploidy can display distinctive anatomical features such as choroid plexus cysts and shortened long bones, which can be detected by prenatal ultrasound[37]. However, not all affected fetus will be identified by ultrasound[34,37]. The presence of anatomical features suggestive of chromosome aneuploidy are referred to as soft-markers[34,38]. A detailed anatomical ultrasound can be performed at 18 to 22 weeks of pregnancy to thoroughly assess the fetus' growth, position, movement, and anatomical features for pregnancies diagnosed or suspected of fetal aneuploidy[37,38,44] . In general, 73% of Down syndrome pregnancies can be detected by ultrasound (at 18-20 weeks gestation) and 90% of pregnancies with trisomy 18 or trisomy 13 can be detected[41,44]. The false positive rate for trisomy 21 detected by ultrasound is approximately 4%[36,37,41].

To continue to improve prenatal detection for common aneuploidies and reduce the false positive rate, maternal serum screenings tests were developed. The first type of maternal serum screening test developed was multiple marker screening. Multiple marker screening measures the levels of: alpha-fetoprotein (AFP), human gonadotrophin (hCG), unconjugated estriol (uE3) and/or inhibin-A in maternal serum samples drawn at 16-20 weeks gestation[43]. Today, multiple forms of multiple marker screening exist: the triple screen, quad screen and pentascreen. The test varies based on the number of biochemical analytes that are measured in maternal serum samples. The detection rates are summarized in **Table 1**.

Subsequent prenatal genetic screening tests were developed following the advent of multiple marker screening. First trimester screening utilizes a measurement of the fetus' nuchal translucency in conjunction with maternal serum levels of pregnancy associated plasma protein (PAPP-A) and total human chorionic gonadotrophin (total hCG) to determine each pregnancy's risk to have a child with trisomy 21 or 18[40]. This test can be performed at 11-14 weeks of gestation[40]. The detection rate of first trimester screening is 90% for trisomies 18 and 21, the false positive rate is roughly 5% (**Table 1**)[40,70].

Integrated screening is comprised of two stages, a maternal serum draw is initially done at 10-13 weeks of pregnancy and the nuchal translucency of the fetus is measured[71]. Then a second maternal serum draw is performed from 15-21 weeks gestation[71]. The results obtained from the analyses conducted on the maternal serum samples are combined to calculate a final risk estimate for trisomy 21[71]. The detection rate for Down Syndrome by integrated screening is 94% with a 5% false positive rate[35]. Patients are only informed of the combined result. The detection rate for Down Syndrome by integrated screening is 94% with a 5% false positive rate(**Table 1**)[35]. Integrated screening detects about 90% of pregnancies with trisomy 18[35].

Table 1. Comparison of Prenatal Screening Test Detection and False Positive Rates

Screening Tests for Common Trisomies (21, 18, 13)	Detection Rate			False Positive Rate		
	21	18	13	21	18	13
Anatomic Ultrasound[36,37,41]	73%	80%	90-100%	4%	n/a	n/a
Multiple Marker Screen[37]	70-75%	60%	n/a	5%	8%	n/a
First Trimester Screen[37]	90%	90%	90%	5%	5%	5%
Integrated Screen[35]	94%	90%	n/a	5%	n/a	n/a

There are two prenatal diagnostic tests, amniocentesis and chorionic villus sampling (CVS). CVS can be performed at 11-13 weeks gestation to collect a sample of chorionic villi for diagnostic testing[24,27,32]. Amniocentesis can be performed any time after 15 weeks of pregnancy. A sample of the amniotic fluid that surrounds the developing fetus is collected and cultured for cytogenetic analysis[25,32]. Both amniocentesis and CVS have an associated risk of complications that could lead to miscarriage or preterm delivery and is reported at approximately 1/1000 in experienced centers[27,32]. The accuracy for detection of aneuploidy for both of these tests is nearly 100%[24].

3.1.3 NON-INVASIVE PRENATAL TESTING (NIPT)

Non-invasive Prenatal testing (NIPT), also known as cell-free fetal DNA, can detect pregnancies with chromosomal abnormalities through next-generation sequencing of maternal serum samples. NIPT can be performed any time after at least 10 weeks, and 0 days of

gestation[14,50]. The risk for fetal aneuploidy is determined by NIPT through massively parallel sequencing or the single nucleotide polymorphism approach, depending on the laboratory used[12,13,15,16,62,72]. The sensitivities and specificities of NIPT reported by each commercial testing laboratory are listed in **Table 2**. Multiple professional organizations have released opinion statements endorsing the use of NIPT[5,9,51,65,66]. ACOG and the American College of Medical Genetics and Genomics (ACMG) have even amended their original position statements of restricting the use of NIPT to high-risk pregnancies to advocating that it should be discussed as an option for all women regardless of risk[5,6,11,65].

Since its release to the U.S. market in 2011, the number of conditions screened for by NIPT continues to rapidly expand. Several testing companies have begun to offer expanded NIPT options that screen for common trisomies (trisomy 16 and 22) and microdeletion syndromes such as Prader-Willi/Angelman Syndrome, DiGeorge Syndrome, and others[12–17]. In addition one laboratory (Natera) is screening for triploidy, and Integrated Genetics now offers a "genome-wide NIPT[12,62]. ACOG does not recommend the clinical use of NIPT to screen for common microdeletions and ACMG specifies that clinical correlation and extensive patient counseling is needed prior to ordering microdeletion testing[6].

Table 2. Comparison of Clinically Available NIPT Sensitivities and Specificities

	MaterniT21/InformaSeq (15,17)		**Verifi** (16)		**Harmony** (14)		**Panorama** (12)		**Qnatal** (13)	
Aneuploidy	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
21	99.1%	99.9%	99.2%	99.9%	>99.9%	>99.0%	>99%	100%	99.1%	99.9%
18	98.3%	99.9%	96.3%	99.87%	97.4%	>99.0%	>96%	100%	99.9%	99.6%
13	98.2%	99.9%	91.0%	99.87%	93.8%	>99.0%	>99%	100%	91.7%	99.7%
X	95.0%	99.0%	n/a	n/a	n/a	n/a	>92%	100%	n/a	n/a
XX	97.6%	99.2%	97.6%	99.2%	n/a	n/a	>99.9%	100%	n/a	n/a
XY	99.1%	98.9%	99.1%	98.9%	n/a	n/a	>99.9%	100%	n/a	n/a
SCAs	96.2%	99.7%	n/a	n/a	n/a	n/a	100%	100%	96.2%	99.7%
Triploidy	n/a	n/a	n/a	n/a	n/a	n/a	>99.9%	100%	n/a	n/a

3.1.4 CLINICAL STUDIES ON NIPT PERFORMANCE

In a blinded study conducted in 2012, over 2000 maternal serum samples from pregnant women undergoing diagnostic procedures at 60 clinical centers across the United States were collected and massively parallel sequenced to assess the detection rate of NIPT[68]. Massively parallel sequencing was performed on all of the samples and then compared to their cytogenetic results. Massively parallel sequencing of the maternal serum samples was able to correctly identify trisomies 21, 18, and 13 in addition to sex chromosome aneuploidies in the 532 serum samples that indicated an increased risk for fetal aneuploidy[10]. No false positives within this sample were observed, however there were 10 false negative results[10]. The largest number of false negative results were seen amongst the samples with sex chromosome aneuploidies[10].

Decreased detection rates for sex chromosome aneuploidies by NIPT have been reported in several other studies. In Petersen et al.'s study that analyzed 712 maternal serum samples that received abnormal NIPT results and had diagnostic testing results, the positive predictive value for Monosomy X within their cohort was 27%, while the positive predictive value for Klinefelter syndrome (XXY) was approximately 85%[19]. A meta-analysis of NIPT validation studies including the study previously mentioned identified a positive predictive value of 88.6% (95% CI 83.0–93.1) for Monosomy X and 93.8% (95% CI 85.9–98.7) for all sex chromosome abnormalities excluding Monosomy X[59].

Failed NIPT results have been associated with an increased risk for adverse pregnancy outcomes and fetal aneuploidy. Some of the factors that contribute to NIPT failure have been discovered. Maternal use of anticoagulants during pregnancy, maternal metastatic malignancies, and maternal weight exceeding 270 pounds have all been determined to increase risk for a failed NIPT result[54,57,60,61]. Test failure rates of less than 2% have been reported for all of the clinical

validation and meta-analysis research for NIPT that have been performed thus far[19,20,54,58,59]. Further research is need to elucidate the biological mechanisms that underlie NIPT failure.

With the rapid evolution and expansion of NIPT, further research on its clinical performance is needed in order for clinicians and genetic counselors to provide patients with adequate information about the test. The false positive rates, positive predictive values, detection rates, test and failure rates are crucial measures to assess the accuracy and clinical validity of expanded NIPT options. Few studies independent of commercial laboratory sponsorship have been published regarding the clinical experience of genetic testing centers with NIPT at this time. The aim of this study is to qualitatively and quantitatively assess the clinical experience of one genetic testing referral center, in order to gather data to assess clinical outcomes of NIPT. The following were the specific aims of the study:

1. To identify women who have received NIPT results through Magee-Womens Hospital from January 1, 2014- December 31, 2016.
2. To classify these women as positive, negative, or failed based on their NIPT result using the hospital's secured Materni21 database, cytogenetic records, and medical records.
3. To determine if any further prenatal or postnatal testing was done on the fetuses of women who received a positive or failed NIPT result. The medical records of these patients were reviewed but no direct patient contact was done.
4. To perform statistical analysis of the data collected.

3.2 METHODS

3.2.1 PARTICIPANTS

Participants were identified utilizing the NIPT results database (entitled MaterniT21) maintained by the Center for Medical Genetics and Genomics at Magee-Womens Hospital of UPMC. Participants had NIPT ordered through the Center for Medical Genetics and Genomics at Magee-Womens Hospital of UPMC between January 1, 2014 and December 31, 2016 as listed in the NIPT results database. If an initial failed NIPT result was obtained, then women were offered to repeat the test at no additional cost, to repeat the test with the same or an alternate clinical laboratory, or to pursue other prenatal screening or diagnostic testing. Women who received non-invasive prenatal testing through Magee-Womens Hospital of UPMC's Center for Medical Genetics and Genomics but were not documented in the MaterniT21 database were excluded from this study. Moreover, only participants who had testing during the previously specified time period *and* received a failed or positive screening result for trisomies 21, 18, and/or 13, were included. A total of 95 women listed in the NIPT results database met those criteria and were subsequently selected for analysis.

3.2.2 PROCEDURES

Prior to the initiation of this study, (PRO17100348) approval by the Institutional Review Board of the University of Pittsburgh was obtained **(Appendix A).** The majority of participants included in this study had non-invasive prenatal testing due to an increased risk for fetal aneuploidy based on age (≥35 years old at time of delivery), pregnancy screening results, and/or

ultrasound findings. No patient contact was involved in this study. A waiver of informed consent and HIPAA authorization to access patients' medical records and genetic testing results were obtained **(Appendix A)**. After the waivers were obtained, the data were gathered through review of patients' medical records and compiled into a password protected database at Magee-Womens Hospital of UPMC.

Collected data included genetic tests ordered as the standard of care, follow up prenatal and postnatal studies/testing, ultrasound reports, in-patient visit summaries, and obstetrician office visit notes. In addition, maternal height, weight, race, medication use, diagnoses, age at delivery, and family history of genetic conditions or birth defects were obtained from medical records. Descriptions of infants' dysmorphic features and/or birth defects as documented on their respective delivery summaries were included in analysis. NICU progress notes and in-patient discharge summaries were analyzed for infants who did not have a dysmorphology exam completed on their delivery summaries. Genetic counseling notes, physician letters, and iGene- a patient record database for genetic testing results- were examined in the absence of genetic testing and/or ultrasound reports to confirm testing results and ultrasound findings documented in patients' obstetrician office visit summaries and progress notes.

Fetal demises that occurred at 20 weeks gestation or earlier were classified as spontaneous abortions, while demises that occurred after 20 weeks and 6 days, as well as stillbirths were classified as intrauterine fetal demises. The selective reduction of multiple gestation pregnancies was categorized as a terminated abortion. Birth outcomes of fetuses were obtained from ultrasound reports, emergency room visit summaries, and delivery summaries. Postnatal follow-up of newborns was tracked through their first year of life.

Only medications that participants were reported as taking during their pregnancy were included in this study. Maternal tobacco, alcohol and/or illicit substance use during pregnancy were also categorized as maternal medication exposures. Maternal conditions/diagnoses were defined as medical conditions that participants were diagnosed with during their pregnancy, including mental health disorders and chronic diseases.

All NIPT ordered at Magee-Womens' Hospital of UPMC from 2014 to 2016 was sent to one of five genetic testing companies: Integrated Genetics, Illumina Inc, Quest Diagnostics, Ariosa Diagnostics, or Natera, Inc. All of these companies utilized a Clinical Laboratory Improvement Act (CLIA)-certified laboratory to perform their non-invasive prenatal testing. With the exception of Natera, Inc, massively parallel sequencing is used to detect fetal aneuploidy at the other laboratories. Natera, Inc employs a single nucleotide polymorphism (SNP)-based approach to analyze NIPT samples. Maternal peripheral blood samples were drawn at the Magee-Womens Hospital Outpatient Laboratory, packaged, and then sent to a clinical testing company.

3.2.3 CYTOGENETIC METHODS

Diagnostic testing was performed at the Pittsburgh Cytogenetics Laboratory within Magee-Womens Hospital of UPMC for patients who received abnormal NIPT results-- positive and failed results. The Pittsburgh Cytogenetics Laboratory is CLIA- certified and located within Magee-Womens' Hospital of UPMC. For FISH analysis the AneuVysion DNA Probe was used to detect suspected chromosome 13, 18, 21, X, and/or Y aneuploidy. FISH analysis was routinely performed in conjunction with chromosomal microarray and karyotype analysis. Karyotyped samples were G-banded at approximately 5-10 Mb resolution and at least 10 metaphase cells or

clones were counted for each sample when available. If indicated, additional metaphase cells were studied for further analysis. Agilent's SurePrint G3 CGH+SNP microarrays (4x180K ISCA design) platform was used for chromosomal microarray analysis by the Pittsburgh Cytogenetic Laboratory during the 2014-2016-time period. Due to technical limitations, balanced chromosomal rearrangements, DNA base pair mutations, low level mosaicism (<20%), balanced insertions, and unbalanced gains in non-covered regions of the genome cannot be detected by SNP or CGH arrays. Sample types submitted for cytogenetic analysis included peripheral blood, chorionic villi, amniotic fluid, cord blood, and products of conception.

3.2.4 DATA ANALYSIS

Participants were categorized by their NIPT results. Positive results were classified as increased risk for trisomy 21, 18, or 13 based on NIPT reports. Fetuses who were positive for a sex chromosome aneuploidy in addition to trisomy 21, 18, or 13 were classified based on the presence of trisomy 21, 18, or 13. NIPT results that were not able to be reported due to insufficient fetal fraction, unacceptable sample quality, or inconclusive were categorized as failed.

Quantitative data analysis was performed using the Stata ® statistical software to determine the positive predictive value and false positive valve for each trisomy in addition to the overall NIPT failure rate. True positive results were defined as participants who received a positive NIPT result (for trisomy 21, 18, and/or 13) and who had follow-up diagnostic chromosome analysis either prenatal or postnatal that confirmed the presence of the predicted trisomy. "'True positive results'"- also included cases of aneuploidy due to chromosome translocations and/or mosaicism. False positive results were classified as participants whose diagnostic testing results

were discordant with their predicted NIPT results. Participants who did not pursue diagnostic testing were excluded from this analysis.

Additional quantitative data analysis was used to compare lab performance and evaluate relationships between other variables collected in this study. In order to assess statistical difference in the accuracy of NIPT results reported by each laboratory, Fisher's exact test was performed. Linear regressions and Pearson's correlation coefficient were calculated to determine whether or not a statistically significant relationship exists between any of the additional variables collected in this study.

3.3 RESULTS

3.3.1 PARTICIPANT DEMOGRAPHICS

A total of 2,589 women had non-invasive prenatal testing (NIPT) through Magee-Womens Hospital's Center for Genetics and Genomics from January 1, 2014 to December 31, 2016. Of those tested, 95 women (3.67%) received a positive or failed initial NIPT result and were included in analysis (**Table 5**). Only one participant had two separate pregnancies during the time period of the study; for both pregnancies she chose to have an NIPT and received failed results. Thereby a total of 95 participants and 96 separate pregnancies were included in our cohort. The majority of the participants were Caucasian. All other participants were of African American descent, Asian descent, and one participant selected 'other' indicating Moroccan descent (**Table 5)**. The average age of our cohort was 35.4 years, with the youngest participant being 16 years old and the oldest being 45 years old at the time of testing (**Table 5).** The average

BMI of participants was 30.3. Four women had dichorionic and diamniotic twins, while all other pregnancies were single gestations. A total of 100 fetuses were included in this study.

Table 5. Participant Demographics

Race	Total (n=95)
Caucasian	75
African American	8
Asian	11
Hispanic	0
Other	1
Maternal Age	Years
Average Age	35.40
St. Dev. Age	5.88
Min Age	16
Max Age	45
Number of gestations	Total (n=100)
Singleton	92
Diamniotic/ dichorionic twins	4
Maternal BMI	
Average	30.3 ± 8.2
Minimum	17.9
Maximum	59.9

3.3.2 NIPT RESULTS

Figure 1 depicts the clinical indications that were reported for NIPT use within this cohort. The majority of the participants (n=95, 55%) had NIPT solely due to advanced maternal age (**Figure 1**). The second most common reason for NIPT was due to joint clinical indications that included advanced maternal age (AMA), abnormal ultrasound findings (US), and increased

risk for fetal aneuploidy by multiple marker screening (MSS) (**Figure 1**). Approximately 15% of participants had NIPT due to abnormal ultrasound findings alone, 6% of participants had NIPT due to abnormal first trimester screens alone, and 2% were solely due to abnormal multiple marker screens (**Figure 1**). One participant had a NIPT ordered by parental request with no indication of increased risk for fetal aneuploidy.

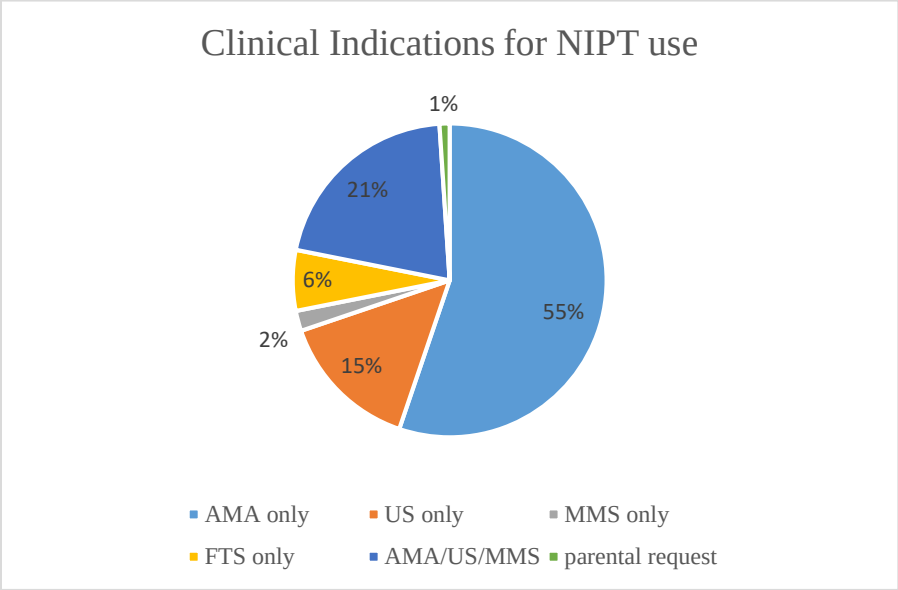

Figure 1. Clinical Indications for NIPT Utilization

The average gestational age at which NIPT was drawn was 14.7 weeks (±4.55). A total of 54 pregnancies (n=96, 27.87%) received a positive NIPT indicating an increased for trisomies 21, 18, or 13; 45 (n=96, 47.87%) pregnancies were positive for trisomy 21, five pregnancies were positive for trisomy 18, and four pregnancies were positive for trisomy 13 (**Figure 2**). The majority of women who received positive trisomy 21 NIPT results were solely AMA or had multiple clinical indications (**Figure 1**). One of the pregnancies that was positive for trisomy 21 also had an increased risk for Monosomy X as reported by NIPT. However, amniocentesis

showed the presence of two X-chromosomes and trisomy 21 (47, XX, +21) in all cells analyzed by FISH and confirmed with karyotyping.

Approximately 44% (n=42/96) of pregnancies received failed NIPT results. The test failure rate is estimated to be 1.62% (42/2589). NIPT failure due to inability of the sample to meet acceptable Quality Control (QC) standards set by each laboratory in order to perform analysis was reported for 24 pregnancies-23 singletons and one set of dichorionic/ diamniotic twins (**Figure 2**). A maternal metastatic malignancy was later detected at 22 weeks gestation for one singleton pregnancy whose reason for test failure was reported as "Did not meet QC threshold" at both attempts for NIPT analysis respectively drawn at 13 and 15 weeks of gestation. Failure due to "Specimen quantity not sufficient"- the extraction of an insufficient amount of cell-free fetal DNA due to a lowered amount of maternal serum sample submitted- was initially reported for three samples. Insufficient cell-free fetal DNA (listed as cffDNA in **Figure 2**) was reported as the reason for test failure in 10 samples. The reason for NIPT failure was not reported by testing laboratories for five failed results (**Figure 2**). One pregnancy that received an unspecified reason for failure was determined to be at a lowered gestational age than the laboratory's minimal threshold. There was no statistically significant association between NIPT results received and the clinical indication for screening ($p>0.120$).

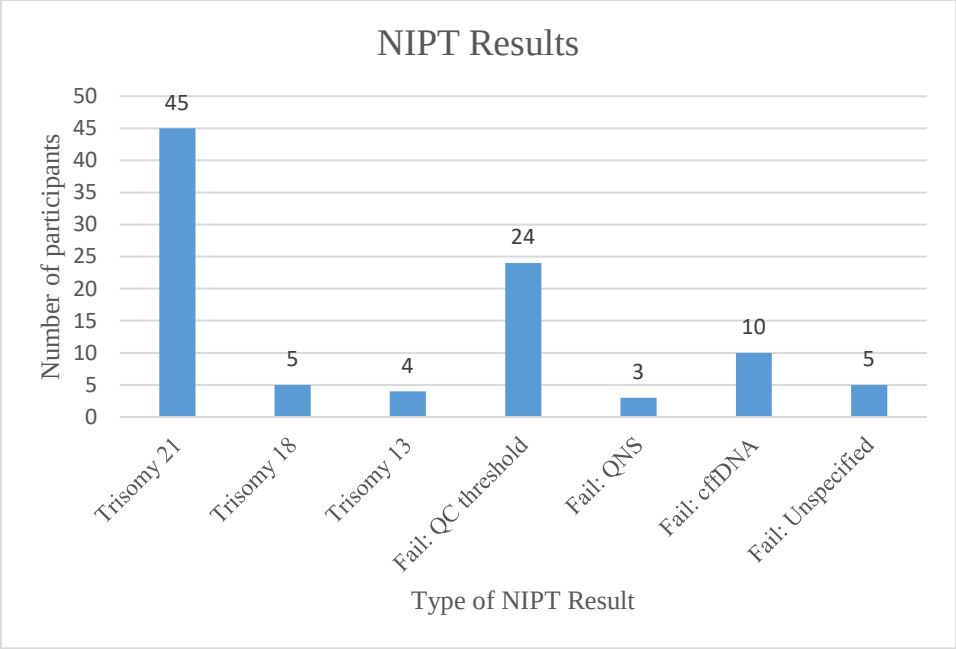

Figure 2. Number of Participants Who Received Each Type of NIPT Result

After receiving an initial failed NIPT result, 26 participants chose to have their sample redrawn for NIPT analysis. All five of the participants who received a failed NIPT result due to unspecified reasons (n=5) elected to have a second NIPT sample drawn. Upon this second attempt, three of these participants obtained inconclusive sex chromosome aneuploidy panels, and were reported as low risk for trisomies 21,18, and 13. Two participants then received negative NIPT results (low risk for all aneuploidies) upon redraw. All three of the participants who received failed results due to "Specimen quantity not sufficient" chose to have their sample redrawn and received negative NIPT results. A total of thee three women elected to have their NIPT sample re-drawn after receiving an initial test failure due to insufficient fetal fraction (30%, n=10) obtained results upon their second draw. One participant was reported to be at an increased risk for Monosomy X, while the other two participants received negative results. A total of 15 participants who initially received a failure NIPT due to inability to meet the testing

laboratory's QC threshold (62.5%, n=24) elected for a redraw. Six participants received a second NIPT failure upon re-draw, two of the failed results were due to insufficient cell-free fetal DNA, three were due to failure to meet the QC threshold, and one participant received a failed result due to lowered gestational age. The remaining nine participants received negative NIPT results upon their second blood draw.

A statistically significant correlation ($p<0.001$, $\alpha=0.05$) between maternal body mass index (BMI) and the reported fetal fraction was observed within our cohort (**Figure 3**). Moreover, a statistical difference ($p=0.0034$) between the average BMI of participants who received positive NIPT results in comparison to participants who received failed NIPT results was observed (**Figure 4**). The median maternal BMI was the highest for participants whose NIPT initially failed due to "Specimen Quantity not Sufficient" errors. While, participants whose NIPT initially failed due to insufficient cell-free fetal DNA extracted from maternal serum samples had the lowest median BMI. The maternal BMIs for participants who received positive NIPT results are displayed in **Appendix B**.

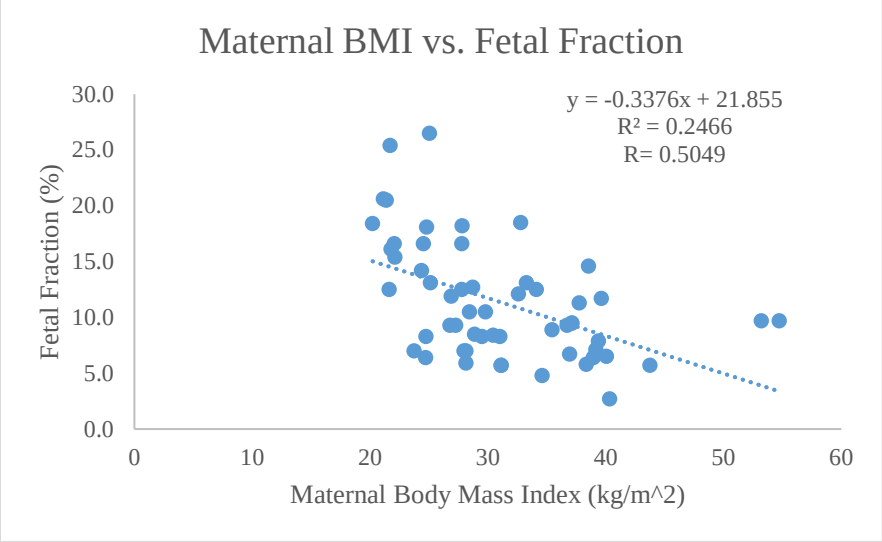

Figure 3. Relationship between Maternal Body Mass Index (BMI) and Fetal Fraction

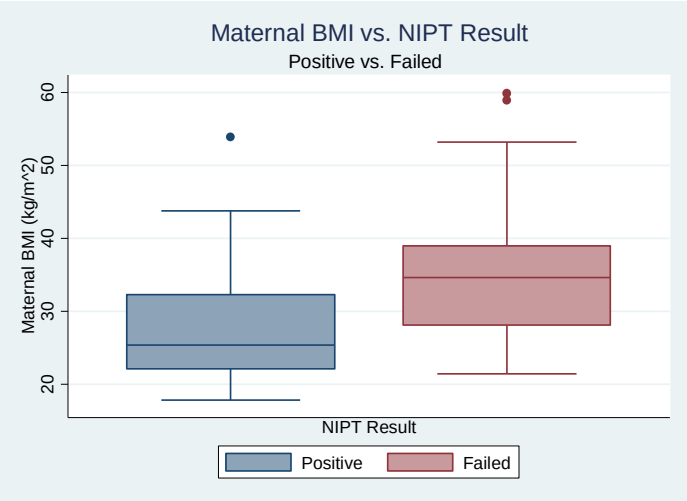

Figure 4. Maternal BMI of Participants Who Received Positive or Failed NIPT Results

All NIPTs that were ordered through the Center for Medical Genetics and Genomics from January 1, 2014 to December 31, 2016 were sent to a total of seven clinical laboratories (**Figure 5, Appendix B**). No statistically significant difference was observed between the number of positive or failed results reported by each laboratory ($p>0.5$) with the exclusion of Ariosa Diagnostic Inc-the most frequently used laboratory within this cohort **(Figure 5**).

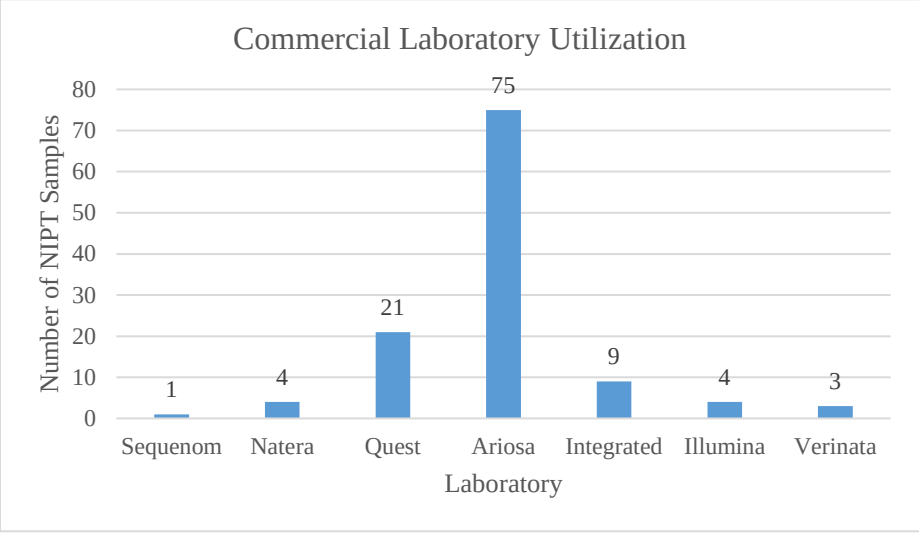

Figure 5. Commercial Laboratories Used for NIPT Analysis

3.3.3 NIPT RESULTS AND DIAGNOSTIC TESTING

Of the 96 pregnancies with an abnormal NIPT result, diagnostic testing was performed for 51 pregnancies (53.1%, n=96). Prenatal diagnostic testing (amniocentesis and chorionic villus sampling) was more commonly performed than postnatal diagnostic testing on cord or peripheral blood samples from neonates (**Figure 6**). Amniocentesis was the most frequently chosen type of diagnostic testing overall (**Figure 6**). Of the 42 participants who initially received a failed NIPT result, only eight women pursued diagnostic testing (19.0%, n=42). Diagnostic testing was not elected by any of the participants who received a failed result due to "Specimen Quantity not Sufficient" or for an unspecified reason (**Figure 6**).

A total of five women (20.8%, n=24) who received a failed result due to failure to meet the laboratory's QC threshold had diagnostic testing. One fetus (20%, n=5) was detected to have trisomy 13 due to a Robertsonian translocation (46,XX,+13, der(13;14)(q10;q10) in all analyzed cells. The other four fetuses (80%, n=5) who underwent diagnostic testing had normal karyotypes. Three women whose NIPT failed due to insufficient fetal fraction (30%, n=10) had diagnostic testing. Two of the pregnancies (66.7%, n=3) received normal karyotypes and one pregnancy was diagnosed with Klinefelter syndrome (47, XXY) (33.3%, n=3). Fetal aneuploidy was suspected in three additional pregnancies that received inconclusive sex chromosome aneuploidy panels upon repeat NIPT analysis after initially receiving a failed result due to reasons unspecified by the testing laboratory. None of these participants elected to have diagnostic testing. In addition, fetal aneuploidy was suspected in one pregnancy that initially received a failed NIPT result due to insufficient cffDNA but was predicted to be at an increased risk for Monosomy X upon NIPT re-draw and analysis. Amniocentesis was performed on the pregnancy and showed 46,XX in all cells analyzed.

Of the total of 45 participants whose initial NIPT was positive for trisomy 21, 38 (84%) chose to have diagnostic testing but results were obtained for only 37 pregnancies (**Figure 6)**. Cytogenetic analysis was not able to be performed on the products of conception from one pregnancy due to lack of placental villi growth. No false positives were detected in this cohort, trisomy 21 was confirmed by diagnostic testing for all of the women with an increased risk for Trisomy 21 by NIPT (**Figure 6, Table 6)**. For both of the dichorionic, diamniotic twin pregnancies that received an NIPT positive for trisomy 21, one fetus was detected to have trisomy 21. The positive predictive value for trisomy 21 was 100% (95% CI: 90.5-100) (**Table 7**).

Diagnostic testing was performed for three out of five participants whose initial NIPT was positive for trisomy 18. Diagnostic testing confirmed the presence of trisomy 18 in two fetuses, however a complex rearrangement resulting in partial monosomy of the p-arm of chromosome 18 was identified in the products of conception from the third pregnancy (**Figure 6, Table 6**). The positive predictive value for trisomy 18 in this cohort is 66.7% (95% CI: 9.4-99.2) (**Table 7**).

The diagnostic testing results were known for two of the four women whose initial NIPT was positive for trisomy 13 (**Figure 6).** One of the results indicated a diagnosis of trisomy 13 and one result was a false positive based on chorionic villus sampling results (**Figure 6, Table 6**). Based on these results the positive predictive value for trisomy 13 within our cohort was determined to be 50% (95% CI 1.3-98.7) (**Table 7**).

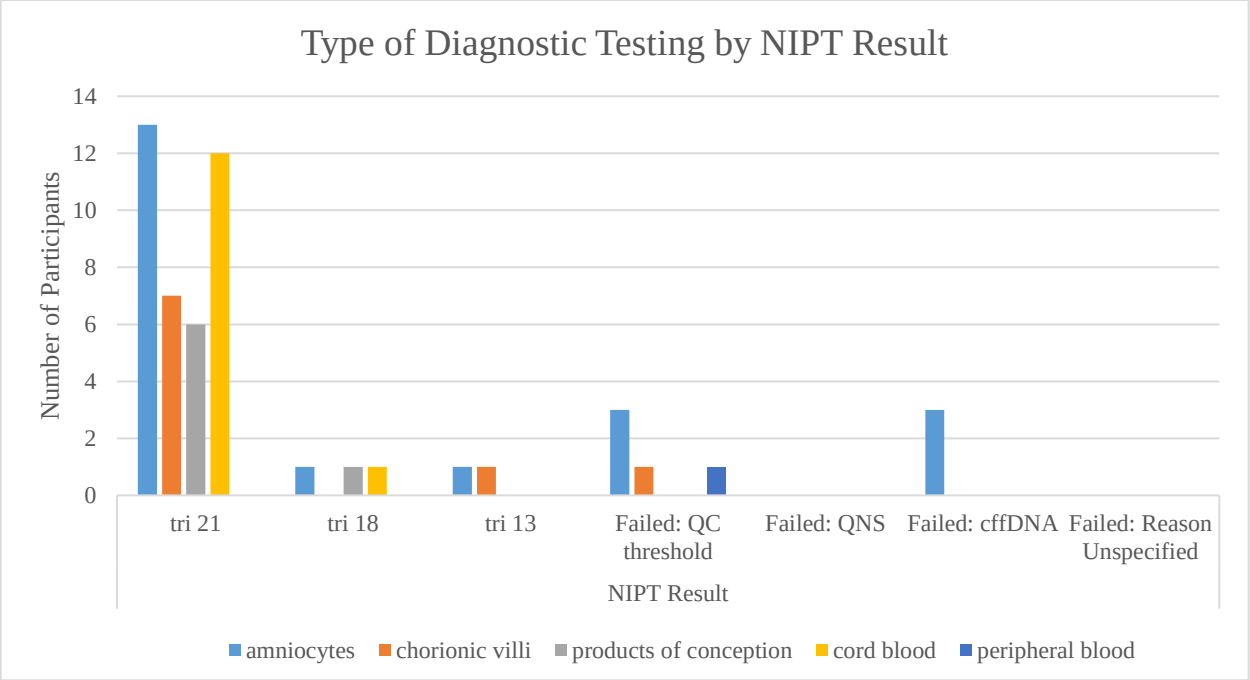

Figure 6. Type of Diagnostic Testing Elected vs. NIPT Result

Table 6. Diagnostic Testing Results Based on NIPT

	Trisomy 21	Trisomy 18	Trisomy 13
47,XX,+21/18/13	19	1	0
47,XY,+21/18/13	16	1	1
unbalanced translocations	2		
46, XX	0	0	0
46,XY	0	0	1
complex structural defect	0	1	0

Table 7. Positive Predictive Value for Each Trisomy

NIPT Result	Number of positive results	Number of positive results with diagnostic testing	Number of true positives	PPV (%)	Confidence Interval
Trisomy 21	45	37	37	100%	90.5-100
Trisomy 18	5	3	2	66.7%	9.4-99.2
Trisomy 13	3	2	1	50%	1.3-98.7

3.3.4 POSTNATAL FOLLOW-UP

The postnatal outcomes were available for 92 out of 100 fetuses as depicted in **Figure 7.** Approximately half of the fetuses in our cohort were delivered (n=52, 57%) (**Figure 8**). The majority of fetuses who were delivered received an initial NIPT that was positive for trisomy 21 or failed due to failure to meet the laboratory's quality control threshold (**Figure 8**). Of the fetuses who were delivered (n=52), the average birth weight was 2.99 ±0.84 kg and the average birth length was 47.34 ±3.57 cm (**Table 8**). The three pregnancies comprised of two singletons and one set of dichorionic/diamniotic twins that initially received failed NIPT results due to insufficient specimen quantity, were delivered. However, one singleton received an abnormal newborn screen indicating Bart's hemoglobin and was subsequently diagnosed with Alpha thalassemia trait. All other newborns in our cohort with available newborn screening results received normal newborn screens.

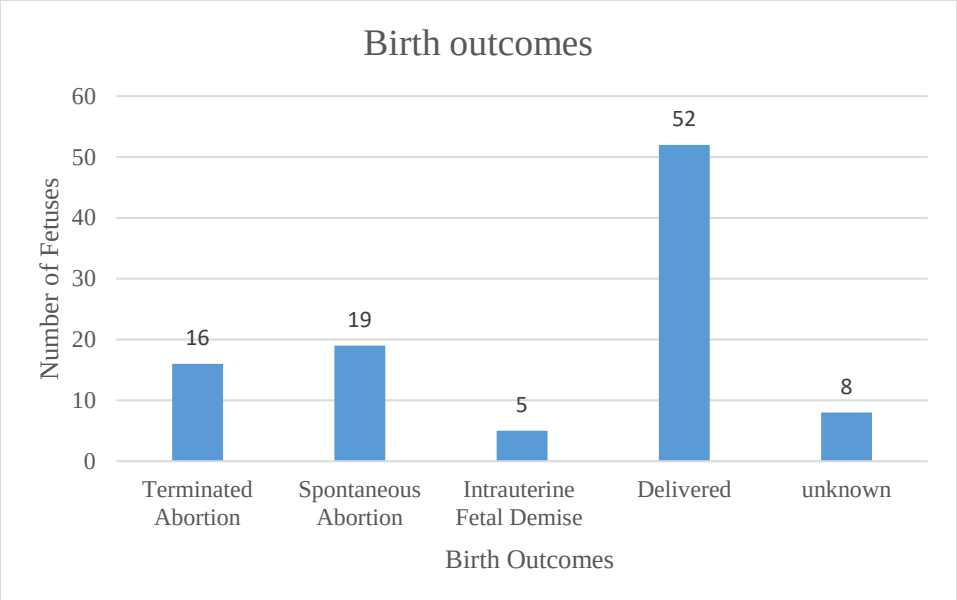

Figure 7. Birth Outcomes of Fetuses

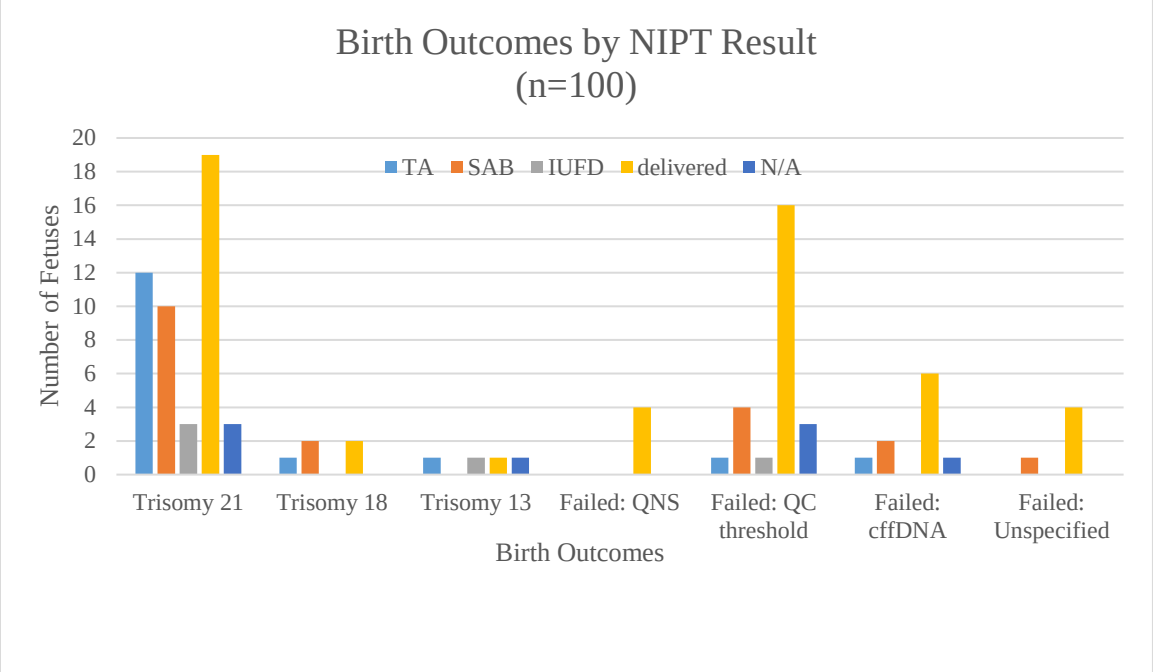

Figure 8. Birth Outcomes of Fetuses based on Initial NIPT Result

Terminated abortions occurred for 16 (17.4%, n=92) fetuses with known birth outcomes (**Figure 7**). No fetuses who received failed results due to an unspecified reason or insufficient specimen quantity, were terminated (**Figure 8**). A total of 19 fetuses (20.7%, n=92) spontaneously aborted, the NIPT results of these pregnancies included trisomy 21, trisomy 18, failed due to lowered fetal fraction, failure to meet the quality control threshold and for an unspecified reason (**Figure 8**). Intrauterine fetal demises (5.43%, n=92) were reported for three fetuses who were positive for trisomy 21 by NIPT, one fetus whose NIPT was positive for trisomy 13, and one fetus whose initial NIPT failed due to failure to meet the quality control threshold (**Figure 8**).

Table 8. Neonatal Characteristics

Birthweight (kg)	Statistic
Average Weight	2.99
Standard Deviation Weight	0.84
Minimum	0.41
Maximum	4.85
Birth Length (cm)	
Average Length	47.34
Standard Deviation Length	3.57
Minimum	41.00
Maximum	53.00

Postnatal diagnostic testing was elected for 21 fetuses (21%, n=100) with known diagnostic testing results. Cytogenetic studies were performed on a single peripheral blood sample (4.76%, n=21), 13 cord blood samples (61.9%, n=21), and seven products of conception (33.3%, n=21) (**Appendix B**). The fetus whom the peripheral blood sample was drawn from initially received a failed NIPT result due to the inability to meet the laboratory's quality control threshold and, the karyotype was normal (46, XY). For the 13 fetuses who had cytogenetic studies performed on their cord blood samples, 12 had a NIPT that was positive for trisomy 21 and confirmed on karyotype, one fetus who received a NIPT positive for trisomy 18 had an abnormal karyotype confirming the presence of trisomy 18 in all cells. Cytogenetic studies on the products of conception from seven pregnancies revealed one false positive NIPT result. One fetus received a positive NIPT for trisomy 18, however microarray analysis revealed a complex structural rearrangement leading to partial monosomy of 18p11.2 and chromosome material of unknown origin attached to the 18p11.2 segment. Trisomy 21 was confirmed by karyotype for all of the fetuses (n=6, 100%) who had a NIPT positive for trisomy 21 and submitted products of

conception for genetic analysis, with the exception of one fetal sample that was unable to grow under the cell culture conditions for karyotype analysis.

3.4 DISCUSSION

Due to its high sensitivity and specificity in combination with its non-invasive methodology, NIPT has become an increasingly popular pregnancy screening option since its launch in 2011. As the clinical uptake of NIPT continues to rise, the rate of invasive diagnostic procedures being performed has gradually declined[18,20]. However, since its release, a limited number of studies on the clinical use and validation of NIPT have been published[19,51]. Clinical assessment of NIPT outcome is essential for the provision of more accurate clinical correlation and interpretation of test results to then guide subsequent pregnancy care and management. The goal of this study was to quantitatively and qualitatively analyze our center's experience with NIPT.

3.4.1 COHORT DEMOGRAPHICS

Our study identified participants from women who had NIPT through the Medical Genetics and Genomics department of Magee-Womens Hospital of UPMC from January 1, 2014 to December 31, 2016. During this time period only women who were classified as high risk for fetal aneuploidy as specified by ACOG and the Society for Maternal-Fetal Medicine (SMFM) in their initial joint statement on NIPT were offered this screening[11]. The array of clinical indications for NIPT use in our cohort reflect this policy. A total of 53 pregnancies (55%, n=96)

in our cohort had a NIPT due to advanced maternal age (AMA) alone, six pregnancies (6%, n=96) had positive first trimester screening results, 14 pregnancies (15%, n=96) had abnormal ultrasound findings, two pregnancies had abnormal multiple marker screens (2%, n=96), and 20 pregnancies (21%, n=96) had multiple clinical indications (**Figure 1)**. A similar study conducted in 2013 by the Prenatal Diagnostics Unit at the University of North Carolina Chapel Hill also reported a similar distribution of clinical indications and a mean maternal age of 37± 7.4 amongst its participants who had NIPT in 2012[20].

3.4.2 POSITIVE NIPT RESULTS

Approximately 83.33% of the positive results (n=45/54) obtained by participants reported an increased risk for trisomy 21. Trisomy 21 has the highest incidence in liveborn infants compared to trisomies 13 and 18[3,23]. In addition, women of advanced maternal age are considered to be at an increased risk for fetal aneuploidy conditions, for example the chance for a 37 year-old woman to have a child with a chromosome abnormality is approximately 1 in 66[73,74]. The average maternal age of participants in this study is advanced maternal age, 35.4 years ±5.88 (**Table 1**).

The positive predictive values for the common aneuploidies calculated for this cohort slightly varied from those reported by prior clinical studies of NIPT performance. Of the 45 participants who received a NIPT positive for trisomy 21 and elected to have diagnostic testing, no false positives were detected and the positive predictive value was 100% (95% CI: 90.5-100)**.** The positive predictive values for trisomy 21 reported by clinical studies ranged from 85-99.9% based on the sample size[19,58,59]. In a meta-analysis of NIPT clinical validation studies conducted in 2014 and a blinded, multicenter study performed at 35 international center, the calculated

positive predictive value for trisomy 21 was 99.0-100%[52,59,75]. There were no false positives detected for trisomy 21 in the 38 pregnancies that were included in the multicenter study conducted by Norton et al[75]. However, the overall false positive rate for trisomy 21 for the studies included in the meta-analysis was approximately 0.15%[59].

The calculated positive predictive values for trisomy 18 and 13 for this cohort were lower than those reported by prior studies. The positive predictive value for trisomy 18 was 66.7% (95% CI: 9.4-99.2%) and 50% (95% CI:1.3-98.7) for trisomy 13. The false positive rate for trisomy 18 was 33.3% (95% CI: 0.08-90.6) and 50% (95% CI: 1.26-98.7) for trisomy 13 within this cohort (**Appendix B**). However, these calculations were limited by the small sample sizes for trisomy 18 and trisomy 13 observed in this study. The positive predictive value for trisomy 18 was approximately 77% as reported by a large referral genetic diagnostic laboratory (n=106) and the false positive rate was 23%[19]. In an international study conducted in 2015, the positive predictive value for trisomy 18 was 74.3% for participants who received diagnostic testing (n=121)[52]. However in the meta-analysis of NIPT clinical validation studies the positive predictive value of trisomy 18 was 96.8% (95% CI 94.5–98.4)[59].

One of the participants who received a NIPT positive result for trisomy 18 was then detected to have a complex structural chromosome rearrangement through diagnostic testing. Microarray analysis of the products of conception from that pregnancy revealed 46,XX,del(10)(?q24q25.2,add(18)(p11.2)ish 10q26.3(D10S2490x2), der(18)(18p11.32-)(D18S552-) in all cells tested. FISH analysis showed evidence of an interstitial deletion on chromosome 10, and an abnormal chromosome 18 with monosomy of the 18p11.2-pter segment and unknown chromatin material at 18p11.2. However, NIPT has not been validated for the detection of complex chromosome structural rearrangements such as the one described[5,51,76]. A

retrospective analysis of 6,388 cases showed that 0.56% (n=258) of pregnancies that receive abnormal NIPT results had a pathogenic copy number variant revealed by diagnostic testing[77]. Moreover, no case reports of fetuses with this or structural rearrangements who received positive NIPT results have been described in the literature.

3.4.3 FAILED NIPT RESULTS

A number of research studies have shown that women who receive failed or inconclusive NIPT results are at an increased risk for fetal aneuploidy[6,19,20,53,54,59,65]. The ACMG encourages healthcare professionals to offer diagnostic testing and continued prenatal surveillance of pregnancies that received failed NIPT results[5]. The test failure rate of this cohort was approximately 1.62%, which is concordant with NIPT failure rates reported by other clinical studies who reported test failure rates of 1-2%[19,20,54]. The test failure rates for most commercial NIPT laboratories are not reported in their performance analytics or reported as >0.1%[12–17].

Fetal aneuploidy was confirmed or suspected in six pregnancies that initially received failed NIPT results. One pregnancy that received a failed result due to inability to meet the quality control threshold of the testing laboratory when the maternal serum sample was initially drawn at 12 weeks gestation, had an amniocentesis that detected trisomy 13 due to a Robertsonian translocation present in all analyzed cells. Throughout the pregnancy, the fetus displayed several features suggestive of trisomy 13 that were detected by ultrasound including but not limited to: cystic hygroma, alobar holoprosencephaly, Dandy-Walker malformation, and bilateral polydactyly of the hands, the pregnancy was terminated at 21 weeks[78,79]. Klinefelter syndrome (47, XXY) was detected by amniocentesis in another pregnancy that initially received a failed NIPT result due to insufficient cffDNA at 15 weeks gestation. The participant received a

second failed result upon sample re-draw and chose to have a third re-draw that resulted in a negative NIPT result with the fetal sex indicated as XY.

One pregnancy that initially received a failed NIPT result due to insufficient cffDNA was indicated to be at an increased risk for Monosomy X upon sample re-draw and NIPT analysis. The participant chose to have an amniocentesis that showed a normal female karyotype (46, XX) and the fetus was delivered liveborn with no noted dysmorphic features. A possible right ventricular myocardium thickening versus thickening of the tricuspid valve was detected by ultrasound, although these findings were not observed in a subsequent fetal echocardiogram. No postnatal diagnostic testing was elected for this infant. In addition, three participants who received failed results due to a reason unspecified by the testing laboratory elected to repeat their NIPT and received negative NIPT results for trisomies 21, 18, and 13 but had inconclusive sex chromosome aneuploidy panels. No prenatal or post-natal diagnostic genetic testing was pursued for these pregnancies. One of the fetuses spontaneously aborted at 12 weeks gestation and two fetuses were delivered at full term with no fetal anomalies detected by ultrasound or postnatal evaluation within the first year of life.

The discrepancies observed between the NIPT and diagnostic testing results and/or postnatal outcomes could be due to an array of potentially confounding factors. Errors in fetal aneuploidy detection by NIPT have been attributed- but not limited to: confined placental mosaicism, fetal somatic mosaicism, and/or technical limitations of NIPT, maternal copy number variants, a vanishing twin, and undiagnosed maternal malignancies[53,54]. Confined placental mosaicism is estimated to occur in 1-2% of all first trimester pregnancies that undergo CVS procedures, although the prevalence of confined placental mosaicism varies based on the exact condition[80,81]. Chromosomal mosaicism is independent of sex however sex chromosome

aneuploidies can generally be better tolerated by developing fetuses due to X-inactivation[81]. Also, somatic mosaicism is commonly observed in individuals with sex chromosome aneuploidies[82,83].

Several clinical studies have observed a higher incidence of false positive and false negative NIPT results for sex chromosome aneuploidy conditions[10,19,59]. Furthermore, the NIPT sensitivity and specificity reported by commercial testing companies for sex chromosome aneuploidy conditions are generally lower than those reported for trisomies 21,18, and 13[12,13,17]. Due to the prevalence of chromosomal mosaicism and numerous biological mechanisms underlying the development of sex chromosome aneuploidies it is recommended that infants who had prenatal diagnostic testing also follow-up with postnatal testing for more thorough assessment[82,83]. The absence of clinical features and/or a normal fetal karyotype created by prenatal diagnostic testing do not rule out the possibility of a sex chromosome aneuploidy[82,84]. Therefore, placental and somatic chromosomal mosaicism cannot be ruled out in the cases of Klinefelter syndrome and Monosomy X observed within our cohort. Moreover, due to the fact that diagnostic testing was declined by the three participants who received failed results then inconclusive sex chromosome aneuploidy panels, the possibility of a sex chromosome aneuploidy cannot be excluded in these cases.

One risk factor for NIPT failure is higher maternal body mass index (BMI)[5,54]. A retrospective clinical assessment of NIPT outcomes in an Australian population showed that women who failed to receive a NIPT result had a higher mean maternal BMI in comparison to participants who received a result upon their first blood draw[54]. In particular, the participants who received a failed result due to a lowered fetal fraction extracted from their sample had a higher mean maternal BMI than all other participants[54]. The maternal mean BMI for participants

who received a failed NIPT due to a lowered fetal fraction (also referred to as insufficient cell-free fetal DNA) was not the highest amongst the groups of women who received failed results in our cohort. The highest mean BMI was observed in the participants whose initial NIPT failed due to insufficient specimen quantity errors. However, a statistically significant difference between the mean maternal BMIs of the participants who received positive versus failed results was observed in this study (**p=0.0034).** Although, discrepancies between laboratory internal quality control and specimen quantity standards could confound results.

Maternal use of anticoagulants during pregnancy has also been associated with NIPT failure due to low fetal fraction[60,61]. Specifically, use of low molecular weight heparin and/or Warfin have been associated with reduction in fetal fraction detected by NIPT analysis[61]. None of the participants (n=10) who received a failed result due to reduced fetal fraction were known to use either of those medications during or prior to their pregnancy.

In vitro fertilization (IVF) and twin pregnancies have also been shown to be independent predictors of lowered fetal fraction[85,86]. IVF was used for three pregnancies within this cohort, two of these were singleton pregnancies while one case resulted in a twin gestation. One of the singleton pregnancies and the twin gestation received a positive NIPT result for trisomy 21. The presence of trisomy 21 was confirmed through diagnostic testing in the singleton pregnancy and one of the fetuses in the twin pregnancy that was conceived from maternal oocytes retrieved at age 36. The other singleton pregnancy conceived from a donor egg received a failed NIPT result due to inability to meet the laboratory's quality control threshold at 12 weeks gestation. The participant chose to have their serum sample redrawn at 14 weeks gestation and received a negative result; the infant was liveborn with no complications or physical anomalies at 37 weeks. Four twin pregnancies were included in this cohort, two of the twin pregnancies received NIPT

results positive for trisomy 21, one of which was conceived through IVF. The other two twin pregnancies received failed NIPT results; IVF was not used for either of these pregnancies. Trisomy 21 was diagnosed in one fetus of the twin pair in both of the twin pregnancies that received positive NIPT results. The reported reasons for test failure in the two twin pregnancies that received failed NIPT results were insufficient specimen quantity and inability to meet the laboratory's quality control threshold. Neither of the twin pregnancies that obtained failed NIPT results elected to undergo diagnostic testing; all four of these fetuses were delivered at full-term (37 weeks gestation or later).

Occult maternal malignancies are a reported incidental finding of NIPT[57]. This screening test was derived from research on tumor DNA detection in the serum of cancer patients[48,50]. In a study conducted by Bianchi et al. seven pregnancies with subsequently diagnosed maternal cancers were detected by Illumina's Verifi NIPT[57]. The NIPT reports for these seven pregnancies showed an increased risk for more than one aneuploidy and bioinformatics analysis of maternal serum samples revealed "nonspecific copy-number gains and losses across multiple chromosomes" suggestive of the presence of a tumor[57]. One participant within our cohort was diagnosed with metastatic synchronous colon cancer at 22 weeks gestation, however this malignancy was not detected by NIPT. The initial NIPT drawn at 13 weeks gestation for this participant received a failed result due to inability to meet Ariosa Diagnostic's quality control threshold, as did her subsequent sample drawn at 15 weeks of gestation and sent to Ariosa Diagnostics. No evidence of non-specific copy number gains or aneuploidy within the samples was reported. However, a rapidly enlarging adnexal mass, and enlarged left ovary (149 mm) with a multicystic appearance was seen on first trimester ultrasounds for the pregnancy. The fetus did

not have any detected physical abnormalities and delivered at 37 weeks of gestations with no complications.

3.4.4 STUDY LIMITATIONS AND FUTURE DIRECTION

This study has several limitations that can be addressed through future research studies. A limited sample size of 100 pregnancies that met the inclusion criteria were analyzed in this study. Due to the small sample size, results generated from this research should be carefully interpreted due to their limited generalizability. Moreover, subtle differences in the data may not have been observed or detected in our cohort due to the limited sample size. Future studies should increase the sample size to determine if there are associations between other risk factors such as maternal medication use and NIPT results.

Another limitation of this research was its retrospective design. Due to the fact that all data was collected at least 2 years after participants had their initial NIPT testing, some participants were lost to follow-up leading to an incomplete data set. In addition, there was limited or incomplete medical records available for a number of participants. Future research could include a prospective analysis to currently assess trends in NIPT utilization and outcomes to minimize loss to follow-up bias and increase the temporal relevancy of results.

Thorough assessment of the clinical experience of genetic testing centers is essential for understanding the clinical utility and limitations of NIPT in order to increase the accuracy of information provided to patients during the informed consent process. Pregnancies that receive abnormal and failed NIPT results are at an increased risk for fetal aneuploidy and adverse prenatal outcomes. As a result, these pregnancies should receive increased surveillance and prenatal care to reduce maternal and fetal mortality and morbidity. Clinical correlation of NIPT results can help

to better direct the provision of appropriate healthcare services to patients, and identify current gaps in knowledge.

3.5 CONCLUSION

The launch of NIPT has made a significant impact on the field of prenatal genetics. The clinical adoption of NIPT continues to rapidly expand as more conditions and panels are offered by laboratories. However, research on the clinical outcomes and follow-up of pregnancies that received NIPT lags behind. Through our assessment of Magee-Womens Hospital of UPMC's experience with NIPT, a test failure rate of 1.62% and positive predictive values of 100% (95% CI: 90.5-100) for trisomy 21, 66.7% (95% CI: 9.4-99.2) for trisomy 18, and 50% (95% CI: 1.3-98.7) for trisomy 13 were obtained. Fetal aneuploidy was detected in two pregnancies that received failed NIPT results. These results can be used to provide more precise pre-test counseling in addition to genetic counseling for individuals interested in pursuing NIPT.

4.0 RESEARCH SIGNIFICANCE TO PUBLIC HEALTH AND GENETIC COUNSELING

The core functions of public health are policy development, assessment, and assurance[87]. These functions provide the framework to achieve public health's primary objective: the improvement of population health through prevention, protection, and promotion. A public health intervention that encompasses all three of these core functions is prenatal screening. Pregnancy screening enables healthcare professionals to assess the occurrence of health conditions such as aneuploidy within the population. Although there are known risk factors for fetal aneuploidy such as family history and maternal age, aneuploidy can also occur in pregnancies with no known risk factors[3,39]. It is estimated that 80% of children with trisomy 21 are born to women under the age of 35 with no prior family history of trisomy 21[39]. By offering pregnancy screening healthcare providers can identify women within the general population who are at an increased risk to have a child with a medical condition and/or experience adverse pregnancy outcomes. Through early detection provided by prenatal screening, pregnancy management plans, specialized medical care and services, in addition to psychological support can be provided to families, improving pregnancy outcomes.

Initial pregnancy screening methods consisted of ultrasound and maternal serum screening tests. Using ultrasound imaging, physicians are able to visually assess the fetus for structural anomalies. However, due to technological limitations, positioning of the baby, and/or maternal weight, ultrasound is not able to detect all structural defects prenatally. Maternal serum screening tests were designed to increase prenatal detection rates for specific birth defects and genetic conditions such as Down syndrome and trisomy 18.

Since its launch, NIPT has quickly been incorporated into prenatal care and multiple professional organization including ACOG and NSGC have endorsed its use[5,6,9,65]. NIPT offers higher detection rates than its predecessors, and is able to detect trisomy 13 and sex chromosome aneuploidies in addition to trisomy 21 and 18 unlike any other prenatal genetic screening tests. However, the positive predictive value of NIPT results varies based on maternal age and the detected condition[5,6,65,88]. ACOG, NSGC, and ACMG maintain that healthcare professionals who order or discuss NIPTs with patients should determine the positive predictive values, false positive rates, and negative predictive values in order to accurately interpret their results for patients who choose to have screening[5,6,9,65]. Alternatively, healthcare providers can also refer their patients to discuss results with genetic specialists like genetic counselors[5,6,9,65]. According to NSGC's 2016 Professional Status Survey, 43% of genetic counselors who counsel patients specialize in prenatal genetics and therefore, likely discuss NIPT with their patients on a regular basis[89].

In order for individuals to receive the most appropriate prenatal care and to make informed decisions regarding prenatal screening and pregnancy management decisions based on screening results, it is imperative that patients receive the most accurate information regarding NIPT. Through analysis of NIPT outcome, genetic counselors and other health care professionals can be equipped with information that has the potential to improve the informed consent process with their patients. For example, while multiple studies have shown that failure to receive a NIPT result is associated with an increased risk for adverse pregnancy outcomes, many of the biological mechanisms that contribute to these failures remain unknown[22,54,56,60]. This study showed that fetal aneuploidy was later confirmed in two pregnancies that initially received a failed NIPT result, one of which resulted in a spontaneous abortion. Follow-up of the clinical

outcomes of abnormal and failed NIPT results can aid healthcare professionals in providing families with more precise pre- and post-test counseling to identify more cases of fetal aneuploidy and improve pregnancy outcomes.

APPENDIX A: INTERNATIONAL REVIEW BOARD APPROVAL

PITT SEAL

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Aleksandar Rajkovic
From: IRB Office
Date: 1/2/2018
IRB#: PRO17100348
Subject : Non-invasive prenatal testing performance in the academic setting

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The University of Pittsburgh Institutional Review Board reviewed and approved the above referenced study by the expedited review procedure authorized under 45 CFR 46.110 and 21 CFR 56.110. Your research study was approved under:

45 CFR 46.110.(5)

The IRB has approved a waiver of informed consent/HIPAA authorization to access, record and use protected patient health information/patient medical record information.

This study has been approved under 45 CFR 46.404 for the inclusion of children.

The risk level designation is Minimal Risk.

Approval Date:	1/2/2018
Expiration Date:	1/1/2019

For studies being conducted in UPMC facilities, no clinical activities can be undertaken by investigators until they have received approval from the UPMC Fiscal Review Office.

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least one month prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

APPENDIX B: SUPPLEMENTARY TABLES AND FIGURES

Table 9. NIPT results vs. Clinical Indication for NIPT Utilization

NIPT Result	AMA	US	FTS	MMS	AMA/US/MMS/FTS	Parental Request
Trisomy 21	17	8	3	1	16	1
Trisomy 18	1	0	1	1	2	0
Trisomy 13	2	1	0	0	1	0
failed	32	5	2	0	2	0
TOTAL	53	14	6	2	20	1

Table 10. Labs used for Non-invasive Prenatal Testing per Year

Year	Sequenom	Ariosa	Integrated	Quest	Illumina	Natera	Verinata
2014	1	1	8	9	4	0	3
2015	0	18	0	12	0	1	0
2016	0	54	0	0	0	3	0
Total	1	73	8	21	4	4	3

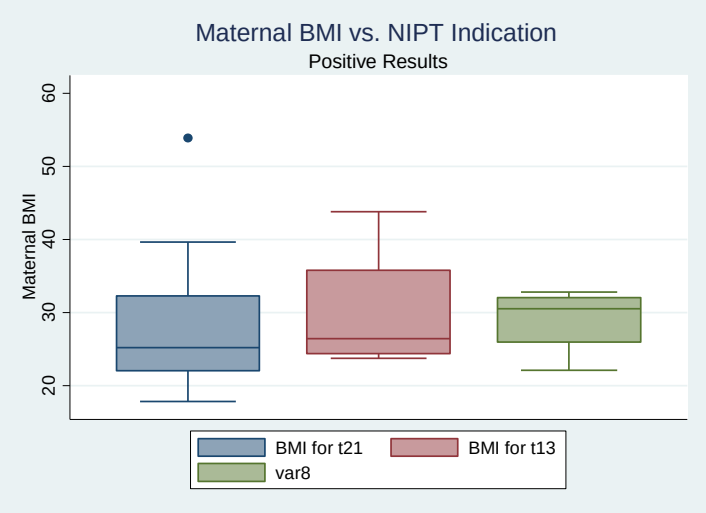

Figure 9. Maternal Body Mass Index vs. Positive NIPT Result:

***** Var 8= trisomy 18

Table 11. Presence of US Findings by NIPT Result

NIPT Result	no US findings	US findings
Trisomy 21	8	37
Trisomy 18	0	5
Trisomy 13	2	2
Failed: Specimen quantity not sufficient	0	3
Failed: Did not meet QC threshold	6	18
Failed: Unspecified Reason	5	1
Failed: Insufficient fetal cffDNA	5	5

Table 12. NIPT Results Received From Each Laboratory

	Positive results			Failed Results			
Lab	Trisomy 21	Trisomy 18	Trisomy 13	QC threshold	quantity not sufficient	cffDNA	Reason Unspecified
Ariosa	26	1	4	21	2	4	5
Quest	12	1	0	3	0	4	0
Natera	0	0	0	0	0	1	0
Illumina	3	0	0	0	0	0	0
Integrated	3	1	0	0	0	1	1
Verinata	1	2	0	0	0	0	0
Sequenom	0	0	0	0	1	0	0

Table 13. False Positive Rates of NIPT Results

Aneuploidy	False Positive Rate	Confidence Interval (95%)
Trisomy 21	0%	N/A
Trisomy 18	33.3%	0.08-90.6
Trisomy 13	50%	1.26-98.7

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