NON-INVASIVE PRENATAL TESTING: PROVIDER KNOWLEDGE AND FUTURE DIRECTIONS

by

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ABSTRACT

Non-Invasive Prenatal Testing (NIPT) is a commercially available technology that analyzes cell-free fetal DNA circulating in maternal blood with the purpose of detecting specific fetal aneuploidies. Currently, this technology can be ordered by any prenatal medical professional including genetic counselors, obstetricians, and midwives. Professional societies recommend that NIPT be used as a screening method that is followed up with diagnostic testing and also that testing be accompanied with genetic counseling. With the increasing accessibility and relatively low cost of NIPT, prenatal medical professionals and labs are considering offering NIPT to all pregnant women.

A questionnaire focusing on knowledge and views of NIPT was distributed to genetic counselors via the National Society of Genetic Counselors. Results were analyzed using descriptive statistics. Results showed that a total of 95% of genetic counselors agreed/strongly agreed with being familiar with NIPT clinical data. 99% are mostly confident/very confident when interpreting or explaining NIPT results to their patients. 89% agreed/strongly agreed they are familiar with microdeletion and microduplication conditions analyzed via NIPT. Although there is a strong understanding of microdeletions and microduplications, 26% agreed that they should be analyzed via NIPT while 35% did not agree. 95% of genetic counselors currently order NIPT for high-risk pregnancies, while 36% order NIPT for average-risk pregnancies. Regarding

monogenic disorder analysis, 32% approved of this addition to NIPT, 47% remained neutral, and 21% disapproved.

In the future, it is likely that NIPT will be offered via a public health-based model to all pregnancies rather than only pregnancies meeting high-risk criteria. This study aims to assess current opinions of genetic counselors regarding their knowledge and comfort level of genetic testing regarding NIPT in order to identify advantages, limitations, concerns, and gaps in knowledge. The public health significance of this research is that it will allow for the creation of appropriate educational materials regarding NIPT. Therefore, providers will become informed and skilled in their interactions with patients. This will in turn lead to a greater level of competency and education among all prenatal providers. Understanding these factors will help streamline patient interaction with the most benefit to the patient.

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PREFACE

I would like to take this opportunity to express my gratitude toward the many individuals who have supported me throughout this project. First and foremost, I would like to thank my family and classmates for their encouragement and continual support as I work to pursue my dream.

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1.0 INTRODUCTION

Prenatal testing throughout the antenatal period is a standard practice of care around the world. The goal of prenatal testing is to monitor a pregnancy, allowing physicians to detect any fetal congenital anomalies that may suggest a particular condition or to detect conditions that may require immediate surgery post-birth. It has been well documented that chromosome abnormalities, such as aneuploidy, constitute approximately 0.65% of all live births and greater than 50% of all clinically recognized early pregnancy losses.[1-3] It is understood that the chance a pregnancy will be effected with aneuploidy increases with maternal age.[3,4] Based on this information, women are routinely offered additional prenatal screening and/or testing to increase the detection of chromosome conditions.

Broadly, prenatal testing has two categories: screening tests and diagnostic tests. Screening tests include the use of ultrasonography and/or maternal serum marker screening during the first and second trimesters of pregnancy. Screening tests during the first trimester include first trimester screening which uses both biochemical markers and ultrasonography. Second trimester screening includes level II ultrasonography, quad screening which involves only biochemical markers, combined screening and sequential/contingent screening which combine first trimester screening and quad screening for more accurate results. Non-invasive prenatal screening analyzes maternal blood for chromosomal markers and is available during both the first and second trimesters.

These screening technologies are offered in all pregnancies to increase detection of birth defects and potential chromosomal aneuploidies.[5] However, these technologies are known to have low detection rates and high false positive rates.[5] If screening tests are pursued and indicate the fetus is at a greater risk for a fetal aneuploidy, invasive diagnostic procedures such as chorionic villus sampling or amniocentesis are recommended to provide a definitive diagnosis.

Diagnostic tests are invasive procedures that give definitive genetic diagnoses via extraction of fetal cells from either the placenta or the amniotic fluid surrounding the baby. Diagnostic procedures are less commonly pursued as they are associated with an increased risk of miscarriage (<0.5%) and have a more limited window of execution.[6-9]

Prenatal screening and diagnostic tests are available to all pregnant women regardless of maternal age or other risk factors. The decision to opt for or decline screening and testing as well as which specific screening or testing to pursue should be a shared-decision between the patient and provider. Although all screening and testing options are viable, certain testing procedures are recommended for high-risk pregnancies. According to the American College of Obstetricians and Gynecologists (ACOG), individuals considered to have a high-risk pregnancy include: 1) individuals of advanced maternal age (35 years of age or greater at the time of delivery), 2) individuals with an abnormal serum screen result, 3) individuals with a personal or family history of aneuploidy, or 4) individuals who have an abnormal ultrasound.[10,11] ACOG also recommends that pretest counseling and informed consent should be performed for each patient.[10] They also state that non-invasive prenatal testing should not be a part of routine prenatal laboratory assessment for average-risk pregnancies, but a woman and her physician can choose any screening test.[10,12]

Recent advancements in technology have increased the number of prenatal screening options available to mothers, particularly those of high-risk pregnancies. In 1997, it was discovered that the fragments of cell-free fetal DNA (cffDNA) circulating in maternal blood can be amplified and analyzed for aneuploidies.[7,13,14] Introduced commercially in 2011, this screening test is called non-invasive prenatal testing (NIPT) and has drastically changed the way prenatal screening tests are viewed. This non-invasive screening test is attractive to both medical professionals and mothers of high-risk pregnancies due to increased aneuploidy detection accuracy and a decreased false-positive rate (as compared to other prenatal screening options).

A recent study assessed genetic counselors experience with NIPT and their perceptions of patient attitudes regarding this screening.[15] Genetic counselors felt all screening options should be available for their patients, which was the biggest reason for offering NIPT as well as feeling confident in NIPT validation studies.[15] Genetic counselors indicated they felt knowledgeable regarding NIPT clinical information (96.1%) and confident offering NIPT as an option for their patients (94.2%).[15] Regarding perceptions of patient attitudes, genetic counselors felt the majority of patients pursued NIPT when it was offered and have indicated a noticeable decline in the uptake of diagnostic testing options.[15] The assumption is that patients are not undergoing invasive diagnostic procedures due to being comfortable with NIPT testing detection rates and accuracies.[15]

Given the rapid adoption of NIPT for both high-risk and average-risk pregnancies as well as the continuing advancements of the test's analytic capabilities, there have been a number of studies to assess providers' attitudes, knowledge, and comfort with NIPT. It is important to understand how all providers feel regarding various aspects of NIPT so that prenatal professional organizations can adequately address these concerns and help streamline patient interaction with appropriate medical professions. Current guidelines recommend that NIPT be accompanied by

genetic counseling to allow families to make informed decisions.[12] Therefore, this study aims to assess the knowledge, practice and professional opinions of genetic counselors regarding NIPT.

1.1 BACKGROUND

1.1.1 Aneuploidy

Aneuploidy refers to the presence of an abnormal number of chromosomes within a cell without loss or gain of an entire chromosome set. Every cell in the human body typically constitutes 23 pairs of chromosomes for a total of 46 chromosomes. The chromosomes are paired together via similarity in banding patterns and are ordered by the size of the chromosome. The last pair of chromosomes are the sex chromosomes, listed as X for female and Y for male. One chromosome in each pair is inherited from our mother while the other chromosome is inherited from our father. Our chromosomes give instructions on how our body is to grow and function, therefore, having extra or missing chromosomes or pieces of chromosomes can affect an individual's health and body functioning.

The majority of aneuploidy cases are due to nondisjunction of chromosomes during cell division (meiosis). Nondisjunction is the failure of homologous chromosomes to separate properly during meiosis resulting in an uneven distribution of chromosomes within the gametes. There are two stages to meiosis. During meiosis I, the chromosomes are copied creating an extra set of DNA, then the homologous chromosome pairs divide into separate cells. During meiosis II, the cells divide again to ultimately end with 23 chromosomes per cell.

Nondisjunction can occur during both meiosis I and meiosis II. When the cells are dividing, an error may occur causing a failure of the homologous chromosome pairs to separate. In this case, the chromosomes from one pair would both go into one cell rather than separating into two cells. Fertilization of a gamete containing an extra or missing chromosome would result in a fetal aneuploidy. Cells with one missing chromosome result in a monosomy, while cells with one extra chromosome result in a trisomy. Fetal aneuploidy is the major cause of miscarriages with the greatest risk of miscarriage in the first trimester of pregnancy.[2] Although nondisjunction occurs spontaneously and cannot be prevented, it is recognized that the risk of aneuploidy increases with maternal age.[2] Most fetal aneuploidies are non-viable with the exception of a few that are commonly seen in live births, these include monosomy X and trisomies 13, 16, 18, 21, and 22.[16-19] There are also live born cases that result from additional sex chromosomes including XXX, XXY, and XYY.[20] In general, approximately 18-19% of oocytes and 3-4% of sperm show aneuploidy.[2]

Although all autosomal chromosomes can result in a fetal aneuploidy, there are a few that are more commonly recognized within pregnancy. These include chromosomes 13, 16, 18, 21, and 22. Errors that occur during maternal meiosis I most commonly involve chromosomes 13, 16, 21 and 22.[21,22] Errors during maternal meiosis II most commonly involve chromosome 18.[21,23-25]

Trisomy 13, or Patau syndrome, occurs in approximately 1 in 4,000-29,000 liveborns and constitutes approximately 2% of first trimester spontaneous miscarriages.[21] 80% of affected fetuses will result in spontaneous miscarriage during the second and third trimesters.[21] Approximately only 4% of trisomy 13 fetuses survive to term.[3] Trisomy 18, or Edwards syndrome, occurs in approximately 1 in 8,000 live births.[21] Approximately 80% of fetuses will result in spontaneous miscarriage during the second and third trimester, while 4% of fetuses with trisomy

18 will survive to term.[3,21] Maternal meiosis II nondisjunction is more frequent among trisomy 18 cases, but this condition can also result from maternal meiosis I nondisjunction.[26] Trisomy 21, or Down syndrome, is the most common live born trisomy with an incidence of approximately 1 in 700-1,000 newborns.[27] Approximately 20% of trisomy 21 fetuses survive to term.[3]

Trisomy 16 occurs in >1% of all clinically recognized pregnancies and is one of the leading reasons for first trimester spontaneous miscarriage.[28] Trisomy 22 is also commonly observed among first trimester miscarriages.[22] Trisomy 16 and 22 fetuses are not observed in liveborns.[22]

Most monosomies are nonviable and result in miscarriage with the exception of Turner syndrome.[20,29] Turner syndrome occurs when there is a single X chromosome. The incidence of this condition is approximately 1 in 2,500 live born females.[29] The expected prevalence of Turner syndrome in all clinically recognized pregnancies is approximately 1-2%.[20,29] Turner syndrome has a high rate of spontaneous miscarriage with approximately only 10% of affected fetuses surviving to term.[20] Approximately 74.2% of the time, this condition results from maternal meiosis II nondisjunction, the remaining 25.8% of the time it results from maternal meiosis I nondisjunction.[20]

Errors involving additional sex chromosomes can also be noted in liveborns. XXX females occur in approximately 1 in 1,000 liveborns.[30] XXX aneuploidy is typically due to maternal meiosis I nondisjunction errors (58%) with approximately only 10% of cases due to paternal nondisjunction errors.[20] Klinefelter syndrome, or XXY males, have a live born prevalence of 1 in 660 live born males.[31] 50% of the time, this aneuploidy originates from paternal meiosis I nondisjunction.[20] Lastly, XYY males occur due to paternal meiosis II nondisjunction 84% of the time.[20] This condition has a live born prevalence of approximately 1 in 1,000 males.[20,32]

During fertilization and embryogenesis there is also an independent risk for mosaicism to occur. Mosaicism results when there is a presence of two independent cell lines within one individual who developed from a single fertilized egg. During early development, errors in mitosis may occur which can result in fetal aneuploidy. Mosaicism, including monosomic and trisomic cell lines, may be difficult to discern as not all body systems may be constituted of the monosomic or trisomic cell line. In order to delineate aneuploidy mosaicism in individuals, multiple tissue samples may be taken to see which, if any, systems are affected.

1.1.2 Microdeletions and Microduplications

A microdeletion is the loss of a small fragment of a chromosome that involves several contiguous genes. A microduplication is a gain of a small fragment of a chromosome. Typically, microdeletions and microduplications are too small to be detected by conventional cytogenetic methods such as light microscopy using high resolution banding.[33] Methods for detecting microdeletions and microduplications traditionally include fluorescence *in situ* hybridization (FISH) or microarray analysis. The risks of microdeletions and microduplications occurring are independent of maternal age, unlike aneuploidies.[34] In comparison to karyotyping, microarray analysis was able to detect genetic anomalies in an additional 1.7% pregnancies presenting with positive screening results and advanced maternal age as well as an additional 6% of cases with an abnormal ultrasound.[33]

Microdeletion and microduplication conditions screened for via NIPT include conditions that can be associated with heart defects, cleft lip and/or palate, learning delays, difficulty with speech, and poor muscle tone.[34-38] Microdeletion and microduplication conditions are rare, therefore impacting the positive predictive values.[39]

Research is ongoing for screening for microdeletion and microduplication conditions with NIPT. Currently, the most studied microdeletion and microduplication conditions are 22q11.2 deletion, Prader-Willi syndrome, Angelman syndrome, 1p36 deletion, and cri-du-chat syndromes.[39,40] These conditions have reportedly high detection rates with low false positives, but providers are cautioned as research for these conditions have been performed on few samples.[39-41] The specificity and sensitivity of these tests have not yet been validated.[42] It is strongly recommended that individuals with an abnormal microdeletion or microduplication result follow-up NIPT with diagnostic testing for confirmation.[41,43]

1.1.3 Monogenic Disorders

Monogenic disorders are conditions that are due to a genetic change in a single gene. Carrier screening for medical conditions is widely available for individuals to detect if they carry a single gene mutation for numerous conditions. Currently, NIPT researchers are looking into the possibility of including common monogenic disorders onto their panel of testing.[44] The utility of this addition would allow for the detection of certain autosomal dominant and autosomal recessive conditions. In particular, researchers are looking at the ability to detect skeletal dysplasia's due to *FGFR* mutations in likely affected fetuses.[45] The addition of monogenic disorder analysis could allow for parents to have more information about whether their child has a specific condition antenatally without the use of invasive diagnostic procedures or waiting until after their child is born to perform genetic testing.

Currently, with regards to monogenic disorders, NIPT is most commonly used to establish the fetal rhesus-D (RhD) status.[46] Approximately 15% of Northern European women are RhD negative.[7] The standard therapeutic course during pregnancy is to administer anti-D

prophylactically when the partner was a known heterozygote for RhD. Through research, it has been recognized that approximately 40% of fetuses of RhD negative mothers are also RhD negative which would negate the need for anti-D therapy.[7] It has been noted that NIPT can be used to reliably determine the fetal RhD genotype to better assess the need for anti-D therapy and for better management of the pregnancy.[7,14,46,47] When this testing was performed in the second and third trimester, results were completely concordant with those performed using amniocentesis.[46]

1.1.4 Prenatal Diagnosis of Aneuploidy

1.1.4.1 Non-Invasive Screening for Fetal Aneuploidy

Prenatal screening methods (first trimester screen, sequential and contingent screen and quad screen) create a risk adjustment regarding trisomy detection. The methodology for calculating aneuploidy risk for these screening techniques is performed by taking a mother's age-related aneuploidy risk and adjusting this risk by combining screening results for a more precise aneuploidy risk. Individuals are given an increased risk report if their results fall above a certain risk threshold which is designated by each individual lab. Several national professional organizations recommend that all women should be offered these prenatal screening options during their pregnancies.[41,43] Any individuals with a result detailing increased risk for an aneuploidy are recommended to follow-up their screening test with an invasive diagnostic procedure for confirmation.[41,43]

First Trimester Screening

First trimester screening (FTS) is performed between 11-14 weeks of gestation and constitutes blood work and ultrasonography. The blood work analyzes two biochemical markers, human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein A (PAPP-A).[48,49]

Ultrasonography is used to measure the nuchal translucency (NT), the fluid filled space behind the baby's neck.[48,49] FTS measurements relay risk information regarding trisomy 18 and trisomy 21 with respective detection rates of 82-96% and 62-90% and a false positive rate of 5%.[8,16-19,50-54] Unlike some other prenatal screening options, FTS is not able to detect open neural tube defects (ONTDs).

Quad Screening

Quad screening is performed between 16-21 weeks gestation and is constituted of blood work only. This screening test can help identify pregnancies at increased risk for trisomy 18, trisomy 21, and open neural tube defects (ONTDs). Quad screening may also incidentally detect placental insufficiency, intra-uterine growth restriction, and pregnancy loss via analysis of biochemical markers.[55] The blood work during this stage of the pregnancy analyzes four biochemical markers including human chorionic gonadotropin (hCG), alpha-fetoprotein (AFP), unconjugated estriol (uE3), and inhibin A.[55] Combinatorial analysis of these four markers detects approximately 60-75% trisomy 18, 75-83% trisomy 21, and 75-90% ONTDs.[8,16-19,50,52]

A variation of this screen, known as penta screening, analyzes a fifth biochemical marker. This biochemical marker is known as hyperglycosylated hCG, or invasive trophoblast antigen, and has been found to be sensitive for detecting trisomy 21.[56] Use of this biochemical marker alone detected 38-40% of trisomy 21 pregnancies with a 5% false-positive rate.[56]

Combined First and Second Trimester Screening

Combined screening incorporates the results from first trimester screening and quad screening to allow for a more accurate risk adjustment. The results of this test are not reported until after quad screening is performed.[57] A greater sensitivity is able to be achieved by combining results from both screening tests detecting 87-96% of trisomy 18, 91-95% of trisomy 21, and 85%

of ONTDs.[8,50,52,58] Although combined testing is able to achieve a higher level of accuracy and detection of ONTDs, no result is given until the completion of the blood draw during the second trimester.

Sequential and Contingent Screening

Sequential screening utilizes a stepwise approach, meaning that both first and second trimester tests are performed. The difference between sequential and contingent screening and combined first and second trimester screening is that the results are reported after each screening test is performed and moving on to complete the second trimester screen is contingent upon the results of the FTS. For sequential screening individuals are separated into two categories after FTS is complete, and these are delineated as high or low risk for fetal aneuploidy.[49] If, after FTS is conducted there is a high-risk of aneuploidy, the patient is notified and offered the opportunity of diagnostic testing or NIPT and the second trimester serum screening portion of the test is not performed. If, after FTS is conducted, there is a lower-risk the patient proceeds to the second trimester screening test.[49]

Contingent screening delineates patients into three categories after FTS results are available, and they are classified as having a high, intermediate, or low aneuploidy risk. Women whose results place them in the high-risk category are offered NIPT or invasive diagnostic testing. Women who are placed in the intermediate category are able to move on to the second trimester serum screening. Women in the low risk category are not required to complete any further screening or testing.[49] Both tests have a detection rate of 93.2% for trisomy 18, and 92.9% for trisomy 21. 81.6% of all chromosomal aneuploidies were detected with a false positive rate of 4.5%.[49] Benefits of sequential screening include increased accuracy when combining both first and second trimester results. Individuals are also able to have a more customized screening process as

they are given results and recommendations after FTS results are available. Limitations would be the lack of ONTD analysis if an individual does not require second trimester serum screening and having the ability to only detect trisomies 18 and 21 through biochemical marker analysis.

Ultrasonography

Ultrasonography alone is also an option for prenatal screening and can be a helpful tool for detecting fetal aneuploidy. Level II ultrasounds are performed between 18-20 weeks gestation. At this stage of gestation, certain aneuploidy features are also able to be visualized. These include findings such as heart defects, cleft lip, choroid plexus cysts, echogenic bowel, intrauterine growth restriction, and other physical features. By ultrasound alone, detection rates include 80-100% for trisomy 13 and trisomy 18, 50-87% for trisomy 21, and 80% for ONTDs.[8,50,59-61] Level II ultrasounds are routinely performed on all pregnancies.[41]

Compared to NIPT there are many benefits and limitations of ultrasound analysis alone. Ultrasonography is less sensitive and less specific than NIPT for trisomy 18 and 21 detection. Benefits of ultrasonography include the ability to detect ONTDs and other birth defects as well as its ability to compare measurements over time for assessment of poor growth or suspected skeletal dysplasia syndromes. Through ultrasonography, condition markers for cystic fibrosis and cytomegalovirus may also be detected, which would not be detected via NIPT.

Non-Invasive Prenatal Testing

NIPT technology analyzes cell-free fetal DNA (cffDNA) in maternal blood and in the United States is currently recommended to high-risk pregnancies, although available for all pregnancies.[12,41,43] cffDNA is derived mostly of placental cells and the fraction of cffDNA within maternal circulation increases with gestation.[62] The level of fetal fraction in maternal circulation

necessary for analysis is 4-10% or greater depending on testing method used.[51] NIPT is performed on a maternal blood sample any time after 10 weeks gestation, ideally between 10-22 weeks.[63]

The knowledge that cffDNA was able to be detected and analyzed in maternal blood was discovered in 1997.[13] Understanding that this testing could be of great value to the prenatal community, multiple validation studies were performed from 1997 until NIPTs induction into commercial availability in 2011.[64] Originally, non-invasive tests using cffDNA were utilized to determine trisomy 21, fetal sex, and Rh status.[14] The validation studies performed prior to 2011 all revolved around the detection of trisomy 21. Large cohorts and studies of high-risk pregnancies were analyzed showing sensitivities between 98.58-100%, specificities between 97.97-100%, and negative predictive values of 100%.[65] However, the positive predictive values had a large range of 19.7-100%.[65] Researchers have understood that trisomy 21 was an ideal testing start, as the condition has a higher prevalence as compared to other aneuploidies.[65] Since the discovery of cffDNA, multiple technologies for analyzing fetal aneuploidy have been created including the development of quantitative methods and a single nucleotide polymorphism (SNP) method.[53,66]

Quantitative methods are based on creating random, unique sequence tags, typically 25-36 base pairs in length, then counting for the relative number of chromosome copies present.[53] Targeted sequencing amplifies and reads specific genomic regions of interest, such as chromosomes 13, 18, 21, X and Y.[67-69] There are also other forms of this technology that create and read tags across the entire genome.[67] This technology does not require the differentiation and separation of maternal and fetal cell-free DNA. Determination of ploidy status is conducted by counting the tags within each region. When the number of relative tags is decreased (monosomy) or increased (trisomy) aneuploidy is present.

The non-quantitative SNP method differentiates between the maternal and fetal cells by characterizing their genotype.[71-73] The plasma contains both the maternal and fetal DNA. Therefore, after collecting the maternal blood sample, the sample is treated and separated so the section that contains only maternal DNA is genotyped.[71-73] Genotyping mixed maternal and fetal DNA allows for SNP detection and direct analysis against the maternal-only component.[71-73] Allelic ratios are quantified for aneuploidy detection.[71-73] This technology also allows for the detection of triploidy, which is the gain of an entire chromosome set.[74]

NIPT technology routinely conducts analysis on chromosomes 13, 18, 21, X and Y. Currently, NIPT has the option of expanded screening including assessment of chromosomes 9, 13, 16, 18, 21, 22, sex chromosome aneuploidies, and several specific microdeletion and microduplication syndromes.[40] Accuracy is greatest for chromosomes 13, 18 and 21, at 79-92%, 99%, and 99% respectively.[11,51,53,72,75] Newer, expanded aneuploidy assessment has a variable range of detection and increased risk of false positive reporting. This decreased sensitivity is due to the fact that these aneuploidies, microdeletions and microduplications are less common and less researched within this technology. Therefore, data is not as consistent allowing for a wider range of reported detection rates.

NIPT is classified as a screening test and therefore, like other prenatal screening tests, should be followed up with an invasive diagnostic procedure for confirmation.[41,43] It is also important to follow-up NIPT with amniocentesis due to the fact that the cffDNA analyzed is made up of placental DNA. Since the placenta is rapidly growing and dividing, there is a chance that the aneuploidy will be isolated to the placenta and the baby will have normal chromosomal studies.[77-79]

Although NIPT has great advantages, there are multiple limitations that must be considered when ordering testing. The majority of NIPT panels assess the chromosomes in a targeted manner, meaning that they are only looking at specific chromosomes or chromosome regions versus diagnostic testing which looks at every chromosome.[74,80] Due to the targeted assessment of chromosomes, some chromosomal abnormalities will not be detected by NIPT such as balanced translocations, deletions, and duplications. NIPT is also not able to differentiate between different forms of aneuploidy such as high-level mosaicism, maternal mosaicism, or a Robertsonian translocation. NIPT does not replace ultrasonography, AFP testing for ONTDs, or invasive diagnostic procedures.[78]

When talking with patients it is also important to go over the validity of the test. Although the sensitivity and specificity are high, data regarding the positive predictive value and negative predictive value are not readily available leading to another limitation of NIPT. This information is important as it demonstrates the reliability of the test. These values are impacted by disease prevalence, the less prevalent the disease the higher the negative predictive value and the lower the positive predictive value will be.[81]

As NIPT becomes available for the general population, there is an increased need for research regarding the validity in average-risk patients. Due to the prevalence of chromosomal conditions being fewer in the average-risk population, there is a greater risk for a higher false-positive and false-negative rate which is a significant limitation.[51,82] As the population for testing becomes broader, the need for more in-depth research is necessary. This increase in research will allow for providers to accurately cite information to patients as well as feel comfortable ordering this testing for their patients.[75]

Another limitation of NIPT is the risk of obtaining a non-informative result. There are many instances for which this result can happen. One known risk factor is maternal weight. There has been a demonstrated association between increased maternal body mass index and decreased fetal fraction.[73] Many tests differ in the amount of fetal fraction necessary to perform the test. Too low of a fetal fraction can result in the need for a re-draw of maternal blood or can cause an inconclusive result. Other factors that could lead to a non-informative result include a sample that does not meet quality control, collection or sampling errors, or having a low fetal fraction.[53,63,68,70,72,83]

Lastly, expanding screening allows for the chance to detect a chromosomal anomaly in a parent, especially the mother since her chromosomes are simultaneously analyzed. This could include the detection of low-level mosaicism or a microdeletion/microduplication disorder. Many of the microdeletion and microduplication conditions lie on a spectrum whereas individuals may have the condition and show no symptoms or they could be severely affected. Since the outcome and penetrance of some conditions are not fully understood, there is difficulty assessing the impact a condition could have on the fetus if they are also found to have the chromosomal anomaly. There is also the chance of learning, after additional parental testing, that one of the parents is a balanced translocation carrier and the burden that may persist due to learning this information. It is important that patients and providers understand all aspects of testing and the potential results that may arise.[78,84]

Comparison of Screening Tests

NIPT compared to all other maternal serum screening tests has an improved sensitivity and specificity with lower false-positive rates. In comparison to FTS, both FTS and NIPT have the ability of screening early in pregnancy. One benefit of FTS is that it has a faster turn-around time

of 1 week, when compared to NIPT.[51] NIPT turn-around time ranges with an average of 8-12 days.[51,85] Another benefit of FTS is that a full ultrasonography scan is performed which allows for the most accurate dating of the fetus. A benefit of NIPT as compared to FTS is that it has the capability of screening for trisomy 13 and sex chromosome aneuploidies.

When compared to Quad screening, NIPTs disadvantage is that it is unable to screen for open neural tube defects, placental insufficiency, intra-uterine growth restriction, and pregnancy loss.[55] NIPTs advantage would be the fact that it can be ordered during the first trimester, providing patients with information earlier in their pregnancy and allowing more time to make decisions regarding diagnostic testing and/or pregnancy management. NIPT can also screen for sex chromosome aneuploidies. Both screening tests assess risk for trisomies 13, 18, and 21. NIPT benefits when compared to sequential or combined screening is the fact that this test can be performed during the first trimester and does not include the need for multiple blood work-ups. Benefits of sequential and combined screenings would be the ability to rule out the risk of open neural tube defects. These tests are also combined with FTS which allows for analysis of the fetus via ultrasonography for proper dating.

When compared to ultrasonography alone, NIPT has the benefit of assessing with greater sensitivity and specificity the risk for chromosomal aneuploidies. Ultrasonography alone is not very sensitive or specific in regard to all chromosomal anomalies, but is very accurate at detecting open neural tube defects. The ability to compare ultrasound images and measurements over time also allows for the benefit of assessing the fetus' growth to determine if there are any skeletal dysplasias. There are other conditions that are able to be discerned by ultrasonography such as cystic fibrosis and cytomegalovirus, which cannot be detected via NIPT.

1.1.4.2 Current State of Non-Invasive Prenatal Testing

Commercially Available Testing

First, it is important to understand the measures used to compare the different testing platforms and their validity. Validity describes the effectiveness of producing accurate results and is measured using sensitivity and specificity. Validity is measured using four factors: true positive (those who have the condition and tested positive), false positive (those who do not have the condition yet tested positive), false negative (those who have the condition yet tested negative), and true negative (those who do not have the condition and tested negative). If a test is able to correctly define an individual as having a condition, it is classified as being sensitive. If a test is able to correctly define a non-affected individual as being condition free, it is classified as being specific.[81]

Sensitivity is calculated by dividing the number of true positive individuals over the total number of true positive individuals and false negative individuals. Specificity is calculated by dividing the true negative individuals over the total number of true negative and false positive individuals. These two factors, sensitivity and specificity, work inversely of each other. Therefore, as the sensitivity increases, the specificity of the test will decrease and vice versa.[81]

Positive predictive value is another measurement used in validity measures. The purpose of this measurement is to understand how many positive test results are true positives. Positive predictive value is calculated by dividing the number of true positives over the total number of true positive and false positive individuals. This result will give the probability that an individual will have the condition when a positive test result occurs.[81]

There are currently five commercially available NIPT panels in the United States. Three tests use the quantitative method; MaterniT21 by Sequenom, Verifi by illumina, and Harmony by

Ariosa. One test uses the SNP method, Panorama by Natera. Sensitivity, specificity, and false positive rates as obtained through provider websites are listed in **Table 1**.[42,86-89]

Although lab companies are willing to disclose their detection rates, not all labs are willing to disclose the specificity, false positive rates, or positive predictive values. The lack of this information makes effective conversations regarding test accuracy and comparison with patients difficult for prenatal providers. Without this information, providers are unable to discern the number of true positives a test captures as well as the tests overall accuracy. The lack of full disclosure regarding all aspects of a tests validity also makes comparison of tests difficult, especially when a provider is attempting to decide which test would be of most value to their patient population.

Table 1. Comparison of currently available Non-Invasive Prenatal Testing screening panels.

	MateriT21				Verifi				Panorama				Harmony				InformaSeq			
	Sens	Spec	FPR	PPV	Sens	Spec	FPR	PPV	Sens	Spec	FPR	PPV	Sens	Spec	FPR	PPV	Sens	Spec	FPR	PPV
13	91.7	99.7			97.5	>99.9			>99		0		93.8		<0.1		98.2	99.9		92.0
18	>99.9	99.7			97.4	99.6			96.4		<0.1		97.4		<0.1		98.3	99.9		
21	99.1	99.9			>99.9	99.8			>99		0		>99		<0.1		99.1	99.9		96.8
XX	99.4	99.4			97.6	99.2		98.4	>99.9		0						97.6	99.2		
XY					99.1	98.9		99.0	>99.9		0						99.1	98.9		
Sex Aneuploidy	96.2	99.7							>99											
Monosomy X					95.0	99.0			92.9		<0.1						95.0	99.0		
Triploidy									>99											

Sens = sensitivity; Spec = specificity; FPR = false positive rate; PPV = positive predictive value

Provider Experience with NIPT

Since the uptake of NIPT, there have been a number of studies analyzing provider experience, knowledge, comfort, and attitudes toward NIPT. A recent study by Horsting, et al. looked specifically at genetic counselors to better understand their personal opinions, experiences, thoughts, and concerns regarding all aspects of NIPT.[15] This study found that genetic counselors value cffDNA as a screening option and feel it should be made available to their patients. Counselors offered testing mostly to high-risk pregnancies, but considered average risk pregnancies on a case-by-case basis. Counselors indicated that they felt more comfortable accepting a negative cffDNA test as diagnostic than a positive result. Overall, the counselors felt comfortable offering this screening test to their high-risk patients.[15]

A study looking at Maternal-Fetal Medicine (MFM) fellows who were active members of the Society of Maternal-Fetal Medicine indicated that fellows reported being comfortable using NIPT 75.6% of the time.[90] There was a high knowledge level (>70% accuracy) demonstrated regarding using NIPT to screen for aneuploidy, however, knowledge accuracy decreased when queried about recent additions to NIPT panels.[90] 78% of patients indicated that they believe patients should see a genetic counselor when their pregnancy had an indication for NIPT use.[90] A majority of MFM fellows (82%) also expressed their preference of having a patient discuss all prenatal testing options with a genetic counselor or other prenatal provider.[90]

Multiple studies have been performed with respect to obstetrician attitudes regarding NIPT. In a study performed in 2013, 22% of respondents indicated that they are familiar with NIPT clinical data.[91] In a study performed in 2015, 91% of obstetricians expressed the need for more information regarding screening options.[92] 32% of obstetricians indicated they currently offered NIPT to high-risk patients.[91] Of the respondents 86.1% felt they would offer NIPT to high-risk

pregnancies by October 2013, and 76.2% predicted they would offer NIPT to average-risk pregnancies by October 2013.[91] As of 2015, 33% of surveyed obstetricians indicated they were ordering an expanded version of NIPT.[92] Of those individuals, 83% indicated they felt at least somewhat comfortable explaining the expanded version of the test to their patients.[92]

Obstetricians most commonly felt that patient anxiety, risks of follow-up invasive testing, and high false positives were the greatest limitations of NIPT.[91] The greatest advantages were recommendation by professional societies, no risk to the pregnancy, and long history/experience with the test.[14] Common themes among the studies show that respondents do not feel they have a high level of knowledge regarding NIPT, but they feel positive regarding the utility of the test. Many providers felt most comfortable ordering this test for chromosomal aneuploidies (85%), while many others were still comfortable offering this test for specific condition analysis (cystic fibrosis 82%, congenital adrenal hyperplasia 55%) and sex testing (21%).[14] 5% of obstetricians indicated they always refer their patients to an MFM specialist or genetic counselor for NIPT.[92] Overall, obstetricians indicated a favorable view toward the utility and future of NIPT.[14,91,92]

NIPT Informed Consent

There are a limited number of studies that assess the informed consent process, overall patient understanding, and patient thoughts regarding the informed consent process. A recent study involving multiple hospital locations analyzed the informed consent process regarding NIPT and patient's understanding after receiving a negative result. Patients indicated they understood that their normal NIPT test did not guarantee a healthy baby (27%) and still left a chance of having a child with Down syndrome (41%).[64] Overall there was a lack of knowledge, with only 10% of patients accurately answering all of the knowledge-based questions.[64] With regard to their experience, 32% reported a desire to have some element of their counseling session changed.

Broadly, patients requested more NIPT information, such as benefits and limitations, or for changes in the NIPT process. Regarding potential changes, participants indicated they would want a written copy of their results, to have additional NIPT options, to be offered NIPT in place of maternal serum screening, or they indicated that too much information was received at once.[64] This last point is especially important to understand because patients need to be fully informed yet comfortable with the information and their decision.

1.1.4.3 Invasive Prenatal Diagnosis of Fetal Aneuploidy.

Prenatal diagnosis of a fetal aneuploidy occurs via analyzing fetal cells directly. Fetal cells are collected using invasive procedures, either chorionic villus sampling or amniocentesis depending on gestational age. Due to the invasive nature of these procedures there is an inherent risk for pregnancy loss which is estimated to be <0.5%.[6-8,50] These procedures are offered to all women and recommended to women over 35 years of age at the time of delivery or women identified as high-risk based on screening test results or family history.[41,43]

Chorionic villus sampling (CVS) is a diagnostic procedure performed between 10-13 weeks gestation that extracts cells from the placenta. The placenta surrounds the baby and hosts its environment for growth and development. The placenta also contains the baby's DNA, allowing for a more accurate diagnosis as compared to non-invasive screening options. This procedure analyzes all 23 pairs of chromosomes via traditional cytogenetic methods such as FISH and karyotyping. CVS is able to detect and diagnose >99% of numerical or structural chromosome anomalies larger than 5-10 megabases.[93] Since the placenta is developing at a faster rate than the fetus, there is a chance that any aneuploidies detected during this procedure are isolated to the placenta due to replication error.[77-79] Amniocentesis is available for follow-up determination if a positive result occurs.[41,43]

Amniocentesis is an invasive procedure that extracts amniotic fluid for testing. Amniotic fluid surrounds the baby and contains only the baby's DNA material. This procedure is performed after 15 weeks gestation and ideally between 16-20 weeks gestation for detection of aneuploidy.[93] Amniocentesis uses traditional cytogenetic methods to detect and diagnose >99% of numerical or structural anomalies larger than 5-10 megabases in pregnancies.[93] For both amniocentesis and CVS, microarray and single gene testing can be performed on the collected samples.[2,15,43]

1.1.4.4 Current Guidelines

National Society of Genetic Counselors Position Statement, 2012

The National Society of Genetic Counselors (NSGC) supports the use of NIPT as a screening test in pregnancies that are considered to be at an increased risk for chromosomal anomalies. Currently, NSGC does not support the use of NIPT in average-risk populations. At the time of testing, NSGC recommends counseling by a qualified provider who is able to offer informed consent, counseling regarding the benefits and limitations of NIPT, and education regarding this screening test and all possible outcomes. NSGC recommends that individuals who receive an abnormal result should be offered the opportunity to pursue diagnostic testing for confirmation.[12]

National Society of Genetic Counselors Practice Guidelines, 2012

The majority of current studies focus on NIPT use in high-risk populations where a pregnancy was at an increased risk for a chromosomal anomaly. Therefore, NIPT is recommended for high-risk pregnancy populations including individuals who have had a positive screening test, an abnormal ultrasound, pregnancy history of chromosomal aneuploidy, or are of advanced maternal age. If an abnormal NIPT result is to occur, it is recommended that diagnostic testing be

offered for confirmation. If an individual opts for NIPT, it is not necessary to perform additional serum screening.[94]

American College of Obstetrics and Gynecologists Screening Practice Guidelines, 2016

ACOG recommends that all women be offered all screening and diagnostic testing options for genetic disorders, ideally at their first prenatal appointment. Each prenatal test has advantages and limitations that should be discussed thoroughly with each patient. The decision of which prenatal test is best to be performed should be based on an informed choice and via shared patient-provider decision making. Post-test counseling should be available for individuals who test positive or receive an inconclusive result. If a screening test is performed and results negative, additional screening testing should not be offered. All screening tests should be followed up with diagnostic testing options for confirmation.[41]

American College of Obstetricians and Gynecologists Diagnostic Practice Guidelines, 2016

ACOG recommends diagnostic testing options be available to all pregnant women and ideally discussed during their first prenatal appointment. Individuals electing to undergo diagnostic testing should be offered the option of chromosomal microarray in conjunction with their diagnostic test. Chromosomal microarray is recommended as the primary test when a fetus is presenting with an abnormal prenatal ultrasound examination. If the anomalies are strongly suggestive of a certain aneuploidy, karyotype with or without FISH may be performed before chromosomal microarray analysis. Post-test counseling should be available to all individuals.[43,78]

Statement of Purpose

The purpose of this research is to better understand genetic counselors' knowledge, opinions, and current ordering practices regarding NIPT given the recent advances of the screening test and possible future uses for this technology. This will allow for a greater insight into the utility

of this technology from a genetic counseling profession standpoint and will contribute to the body of knowledge that will allow researchers, prenatal professional organizations, and insurance companies to create comprehensive guidelines regarding NIPT. As NIPT continues to evolve and become available for all pregnancies, it is also important to create an inclusive public-health model for effective implementation.

1.2 SPECIFIC AIMS

Aim 1: To assess genetic counselors' current knowledge of NIPT.

Aim 2: To assess the readiness of genetic counselors in regard to the expansion of NIPT to all pregnancies and acuity toward the evolution of NIPT.

2.0 PUBLIC HEALTH

The value of NIPT has created its own public health significance and has changed the course of prenatal screening for high-risk pregnancies. Technology for this test has increased while still allowing for safe, non-invasive analysis of small genetic changes in fetal chromosomes with greater sensitivity and specificity than current screening options. Currently, this testing is recommended and most understood in pregnancies deemed at high-risk for a fetal aneuploidy, although available for all pregnancies.[41,43] Further research of this technology in average-risk individuals could increase detection rates of fetal aneuploidy and microdeletion and microduplication disorders while providing women a more accurate option for prenatal screening.

Overall, this non-invasive screening option has the highest detection rates among screening tests that do not carry a risk of miscarriage. There are advantages and limitations to women undergoing NIPT during pregnancy. One benefit is that most other non-invasive screening tests are only able to adjust a woman's risk which could lead to additional unnecessary screening and testing as well as patient anxiety. After completion of NIPT, follow-up with invasive diagnostic testing procedures is recommended, but additional serum screening is not warranted. NIPT also has the ability to give much more information which would otherwise require multiple methods of testing for the same results as well as an increased risk to the pregnancy. However, NIPT does not provide information on all the conditions that are included as part of maternal serum screening. NIPT is only able to detect certain chromosomal anomalies and is not able to detect the risk of open neural tube defects (ONTDs) or able to define physical characteristics which would be detected through second trimester screening methods. Also, since NIPT is targeted in nature, it

would not be as comprehensive with regard to chromosome analysis as compared to invasive diagnostic methods.

Although invasive diagnosis will remain necessary for some conditions, cffDNA has the potential to reduce the number of unnecessary invasive procedures.[53] One current application of this testing allows for the analysis of blood group incompatibility, particularly those caused by the rhesus blood antigen (RhD). Testing of cffDNA may also be useful in women who have a child with a known sex-linked condition.[14] Rather than having to go through invasive diagnostic procedures to produce a karyotype to learn the baby's sex, women can use cffDNA through non-invasive testing to learn the baby's sex. Although male fetuses would need to undergo invasive testing to see if they carry the harmful gene, initiating cffDNA testing beforehand could halve the number of invasive diagnostic procedures as female fetuses would not need to be tested until after birth and only when the female is ready to learn of her genetic status. This testing is currently being researched to be extended to covering a number of monogenic disorders.

Utilization of cffDNA analysis can benefit many individuals. The ability to use this screening test as early as 10 weeks gestation allows for physicians to more accurately follow patients as well as have a more productive conversation with their patients. Information provided by NIPT can give clear information about the fetus that could have significant clinical benefits, particularly where that information can be used for ongoing management throughout the pregnancy or for immediate medical intervention services after birth. For example, if monogenic disorder analysis were capable to detect female fetuses with congenital adrenal hyperplasia, these individuals would be able to receive earlier administration of antenatal dexamethasone which can reduce genital ambiguity.[14]

The ability to establish genetic information earlier in a pregnancy can also help families who would seek termination if a fetus is affected with an aneuploidy. Having the time to reflect on the result may help ease a family's emotional concerns and reduce psychosocial sequelae if they choose termination. Knowing the fetus's genetic status early in the pregnancy may also help reduce psychological trauma if the procedure is able to be performed much earlier in the pregnancy. Lastly, there is also a lower risk for complication when termination is performed earlier in gestation. The reverse side of knowing this information is that there may be increased genetic testing accompanied by a higher rate of terminations due to gender selection or selection against fetuses with aneuploidies.[95]

It is important to note that NIPT is a targeted test that is only looking at specific chromosomes. If a fetus were to have an abnormal ultrasound finding and the mother opts for NIPT and results are negative, there could be a false reassurance. One study found that if only NIPT were performed, approximately 23.4% of abnormal karyotypes would have been missed.[96] It is also important to understand the mothers' standpoint on termination if an abnormal result were to occur as the timeframe to receive NIPT results could interfere with amniocentesis testing and termination procedures.

Identification of aneuploidies, microdeletion conditions, or microduplication conditions prenatally can reduce the number of families going through a diagnostic odyssey which in turn can help reduce unnecessary medical costs. Families with microdeletion and microduplication syndromes are typically not noticed until birth or childhood unless a birth defect is noted on fetal ultrasound. Since microdeletion and microduplication conditions are often associated with intellectual disability and low muscle tone, infants and families can benefit from early intervention services. Prenatal detection can allow for optimal management and therapeutic benefit.

There are companies who have NIPT platforms that conduct whole genome sampling and only report specific selected conditions. The difficulty of reporting all conditions picked up via genomic NIPT is that we could have a great influx of variants of uncertain significance. This makes counseling patients on their options difficult, especially as we do not fully understand the impact of the microdeletion or microduplication clinically. Invasive diagnostic procedures would still need to be performed to confirm that these microdeletion or microduplication conditions are not isolated to the placenta, which would cause no harm to the growth and development of the fetus.[39]

2.1 FROM A MEDICAL MODEL TO A PUBLIC HEALTH MODEL

Originally, NIPT was a test offered only to individuals who met high-risk clinical criteria. This included women of advanced maternal age (≥35 years at the time of delivery), individuals who had an abnormal maternal serum screen result or an abnormal ultrasound finding, and individuals who had a family or personal history of aneuploidy.[10] Currently, prenatal professional organizations have released guidelines stating that all women, regardless of risk, should be offered the opportunity of NIPT and invasive diagnostic procedures. This transforms NIPT from a medical model, where high-risk criteria is needed for testing to be performed, to a public health model, where testing is available to all individuals.

To date, there are a few other screening tests that originated as a medical model and are currently successfully utilized within a public health model. One example would be the use of maternal serum screening among all pregnant women. Although it is understood that the risk for fetal aneuploidy increases with maternal age, fetal aneuploidy can occur in pregnancies of women of all ages. Through preliminary assessment researchers were able to show that when match

controls were performed, they were able to detect pregnancies with an increased risk for fetal aneuploidy for all maternal age groups.[97-98] This led to the current guidelines where screening tests are available to all pregnant women, regardless of maternal age. Maternal serum screening was also able to be successful because of its non-invasive nature and its cost effectiveness when compared to using invasive diagnostic procedures alone.

Since ultrasonography and prenatal visits were already standard of care for all pregnancies, providers were able to integrate the conversation of maternal serum screening into all appointments. Having screening offerings become the new standard of care also allowed for providers to have more effective conversations with their patients at a younger maternal age. Originally, these individuals were unaware of a fetal aneuploidy until an ultrasound later in gestation was able to be performed. By incorporating screening practices into all pregnancies, providers were able to better counsel patients regarding the management of their pregnancies.[97-98]

Until recently, NIPT was viewed within the context of a medical model, whereas the patients needed to meet criteria in order to qualify for testing. New guidelines have stated that all prenatal testing options should be available to all pregnancies, regardless of risk. Due to this change, it is important to evaluate the readiness of providers to expand testing options as well as their current knowledge base with regard to NIPT.

2.2 PUBLIC HEALTH RELEVANCE AND DISPARITIES

With the influx of patients eligible for NIPT, it is important to understand all providers' educational level and attitudes toward this expansion. By surveying additional provider populations in the future (midwives and obstetricians), we can accurately assess how each provider population

perceives NIPT allowing provider organizations to more adequately address any concerns or educational needs within their provider population. By understanding each provider population's involvement with NIPT and their specific needs, organizations can also help to streamline patient interaction with necessary providers. This will allow for the creation of appropriate guidelines in combination with other organizations, directly with laboratory companies creating NIPT, and with insurance companies, which will benefit patients and promote a safe testing environment. By creating professional guidelines, we can also be sure that patients are receiving the informed consent necessary to understand all of their prenatal screening options in general as well as their many options within NIPT screening alone.[99]

Given that patients could undergo NIPT under the guidance of a number of medical providers, it is very important that similar information is presented to patients in all of these scenarios so that patients understand all aspects of testing. Some patients hear that they will be able to know the sex of their baby at 10 weeks gestation, which is 8 weeks earlier than patients would normally learn during their level II ultrasound, and may decide to undergo NIPT solely for this information. It is very important that the providers emphasize all of the information that can be revealed during this testing so that patients are not unexpectedly told news. It is also important that patients understand that although this testing has a high detection rate, if any abnormal result were to arise, invasive diagnostic testing would be recommended to confirm this information.

There are many benefits when applying NIPT to a public health model. NIPT has the availability of early detection of many chromosomal anomalies which are undetectable in current prenatal screening methods. NIPT also has a greater sensitivity, specificity, and false positive rate as compared to other prenatal screening tests. This will in turn allow for better pregnancy management for all women. The higher detection rates of NIPT will also decrease the number of

unnecessary diagnostic tests that would otherwise be caused by the high false positive rates seen in other prenatal screening tests. In a recent study, it was found that 4.5% of women who screened positive with FTS simultaneously screened negative via NIPT.[51] There was also an overall lower trisomy risk score when using NIPT as compared to other prenatal screening methods.[51] This could decrease the number of unnecessary procedures as well as reduce patient anxiety.

Although there are great benefits, there are also many limitations to implementing NIPT as a population based model. To date, the majority of research regarding NIPT validity has been performed in high-risk pregnancy populations. There has also been a rapid increase in the number of conditions screened for via NIPT. As some of these conditions are less common, there is the risk of having a greater rate of false-positive and false-negative results with a lower positive predictive value.[51,82] The research that has been performed regarding NIPT validity in average-risk populations have not used karyotyping to confirm NIPT results.[82] Other limitations include those associated with NIPT testing within itself, such as having a greater number of non-informative results or increasing the number of maternal incidental findings with regard to chromosomal anomalies. [53,63,68,70,72,73,83]

There are also barriers with regard to implementing NIPT in a public health model. Current studies have indicated that providers feel they need more education regarding NIPT.[92] One major barrier is the lack of available formal provider education. If providers are not educated about NIPT they are less likely to implement screening using NIPT within their practice. A second major barrier is the increase in ordering volume and the need to provide pre- and post-test counseling regarding NIPT to a larger population. Informed consent is a vital step when ordering prenatal screening tests, providers will now have to talk to all patients about NIPT while still maintaining a similar patient load. Conversations regarding NIPT are comprehensive and the need for proper

post-test counseling will also take additional time. Therefore, the final barrier would be patient accessibility and availability to providers for all aspects of testing.

As genetic testing expands within all medical practices, pre-test counseling has broadened from genetic counselors to other medical providers. For example, many oncologists now provide pre-test counseling regarding hereditary cancer gene testing and neurologists provide pre-test counseling regarding genetic epilepsy disorders. Provider education and understanding of genetic tests are essential as they integrate these conversations into their practice.

2.3 PUBLIC HEALTH INTERVENTION

Due to ACOG recently releasing a statement that all prenatal testing options should be provided to all pregnancies, the striking number of obstetricians who indicated more education regarding NIPT was desired, and the lack of information regarding all prenatal providers' education level surrounding NIPT, the need for an inclusive educational material is apparent.

Continuing education credits are necessary for many medical professions. Although there are some educational resources available, providers are not guaranteed the ability to attend all continuing education courses involving NIPT. Therefore, an applicable public health intervention would be to provide educational materials for all prenatal providers. This educational material could be accessed by providers via online access through professional organizations. All providers would have access to this information, regardless of being a member to that organization or not. By providing this educational material, provider aptitude could increase which will in turn improve the interaction between the provider and patient as well as overall provider comfort when explaining and interpreting NIPT results.

This research project seeks to understand current provider knowledge and opinions toward NIPT. By assessing this information now and understanding that educational materials regarding NIPT is desired by prenatal providers, we can later perform another assessment to understand providers' gain in knowledge and adapt educational materials as necessary over time and as testing options evolve. The long-term goal would be to increase provider knowledge which will in turn increase provider competency within all aspects of NIPT as well as adapting its use within their practice. A detailed logic model regarding this project and further explanation is provided below and in **Figure 1.**

The inputs of this project would be to obtain funding to support the development of the educational materials and creating an open online website which will include educational materials for all prenatal providers. Stakeholders for this project would include all prenatal professionals (obstetricians, midwives, genetic counselors, and maternal-fetal medicine physicians), professional organizations for prenatal providers, policymakers, payers, and public health experts. Outputs would include the collaborative educational material and assessment of provider knowledge after implementation of these educational materials. Assessment of providers would be performed by surveying random practicing prenatal professionals. The first assessment would provide as a baseline and be conducted before educational materials are administered. All following assessments of individuals who access the educational materials would be compared to this baseline. The goal would be to reach currently practicing obstetricians, genetic counselors, and midwives with a long-term objective of >70% of providers indicating competency within multiple aspects regarding NIPT (patient education, ordering testing, comfort interpreting results, and comfort explaining results to patients).

Short-term goals (0-12 months) would be to create and provide these educational materials and re-assess provider knowledge and opinions regarding NIPT. Providers will be required to submit their e-mail address to obtain the educational materials. This e-mail address will be hosted on a confidential server with the only purpose of being able to re-contact providers for further survey follow-ups. Medium-term goals (12-36 months) would be to continue assessment of provider knowledge, to work with prenatal professional organizations to create standard guidelines regarding prenatal testing options, and to work with prenatal professional organizations and lab companies to create and distribute educational materials. Long-term goals (36-60 months) would include assessing provider competency via questionnaire with the objective of >70% of providers indicating competency and comfort ordering NIPT.

With regards to the educational material, there are a few essential matters to cover. First and foremost, it would be ideal to create a slide set covering NIPT information. Creation of this slide set would be a collaborative effort between multiple professional prenatal organizations and NIPT laboratory companies and researchers. These include:

- Overview of basic information:
    - Genes and chromosomes
    - Aneuploidy
    - Microdeletion and microduplication disorders
- Information regarding available screening and diagnostic tests:
    - First trimester screen
    - Quad screen
    - Contingent/Sequential Screening
    - Combined Screening

    - Ultrasonography
    - Chorionic Villus Sampling
    - Amniocentesis
- The history of NIPT
- Indicating what fetal source NIPT analysis is derived from
- Differentiating between the different commercially available platforms and how different platforms affect results disclosures
- Explaining the many different testing options including a further discussion about microdeletion and microduplication disorders
- Sensitivity, specificity, and positive predictive values of available tests
- Detailed information regarding the benefits and limitations of NIPT as compared to other prenatal screening options
- Testing specific information:
    - Applicable patient samples
    - When it is appropriate to draw samples
    - How to contact testing companies
- Possible test results

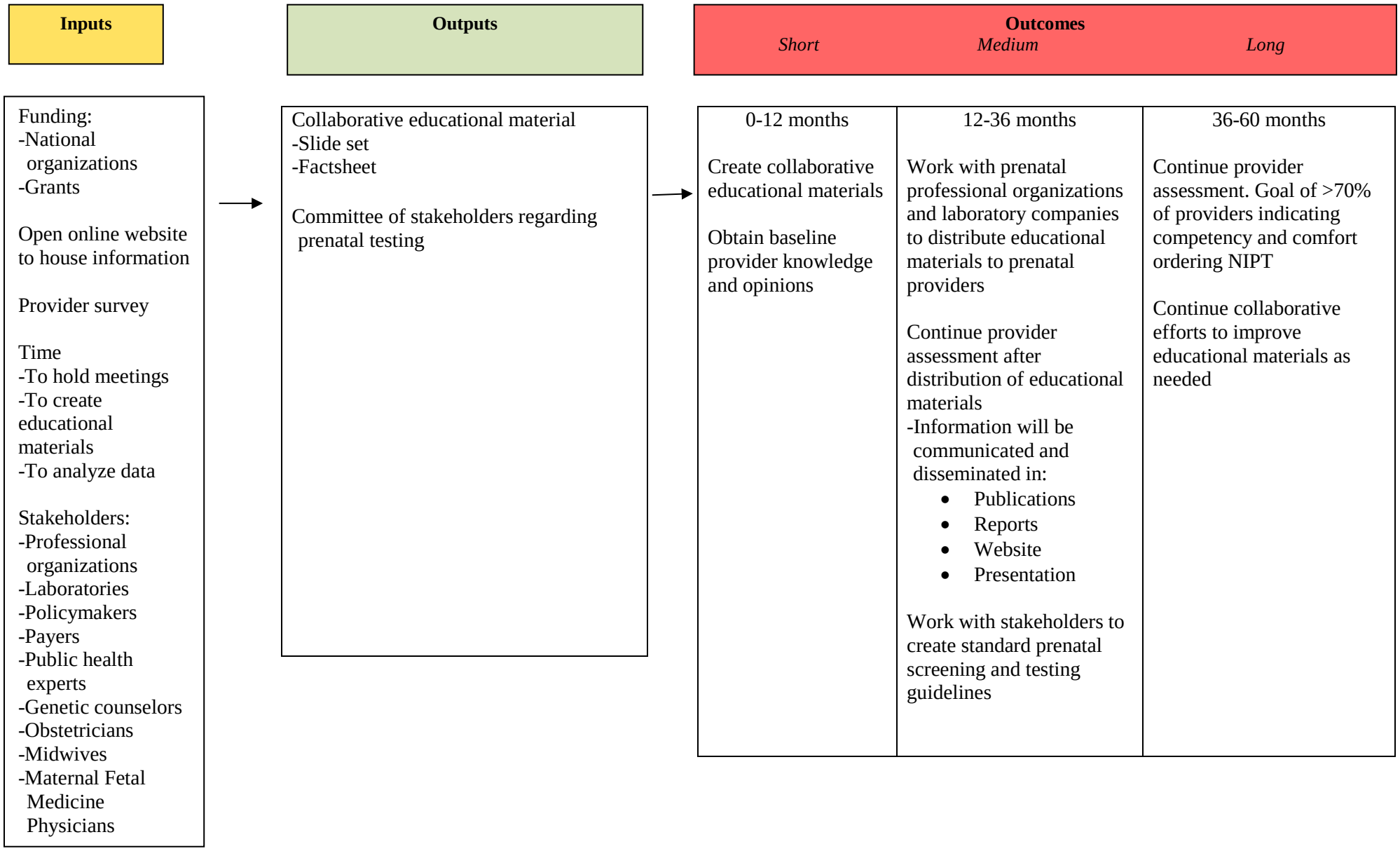


Figure 1. Proposed public health intervention regarding the creation and assessment of prenatal educational material.

3.0 METHODS

3.1 QUESTIONNAIRE DESIGN AND DISTRIBUTION

This study and questionnaire (ID: PRO15090107) is currently approved by the Institutional Review Board of the University of Pittsburgh (**Appendix A**). The study questionnaire was built electronically in Qualtrics and contained 34 questions. The questionnaire was adapted from a previously developed questionnaire on provider knowledge regarding genetic testing.[14] The questionnaire begins with a brief description of NIPT and its current utilization within the clinic setting. The questionnaire contains both set choice and open-response questions and was broken into three sections: 1) gauging current NIPT knowledge of participants and to determine what prenatal population they are ordering this test for, 2) understanding provider opinions of current and future NIPT testing directions including comfort in ordering microdeletion, microduplication, and monogenic disorder NIPT panels and, 3) participant demographics. The questionnaire was designed to be anonymous with no questions revealing identifying information. This questionnaire was assessed by multiple individuals with a specialty in prenatal genetics, genetic counseling, obstetrics/gynecology, and an NIPT researcher prior to distribution.

3.1.1 Participant population.

The participant population consists of practicing genetic counselors who were members of the NSGC in 2015. A cover letter and the questionnaire was distributed electronically via the National

Society of Genetic Counselors e-mail listserv during December 2015. **Appendix B** contains a copy of the cover letter and questionnaire distributed to the members of the National Society of Genetic Counselors. All data was collected through Qualtrics and is saved in a secured electronic file.

3.2 DATA ANALYSIS

Descriptive analysis of all data collected via the questionnaire was performed to generate count data for the responses to each question. Data was analyzed using Microsoft Excel. Open ended responses were analyzed as a separate category, typically denoted as "other". The only instance this did not occur was when analyzing individuals who designated a sub-specialty, these individuals were categorized with other similar answers. Due to the nature of this project, it was required that all questions be answered in order to move forward in the survey. Therefore, those who did not complete the survey were not included in the analysis of the survey questions.

This questionnaire was distributed prior to the May 2016 ACOG bulletin release stating that all prenatal testing options should be available to all pregnancies, regardless of risk. This update allows the availability of average-risk patients to obtain NIPT, CVS, and amniocentesis through their prenatal providers without indication of a high-risk for fetal aneuploidy. Therefore, genetic counselors were surveyed regarding information and guidelines available before the ACOG May 2016 bulletin release.

4.0 RESULTS

A total of 180 participants clicked on the survey link included in the email notification and began the survey. Of these, 113 individuals completed the survey (62.8%). All individuals who completed the survey identified themselves as genetic counselors. Of the 113 respondents 52.2% reported a sub-specialty that involved the field of prenatal genetics. The majority of respondents (54.0%) reported working in a hospital-based setting. The number of years practicing had a fairly even distribution with individuals of each category participating. The majority of individuals (36.3%) had been practicing for 10 or more years. As NIPT was commercially available in 2011, 51.3% of the respondents began practicing prenatal genetics after the incorporation of NIPT into clinical practice. Demographic information for study participants is summarized in **Table 1.**

Table 2. Demographic summary of participants.

	Total (n=113)	
	n	%
Sub-specialty		
Yes	69	61.1
Prenatal	51	73.9
Prenatal & Other	8	11.6
Other	10	14.5
No	41	36.3
Did not answer	3	2.4
Primary Work Setting		
Academic	27	23.9
Private Practice	21	18.6
Hospital Based	61	54.0
Lab	4	3.5
Number of Years Practicing		
0-2	31	27.4
2-5	27	23.9
5-10	14	12.4
10+	41	36.3

Participant opinions regarding their current knowledge of NIPT was assessed over multiple questions. Participants were first asked to what extent they agreed or disagreed with the following statement: I am familiar with published NIPT clinical data. A total of 95% of respondents indicated they agreed or strongly agreed with the statement. A more thorough breakdown of this information is illustrated in **Figure 2.**

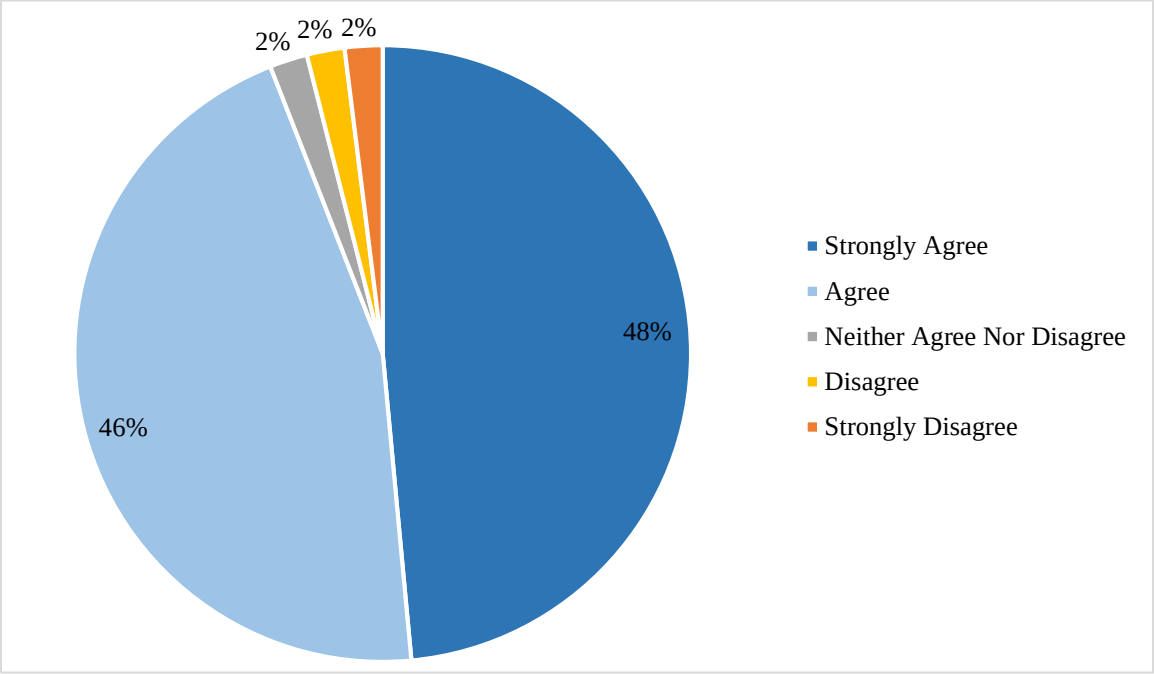


Figure 2. Participant knowledge of Non-Invasive Prenatal Testing (n=113).

Participants were queried on how they learned about NIPT by checking all methods that applied to them. There was a wide distribution regarding all educational standpoints. This information was analyzed from a general standpoint (**Figure 3**) among all genetic counselors queried as well as broken down to analyze the differences in methods utilized by years practicing (**Figure 4**). Individuals who indicated they had not learned about NIPT were circumvented to the demographics section of the questionnaire and were not able to complete the rest of the survey.

In general, the methods most commonly used to learn about NIPT among genetic counselors included discussion with peers (n=103, 91.2%), literature review (n=97, 85.8%), discussion with a lab representative (n=87, 77%), and at a conference (n=83, 73.5%). Methods of learning about NIPT among all participants is depicted in **Figure 3.** Of the "other" column, individuals stated via clinical practice working in a prenatal position (3), through their prenatal clinical rotation in graduate school (1), and that their state provides data comparing labs (1).

This information was also broken down by years practicing. Individuals who have been practicing for 0-2 years reported formal education (n=29, 53.7%) as their primary factor for learning about NIPT. Individuals practicing 2-5 years most commonly reported via lab company advertisements (n=15, 28.8%), discussion with a lab representative (n=23, 26.1%), and through formal education (n=14, 25.9%). Individuals practicing 5-10 years had an equal distribution between all methods with the exception of formal education being the lowest method of learning about NIPT (n=3, 5.6%). Individuals who have been practicing for 10 or more years most frequently reported literature review (n=37, 37.8%), at a conference (n=31, 36.9%), through a continuing education course (n=22, 36.1%), via discussion with peers (n=38, 36.2%) or via discussion with a lab representative (n=31, 35.2) as their major factors for learning about NIPT. Further breakdown of this information is illustrated in **Figure 4.**

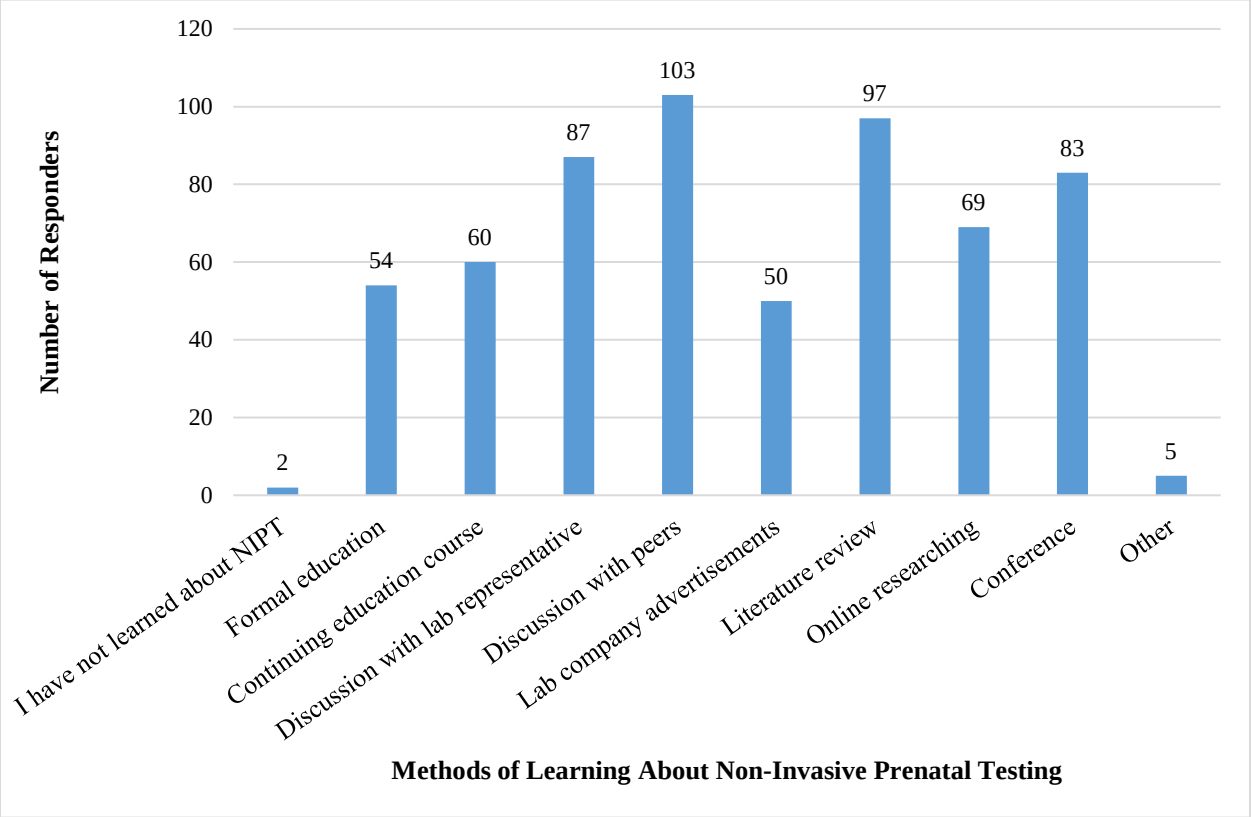


Figure 3. Participant reported methods of learning about Non-Invasive Prenatal Testing.

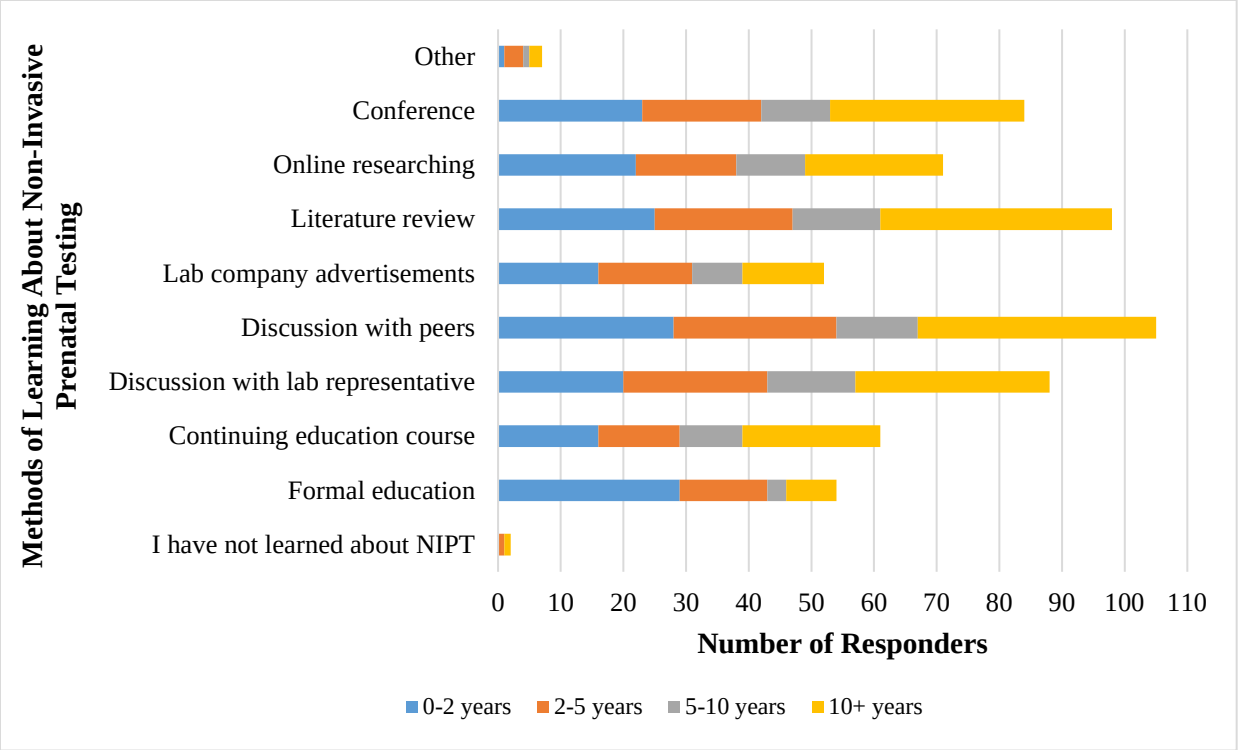


Figure 4. Reported methods of learning about Non-Invasive Prenatal Testing broken down by years practicing.

After the introduction of NIPT into the prenatal clinical setting, it was recommended for only those in the high-risk pregnancy category. Although some recommendations indicate that this testing now be offered to every pregnancy, participants were queried on their knowledge regarding high-risk pregnancy criteria given that it is still recommended by NSGC that use of NIPT be limited to high risk pregnancies (**Figure 5**). With regards to determining if a patient is at high risk-for carrying an aneuploid pregnancy, respondents indicated 100% for maternal age, 98% for an abnormal first trimester screening, 96% for an abnormal maternal serum screening result, 98% for an abnormal ultrasound finding, 60% for a family history of aneuploidy, 95% for a previous pregnancy with aneuploidy, and 10% indicated other. This information falls closely in line with the American College of Obstetricians and Gynecologists (ACOG) recommendations.

The other category encompassed answers including family history of a known balanced translocation (5), if the NIPT resulted unreportable (2), if the patient was average-risk with no previous screening (2), and 2 individuals reported that they are not a prenatal genetic counselor and do not order this testing. Further delineation of classification of a high-risk aneuploidy pregnancy is illustrated in **Figure 5.**

Of the 111 respondents who were able to continue the full survey, 100% accurately stated that NIPT for aneuploidy could be performed as early as 10 weeks gestation. Respondents also indicated that they are mostly or very confident interpreting NIPT results (99%) and mostly or very comfortable explaining NIPT results to their patients (99%).

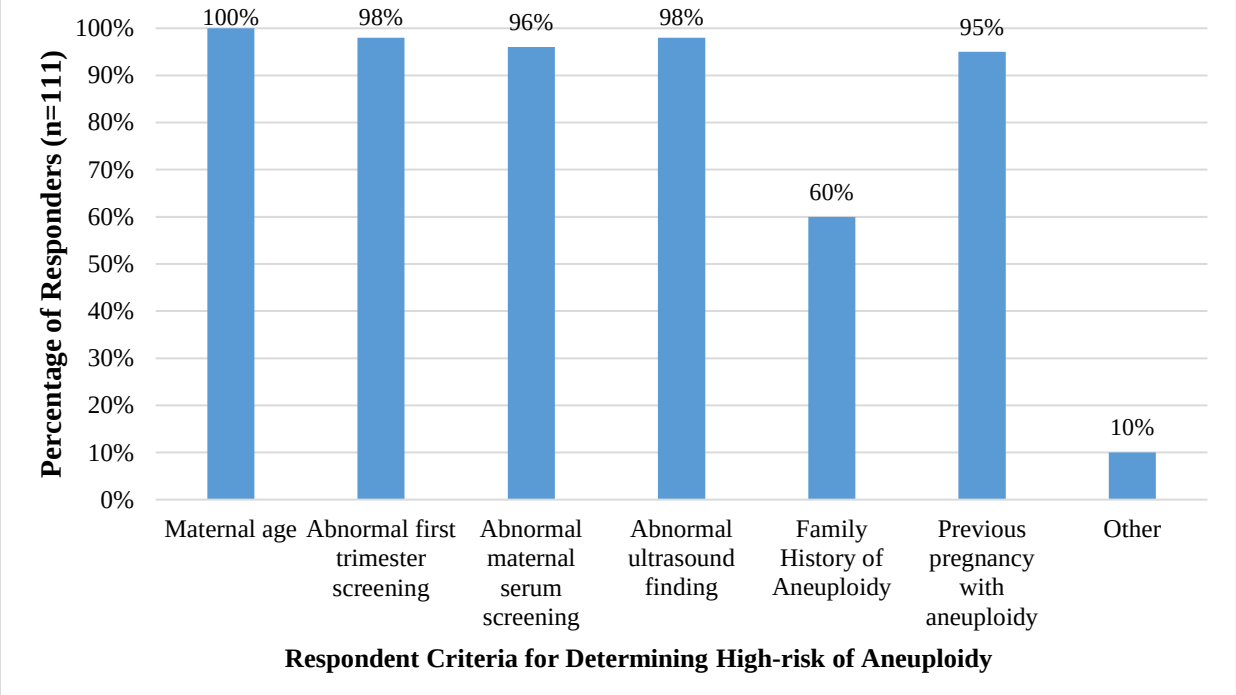


Figure 5. Criteria participants use to classify a patient with having a high-risk for fetal aneuploidy (n=111).

There are many factors involved in deciding which screening test will be the most beneficial for a patient. Participants were queried regarding the many factors of screening tests and what they believed constituted as an advantage, limitation, or neutral factor regarding NIPT compared to other prenatal screening options (**Figure 6**). Respondents felt the greatest limitations were availability of genetic counseling (n=23, 23%), the false positive rate (n=32, 29.3%), and coverage by insurance companies (n=51, 47.7%). The greatest advantages were availability during gestational age (n=88, 80.7%), detection rates (n=104, 95.4%), and patient acceptance (n=84, 77%). Within the "other" column, individuals stated the targeted nature of NIPT (1), cost (1), positive predictive values in average-risk populations (1), the high amount of non-genetic counselors ordering this test without properly counseling the patient (1), the risk of sex selection (1), and 2 individuals indicated they did not understand the question.

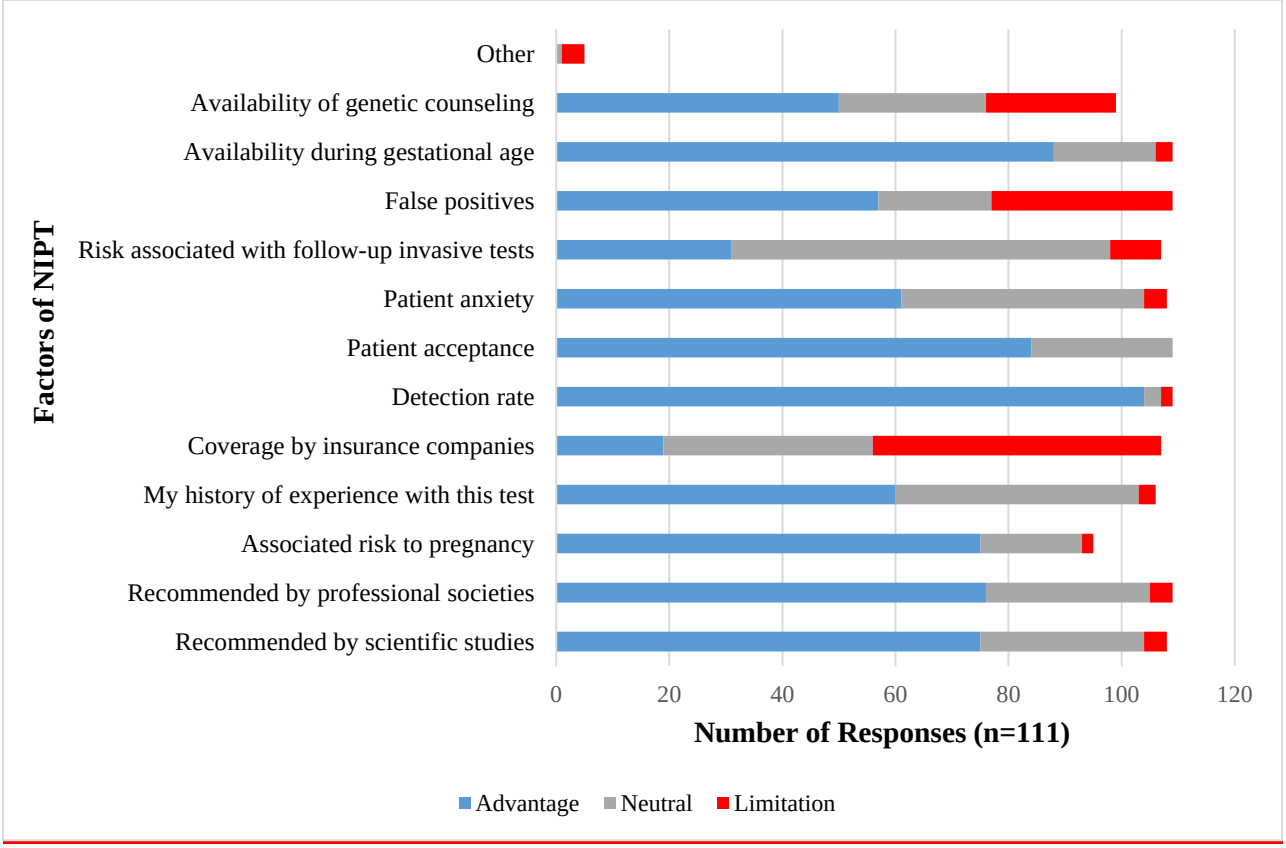


Figure 6. Participant perception of multiple Non-Invasive Prenatal Testing factors (n=111).

Over the years, NIPT has evolved to include select microdeletion and microduplication condition analysis. The questionnaire enquired about participant knowledge of and opinions toward the inclusion of microdeletion and microduplication disorders in the context of NIPT. In total, 89% of respondents agreed or strongly agreed that they were familiar with published clinical data within this realm of NIPT. Of the 111 respondents, 27% agreed or strongly agreed that microdeletion and microduplication analysis should be offered in NIPT while 38% disagreed or strongly disagreed with the statement. More information regarding the offering of microdeletion and microduplication is illustrated in **Figure 7.**

Information regarding the offering of microdeletion and microduplication analysis in NIPT was also broken down by the number of years practicing to assess if there was a difference in

opinion. Information was grouped into disagree, agree, and neutral for comparison. Individuals who have been practicing 0-2 years most strongly identified with neither agreeing nor disagreeing (n=16, 50%) to the addition of these conditions for analysis. Individuals practicing 2-5 years (n=14, 53.8%) and 10 or more years (n=19, 45.2%) most commonly disagreed to the addition. Individuals practicing for 5-10 years equally remained neutral (n=6, 40%) and agreed (n=6, 40%) to the addition of these conditions for analysis. Greater detail of this information is depicted in **Figure 8.**

Participants were also queried regarding their microdeletion and microduplication ordering practices. In general, 16% of respondents indicated that they currently offer microdeletion and microduplication analysis to all pregnancies, 20% offer analysis only to high risk pregnancies, 45% do not offer analysis to any pregnancies and 19% selected the option “other”. Within the “other” category, 16 individuals indicated that they allow this additional analysis on a case by case basis and typically only if there is an indication for a microdeletion or microduplication disorder (family history, ultrasound finding). Two individuals stated that the lack of data makes this offering inappropriate, three individuals stated they do not order this testing as they do not work in a prenatal setting, and 3 individuals indicated they talk about microdeletion and microduplication testing differently than they would talk about aneuploidies or only when using certain testing companies as related to insurance.

As microdeletion and microduplication analysis is available, participants were asked questions regarding their confidence interpreting results and comfort level explaining these results to patients. In total, 86% of respondents indicated they are mostly confident or very confident interpreting patient microdeletion and microduplication results and 87% of respondents are mostly comfortable or very comfortable explaining microdeletion and microduplication results to their

patients. If a patient's results return indicating a microdeletion or microduplication, 92% of individuals indicated that they would offer invasive diagnostic testing, 1% would not offer testing, and 7% stated there is not enough information at the present time to indicate invasive diagnostic testing.

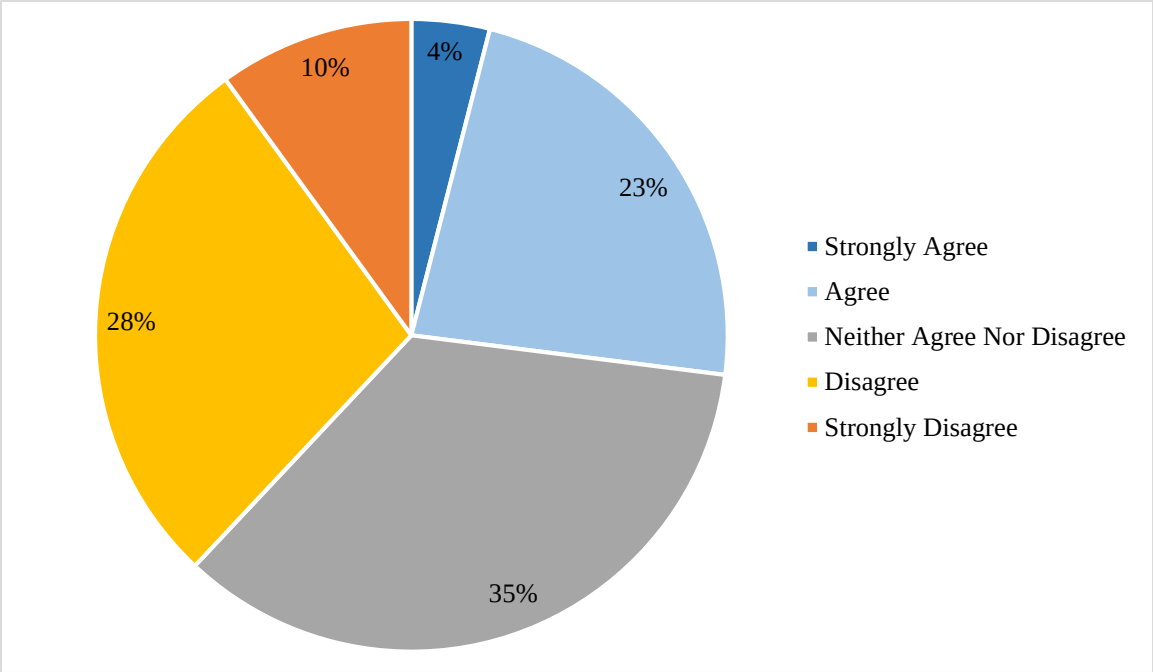


Figure 7. Respondent perception toward Non-Invasive Prenatal Testing inclusion of microdeletion and microduplication disorders (n=111).

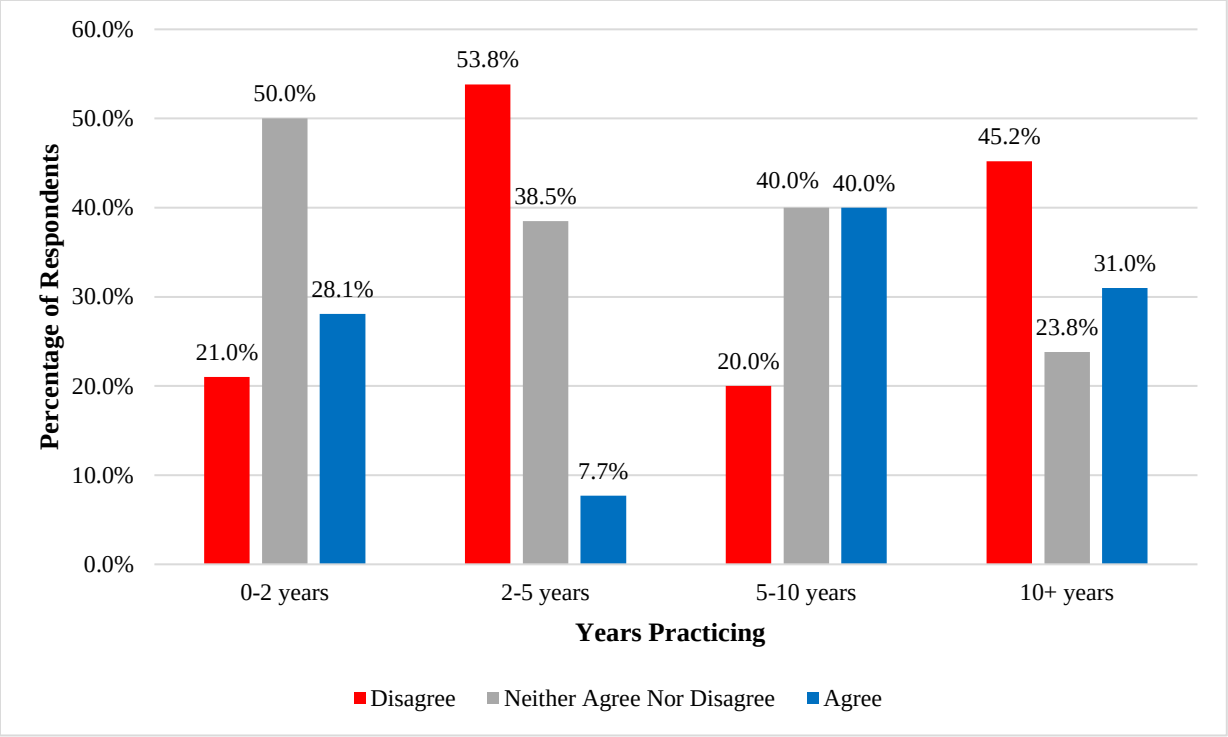


Figure 8. Respondent opinions regarding the inclusion of microdeletion and microduplication analysis in Non-Invasive Prenatal Testing delineated by years practicing.

For greater understanding of ordering practice, participants were asked to what population of pregnancies they currently offer NIPT. Previous ACOG recommendations stated that only high-risk pregnancies should be offered NIPT as they have the greatest risk for carrying an aneuploid pregnancy and are the best studied population for this screening test. It has only recently been recommended that NIPT be available for all pregnancies and not reserved for high-risk pregnancies. This enquiry allowed for a more thorough analysis of how many practicing genetic counselors are currently offering NIPT in general and how many are offering NIPT with microdeletion and microduplication analysis to only high-risk pregnancies versus those offering to all pregnancies.

In general, it was found that 95% of participants currently offer NIPT to high-risk pregnancies, the other 5% of participants identified as non-prenatal genetic counselors. With regards to average-risk pregnancies with no significant risk factors for aneuploidy, 36% of individuals responded that they offer NIPT. The remaining 64% indicated they do not currently offer NIPT to average-risk pregnancies and of these individuals, 81% stated they do not plan to offer NIPT to average-risk pregnancies in the next 12 months.

Of the 95% of participants offering NIPT to high-risk pregnancies, 34% of respondents indicated they are also offering microdeletion and microduplication analysis for those pregnancies. Of the 36% of respondents who indicated they order NIPT for average-risk pregnancies, 16% also indicated they offer microdeletion and microduplication analysis for those pregnancies. This information is illustrated in **Figure 9.**

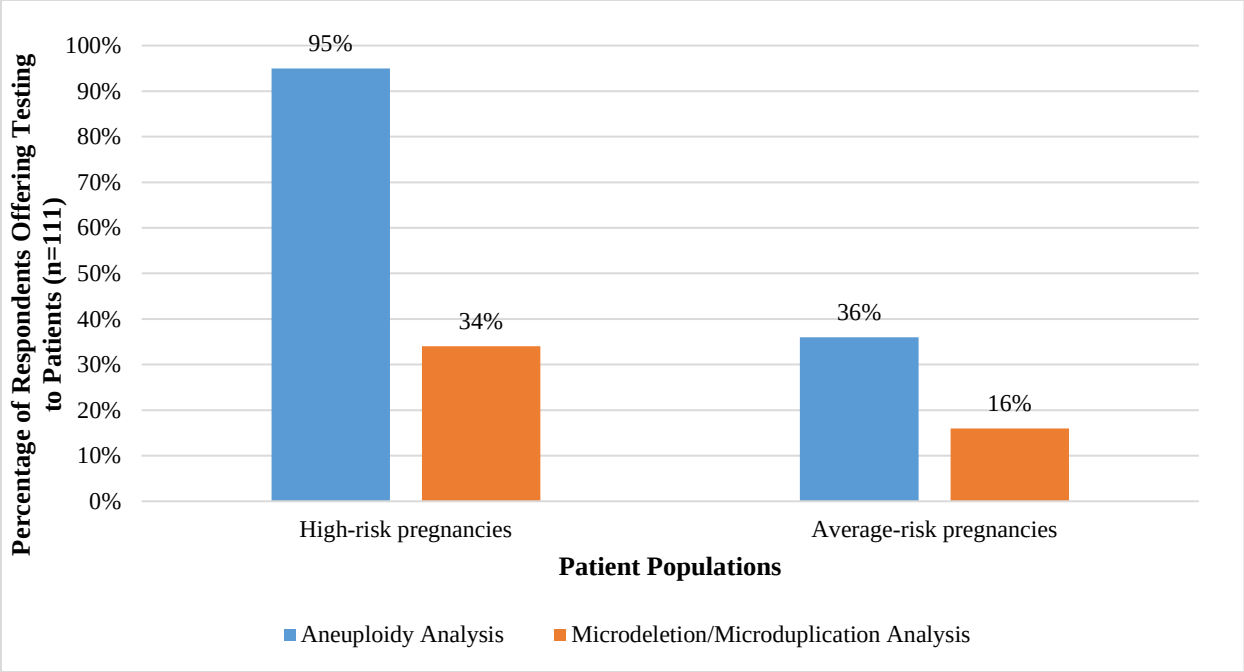


Figure 9. Respondent offering of Non-Invasive Prenatal Testing categories to high-risk pregnancies vs. average-risk pregnancies (n=111).

As research progresses with respect to NIPT, laboratorians are researching the possibility of adding monogenic disorder analysis to the testing panel. Therefore, respondents were queried regarding their opinions toward this potential addition to NIPT panels. In total, 32% mostly or highly approved of this offering, 47% of respondents remained neutral, and 21% of respondents mostly or highly disapproved of this offering. Information detailing this specific breakdown is summarized in **Figure 10.**

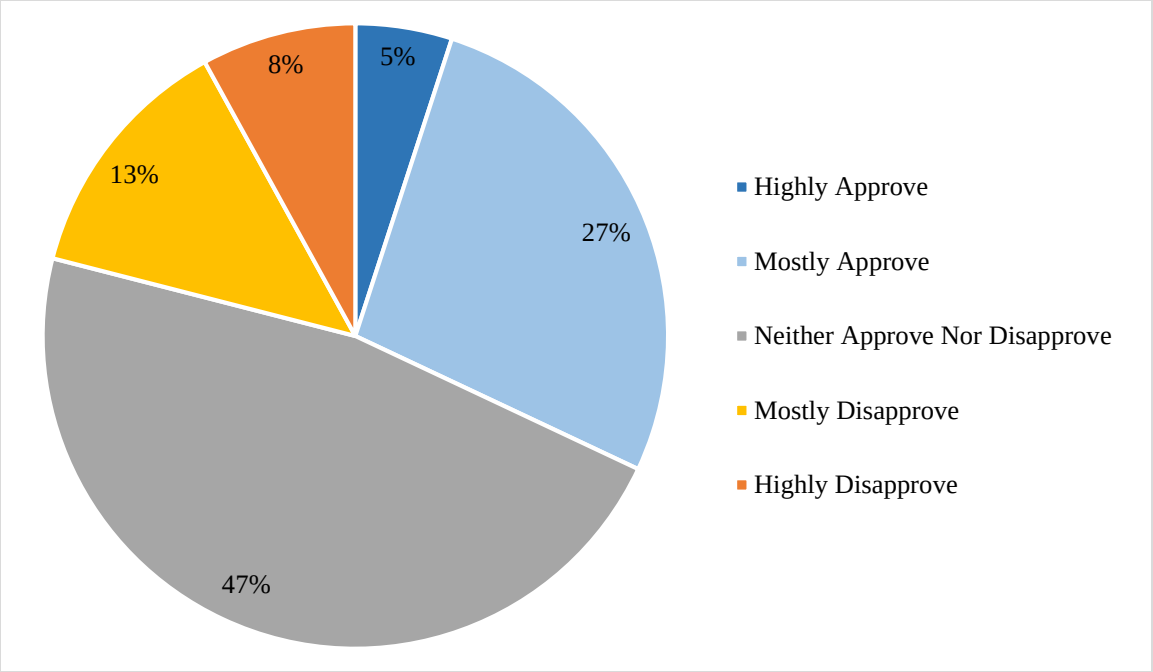


Figure 10. Respondent perception toward future Non-Invasive Prenatal Testing inclusion of monogenic disorders (n=111).

Since there are multiple types of testing that can be offered through NIPT (aneuploidy, microdeletion and microduplication, and monogenic disorders), respondents were asked if they would want the option to opt for specific testing categories or if they would be content with ordering all categories for every patient without the option of limiting testing categories. All 111 (100%) respondents indicated that they would want the option to opt for specific testing as indicated for the pregnancy.

5.0 DISCUSSION

NIPT is a newer technology that has been rapidly evolving since it became commercially available in 2011. Information regarding provider knowledge of this technology is limited, most previous research revolves around obstetrician perceptions toward this technology.[90,91] Due to the number of professions that are able to order this testing, the changing guidelines of NIPT ordering regarding accepted populations for testing, and the quick evolution of this technology, it is important to understand current provider practice as well as how providers feel about new NIPT options and the testing in general. This study represents the genetic counseling population and provides information regarding their comfort with ordering the test and their perception regarding the increased availability in the types of results patients can receive as the testing technology expands.

This information is not only helpful for creating educational materials but is also beneficial for testing companies who are creating more advanced technologies and testing requisition forms. Guidelines are important for patient safety and equality as well as for insurance purposes as these testing technologies can be expensive and are sometimes covered under certain insurance criteria.

5.1 ANALYSIS OF QUESTIONNAIRE

Questions throughout the survey showed that genetic counselors are knowledgeable about NIPT and are highly confident interpreting NIPT test results (99%) and comfortable explaining them to their patients (99%). **Figure 5** results show that genetic counselors criteria for determining an

individual as having a high-risk for aneuploidy closely reflect current ACOG guidelines. The only deviation from ACOG guidelines was that 60% of respondents felt that a family history of aneuploidy constituted high-risk criteria. Further delineation of this question may show there is ambiguity regarding family history of aneuploidy as most prenatal providers are not concerned unless an individual in the family has an immediate relative with a genetic condition or there is a known balanced translocation in one of the parents.

Genetic counselors indicated a wide range of resources for learning about NIPT (**Figure 3**). The most common methods of learning about NIPT included discussion with peers (91.2%), literature review (85.8%, discussion with a lab representative (77%), and at a conference (73.5%). This information was further broken down to delineate which methods are more commonly used based on the number of years practicing (**Figure 4**). Individuals practicing 0-2 years most commonly reported learning about NIPT via formal education (53.7%). Individuals practicing more than 2 years reported a great variation in ways of learning about NIPT. These diverse methods of learning about NIPT allow for individuals to create a more in-depth understanding by talking to peers and lab representatives who can explain nuances of testing. Genetic counselors are also learning about the technologies via conferences where they are able to participate in group discussions regarding ethical issues, and advantages and limitations of the technology platforms offered.

Figure 9 captures a general overview of NIPT ordering in regards to the high-risk versus average-risk pregnancy population, and with respect to microdeletion and microduplication analysis between the two populations. Current guidelines state that NIPT be available for high-risk pregnancies as they are at greater risk for fetal aneuploidy. High-risk pregnancies are also the best studied population with respect to NIPT, making the test most accurate for the high-risk

population.[7,84] 95% of genetic counselors indicated they currently offer NIPT to high-risk pregnancy populations. Many genetic counselors have not yet started offering NIPT to average-risk populations, with only 36% of individuals stating they offer NIPT to this population. In part, this is due to lack of research in individuals carrying an average-risk pregnancy. These results are consistent with current guidelines as NIPT is available for all pregnancies and recommended for high-risk pregnancies.

Genetic counselors showed a significant knowledge of microdeletion and microduplication analysis offerings available within NIPT (**Figure 7**). Through analysis, it is noted that 38% of providers do not think that analysis of microdeletion and microduplication conditions should be offered as there is not as much research and a higher number of false-positives that can occur. 35% of providers remained neutral regarding microdeletion and microduplication analysis. Although a significant portion of providers do not think microdeletion and microduplication analysis should be offered, providers are knowledgeable about these conditions. 86% of genetic counselors indicated they are confident interpreting results and 87% of genetic counselors are comfortable explaining results to patients.

This information was further broken down by the number of years practicing to distinguish if there was a difference regarding opinions toward microdeletion and microduplication analysis (**Figure 8**). Individuals practicing 0-2 years mostly commonly identified with remaining neutral regarding the inclusion of microdeletion and microduplication disorders (16, 50%). Individuals practicing 2-5 years or greater than 10 years most commonly disagreed with this inclusion (14, 53.8% & 19, 45.2% respectively). Individuals practicing 5-10 years were equally divided between including microdeletion and microduplication disorders within NIPT panels and remaining neutral. One thought as to why genetic counselors seem less supportive of the addition of

microdeletion and microduplication disorders as their experience increases could be due to their general experience encountering these conditions. It is known that these conditions are less common than aneuploidies and many genetic counselors feel that validation studies of these conditions have not been fully explored. A common theme among surveyed genetic counselors included that they value research studies when deciding what testing they would offer and to which population they would offer testing to. Therefore, more experienced genetic counselors may be biased toward their exclusion of microdeletion and microduplication disorders due to both the lack of research data and due to the conditions being generally uncommon among the population.

Microdeletion and microduplication disorders are newer options within the NIPT panel with new conditions being added regularly. Many providers stated this analysis is typically offered on a case by case basis when there is an indication for a microdeletion or microduplication condition. 34% of providers indicated they offer microdeletion and microduplication analysis to high-risk pregnancies, 17% indicated they offer this analysis to average-risk pregnancies (**Figure 9**). This data indicates a difference in ordering practice among genetic counselors. This could occur for many reasons including clinics being early adopters of new technology, a lack of guidelines stating when specific tests should be ordered, due to insurance coverage or lack of coverage, due to available research regarding these test offerings, or due to a clinic specific decision to offer or not offer this testing.

As research expands within the field of NIPT, laboratorians are working to identify monogenic conditions via NIPT methods. Although this testing is not currently commercially available, participants were queried regarding the inclusion of monogenic disorders within NIPT (**Figure 10**). Many genetic counselors were skeptical regarding this addition as laboratorians are still working to streamline microdeletion and microduplication disorders. Overall, 32% approved

of adding monogenic conditions, 47% remained neutral, and 21% disapproved of this potential addition to NIPT panels.

With the availability of so many testing options within NIPT, participants were asked how they felt about ordering testing. Many lab companies allow patients to "opt-in" or "opt-out" of additional testing. In general, there are 3 testing categories: aneuploidy, microdeletion and microduplication, and monogenic disorders. Although monogenic disorders are not currently available on NIPT panels, participants were still queried on this information as it impacts future laboratory company possibilities with regard to testing options. All 111 (100%) respondents indicated that they would prefer the ability to opt for specific categories for patient testing as indicated for the pregnancy. This response likely indicates that genetic counselors value choice for their patients when ordering NIPT. This information is especially powerful for laboratory companies creating requisition forms as they will need to distinguish between the different categories and potentially create sub-category options.

Genetic counselors were queried regarding multiple factors of NIPT in order to delineate what genetic counselors found to be advantages or limitations of NIPT (**Figure 6**). Participants clearly found that the detection rates of NIPT were its greatest advantage. Other advantages included availability during gestational age and patient acceptance. NIPT is able to be ordered any time after 10 weeks gestation, most accurately between 10-22 weeks gestation. This allows for a greater opportunity for expectant mothers to pursue testing and feel comfortable with the non-invasive technique due to high detection rates. A similar question was asked of obstetricians, they found that obstetricians ranked recommendation by professional societies, no risk to the pregnancy, and long history/experience with the test to be the top advantages.[91] Limitations of NIPT indicated by genetic counselors included coverage by insurance companies, the rate of false

positives, and the availability of genetic counseling for patients. The top limitations among obstetricians in a similar survey were patient anxiety, risks of follow-up invasive testing, and high false positive rates.[91] In comparison between the survey results, both the obstetricians and genetic counselors found the rate of false positives to be a limitation. Genetic counselors more commonly remained neutral regarding the risks of follow-up invasive testing as compared to obstetricians who most commonly perceived this factor to be a limitation. Overall, both groups differed in regard to what they found to be the advantages of NIPT.

Since this survey is currently only seeking genetic counselor knowledge, the data regarding availability of genetic counseling for patients may be skewed. Genetic counselors are not the only gatekeeper to this specific test, therefore genetic counseling may not be pursued until after an abnormal result is reported. Therefore, genetic counselors may feel that there are not enough genetic counselors to handle the patient load or the ability of other prenatal providers to order NIPT is a limitation. Further delineation of provider feelings toward this topic is warranted.

5.2 LIMITATIONS OF STUDY AND FUTURE DIRECTIONS

There are a number of limitations in this study. The completion rate for this questionnaire was 62.8% (113/180). It is assumed that the non-responders would be individuals without a prenatal background who chose not to continue with the questionnaire, but it is difficult to distinguish if there was a significant difference between responders and non-responders. This can skew the data as it is a general questionnaire of genetic counselors. There is also a low response rate in general, at this time there are over 4,000 certified genetic counselors, but it is difficult to know how many practice within the prenatal profession.

Another limitation is that this study may have selection bias as the questionnaire was only emailed to individuals who are active members of the National Society of Genetic Counselors and was not sent to all practicing genetic counselors. The collected data is also based on self-reported information and may not represent actual practice procedures. Lastly, this questionnaire was created and co-piloted to committee members and supervisors. There were no reliability or internal validity tests performed prior to distribution within the study population.

Although there have been a few studies examining physician attitudes and knowledge regarding NIPT and its incorporation, no studies have looked at all ordering provider information using the same survey for analysis. Also, as NIPT evolves, previous studies have not looked at how providers feel about the future of NIPT regarding its inclusion of so many conditions. One strength of this study is that the questionnaire was created to assess three provider populations (genetic counselors, nurse midwives, and obstetricians) in hopes of understanding their knowledge of NIPT and acuity toward its evolution. By understanding provider knowledge level and opinions toward NIPT, professional organizations can work together to create specialized educational materials and address provider concerns.

Future directions of this study would be to incorporate all prenatal providers capable of ordering NIPT. This would include surveying midwives and obstetricians. Having this data would allow us to understand the differences in perceived knowledge base between the populations and create educational courses or webinars for all three provider groups. Analysis of this data would also be helpful in understanding how the three provider groups feel about ordering testing, the different components of ordering testing, and their comfort in interpreting results and relaying these results to their patients. As genetic counselors are trained specifically in the field of genetic testing and aiding patients in every step of the testing process, this information would also be of

high value to them. Further data would allow genetic counselors to see when midwives and obstetricians find their role to be the most beneficial (ie. before testing, after an abnormal result). This could also help to create a more coherent transition between multiple providers and for greater communication between providers. By understanding information from all provider groups, patients can also be surveyed. This would allow for further comparison between provider groups to see if they are addressing similar information regarding prenatal testing and if patients are receiving comparable care when having testing performed.

5.3 PUBLIC HEALTH IMPLICATIONS

NIPT has changed the prenatal screening field by allowing for increased detection of fetal chromosomal anomalies. Until recently, NIPT has only been available for individuals carrying a pregnancy with an increased risk for fetal aneuploidy. As NIPT is rapidly evolving and is currently moving toward a public health-based model, it is important to ensure all prenatal providers are well educated regarding the test including understanding its benefits and limitations. Although NIPT will be a proficient offering for many pregnancies, other screening and diagnostic tests will also hold valuable information for many pregnancies. Ensuring that providers are able to have a constructive conversation with their patients regarding prenatal testing options will hold the greatest benefit for pregnancy management.

5.4 CONCLUSIONS

Genetic counselor knowledge and opinions regarding NIPT and its evolution were assessed. Specifically, knowledge regarding aneuploidies, microdeletions, microduplications, and monogenic disorders were studied as well as current ordering practices. The majority of genetic counselors were knowledgeable regarding NIPT analyses and comfortable interpreting and conveying results to patients. Genetic counselors had mixed opinions regarding the addition of microdeletion, microduplication, and monogenic conditions to the NIPT panel. There was also a difference between the number of current genetic counselors who offer testing to both high-risk and average-risk pregnancy populations and genetic counselors who offer microdeletion and microduplication disorders.

These results are helpful for prenatal professional organizations to understand the concerns expressed by genetic counselors when ordering NIPT panels. Concerns expressed by genetic counselors included the small amount of research and genetic understanding of some of the conditions placed within NIPT panels. This information is also beneficial for laboratorians who are working to create more analyses within these panels. Laboratories need to understand what providers and their organizations find beneficial in testing and all groups need to work together to create guidelines to improve patient experience and safety.

APPENDIX A: UNIVERSITY OF PITTSBURGH IRB APPROVAL LETTER

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: Kerrianne Morrow

From: IRB Office

Date: 10/19/2015

IRB#: PRO15090107

Subject: Assessment of Provider Knowledge Toward Non-Invasive Prenatal Testing (NIPT)

The above-reference project has been reviewed by the Institutional Review Board. Based on the information provided, this project meets all the necessary criteria for an exemption, and is hereby designated as "exempt" under section

45 CFR 46.101(b)(2)

Please not the following information:

- Investigators should consult with the IRB whenever questions arise about whether planned changes to an exempt study might alter the exempt status. Use the "**Send Comments to IRB Staff**" link displayed on study workspace to request a review to ensure it continues to meet the exempt category.

- It is important to close your study when finished by suing the "**Study Completed**" link displayed on the study workspace.
- Exempt studies will be archived after 3 years unless you choose to extend the study. If your study is archived, you can continue conducting research activities as the IRB has made the determination that your project met one of the required exempt categories. The only caveat is that no changes can be made to the application. If a change is needed, you will need to submit a NEW exempt application.

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

Expiration Date: 12/31/2500

APPENDIX B: NON-INVASIVE PRENATAL TESTING QUESTIONNAIRE

B.1 COVER LETTER

Dear NSGC member,

You are being invited to participate in a research study. All currently practicing genetic counselors are invited to participate.

The aim of this study is to understand current use of Non-Invasive Prenatal Testing (NIPT) among different medical fields (including obstetricians, genetic counselors, and midwives), determine provider understanding of NIPT, and explore provider readiness for the increase in prenatal testing options offered through NIPT.

Participation in this survey is voluntary and survey submissions will be anonymous. The survey should take approximately 15-20 minutes to complete. Due to the nature of this survey, participants must answer all questions in order to submit the survey. Please follow the link below to complete the survey: https://pitt.co1.qualtrics.com/SE/?SID=SV_cYDwB2RPxbyPK73

There are no foreseeable risks associated with this project, nor are there any direct benefits to you. There will not be any payment for participation. This study is being conducted by Kerrianne Morrow, a Master's Degree student in the University of Pittsburgh Genetic Counseling Program, under the direction of Dr. Andrea Durst.

This study has been reviewed and approved by the University of Pittsburgh Institutional Review Board (IRB#PRO15090107).

Sincerely,

Kerrianne Morrow

Genetic Counseling Student
University of Pittsburgh
Graduate School of Public Health
E-mail: kem171@pitt.edu

Andrea Durst
Assistant Director, Genetic Counseling Program
University of Pittsburgh
Department of Human Genetics
Phone: 412-624-3190
E-mail: adurst@pitt.edu

B.2 QUESTIONNAIRE

Assessment of Provider Knowledge Toward Non-Invasive Prenatal Testing (NIPT)

You are being invited to participate in a research study by answering the following survey questions about Non-Invasive Prenatal Testing (NIPT). The purpose of this research study is to understand current use of NIPT among different medical fields, determine provider understanding of NIPT, and explore provider readiness for the increase in prenatal testing options offered through NIPT. For that reason, obstetricians, midwives, and genetic counselors who are members of one of several selected professional organizations in the United States will be asked to complete a brief questionnaire that is expected to take approximately 15 minutes to complete. The questionnaire will include questions regarding demographics, current knowledge of NIPT, and readiness for the evolution of NIPT. There are no foreseeable risks associated with this project, nor are there any direct benefits to you. There will not be any payment for participation. All responses are confidential, and results will be kept in a password protected document on a password protected university computer. Your participation is voluntary, and you may withdraw from this project at any time. Due to the nature of this survey, participants must answer all questions in order to submit the survey. This study is being conducted by Kerrianne Morrow, a Master's Degree student in the University of Pittsburgh Genetic Counseling Program, who can be reached at kem171@pitt.edu, if you have any questions.

Kerrianne Morrow
Department of Human Genetics
University of Pittsburgh

Non-Invasive Prenatal Testing (NIPT) is a new technology that is rapidly evolving. The methodology involves the collection of a blood sample from the mother for analysis of cell-free fetal DNA in maternal plasma. Its most common and current utilization is the quantification of cell-free fetal DNA from chromosomes 13, 18, and 21 to detect the presence of an abnormal number of chromosomes in a cell, referred to as aneuploidy.

1. To what extent do you agree or disagree with the following statement: I am familiar with published NIPT clinical data?
   [ ] Strongly Disagree
   [ ] Disagree
   [ ] Neither Agree nor Disagree
   [ ] Agree
   [ ] Strongly Agree
2. How did you learn about NIPT? (Check all that apply.)

[ ] I have not learned about NIPT
[ ] Formal education
[ ] Continuing education course
[ ] Discussion with a lab representative
[ ] Discussion with peers
[ ] Lab company advertisements
[ ] Literature review
[ ] Online researching
[ ] Conference
[ ] Other

3. NIPT for aneuploidy can be conducted at what gestational age?
   [ ] Anytime
   [ ] As early as 10 weeks
   [ ] As early as 15 weeks
   [ ] As early as 20 weeks
   [ ] I don't know
4. Which of the following criteria do you use in your practice to determine whether a patient is at high-risk for carrying an aneuploidy pregnancy? (Check all that apply.)
   [ ] Maternal age
   [ ] An abnormal first trimester screening result
   [ ] An abnormal maternal serum screening result
   [ ] Abnormal ultrasound finding
   [ ] Family history of aneuploidy
   [ ] Other _____
5. Do you offer NIPT to high-risk pregnancies?
   [ ] Yes
   [ ] No _____
   6. If yes, What percent of high-risk patients do you offer/refer NIPT?
      [ ] 90-100%
      [ ] 75-90%
      [ ] 50-75%
      [ ] 25-50%
      [ ] 10-25%
      [ ] <10%
      [ ] None
   7. If not, do you plan to offer NIPT to high-risk pregnancies in the next 12 months?
      [ ] Yes
      [ ] No

8. Do you offer NIPT to low-risk pregnancies with no significant risk factors?

   [ ] Yes

   [ ] No _____

9. What percent of low-risk patients do you offer/refer for NIPT?

   [ ] 90-100%
   [ ] 75-90%
   [ ] 50-75%
   [ ] 25-50%
   [ ] 10-25%
   [ ] <10%
   [ ] None

10. If not, do you plan to offer NIPT to low-risk pregnancies with no significant risk factors for aneuploidy in the next 12 months?

    [ ] Yes

    [ ] No

11. To what extent do the following factors influence your decision to offer NIPT?

	No Influence	Slight Influence	Strong Influence
Abnormal serum screen result			
Advanced maternal age			
Prior fetus affected with aneuploidy			
Increased nuchal translucency			
Ultrasound markers associated with increased risk of aneuploidy			
Patient presenting late in gestation and past optimal time for screening procedures			
Family history of aneuploidy			
Patient with no indication requested testing			
Other _____			

12. Do you find the following to be advantages or limitations when ordering NIPT as compared to other screening tests such as first trimester screening and quad screening?

	Advantage	Neutral	Limitation	N/A
Recommended by scientific studies				
Recommended by professional societies				
Associated risk to pregnancy				
My history of experience with the test				
Coverage by insurance companies				
Detection rate				
Patient acceptance				
Patient Anxiety				
Risk associated with follow-up invasive testing				
False positives				
Availability during gestational age				
Availability of genetic counseling				
Other _____				

13. How is information about NIPT for aneuploidy provided to your patients prior to testing? (Check all that apply.)

[ ] Patients speak face-to-face with a genetic counselor

[ ] Patients speak with a genetic counselor through telemedicine

[ ] I discuss NIPT with the patient

[ ] Patients speak to a health care provider other than a genetic counselor in my office

[ ] Patients speak to a health care provider other than a genetic counselor who is not part of my office

[ ] Patients read an information handout first

[ ] I do not order this test

[ ] Other _____

14. How confident are you interpreting patient NIPT results?

[ ] Not confident

[ ] Mostly not confident

[ ] Confident

[ ] Mostly confident

[ ] Very confident

[ ] N/A

15. How comfortable are you explaining NIPT results to your patients?

[ ] Not comfortable

[ ] Mostly not comfortable

[ ] Comfortable

[ ] Mostly comfortable

[ ] Very comfortable

[ ] N/A

16. If a patient has an abnormal NIPT result, do you offer invasive diagnostic testing (amniocentesis or chorionic villus sampling)?

[ ] Yes

[ ] No

[ ] It depends _____

17. When would you find access to a genetic counselor helpful during the process of ordering NIPT?

[ ] Before offering NIPT

[ ] After results come back and are abnormal

[ ] Only if results are abnormal

[ ] I do not offer genetic counseling

Recent research has contributed to the development of expanded testing opportunities through NIPT. This testing includes aneuploidy detection involving additional chromosomes as well as assessment of the presence of microdeletions and microduplications, which may be associated with certain genetic conditions. By definition, a microdeletion is the loss of a small fragment of a chromosome that involves several contiguous genes. A microduplication is a gain of a small fragment of a chromosome. Microdeletions and microduplications are typically too small to be detected by conventional cytogenetic methods such as light microscopy using high resolution karyotyping. Detection for microdeletions and microduplications are traditionally done by procedures such as fluorescence in-situ hybridization (FISH) or microarray analysis.

In the future, NIPT may also have the potential to analyze the presence of monogenic disorders. Monogenic disorders such as cystic fibrosis are known to be caused by mutations in a specific gene. Expansion of NIPT to include analysis of monogenic disorders can allow for recognition of these conditions in a fetus early in pregnancy.

18. To what extent do you agree or disagree with the following statement: I am familiar with published clinical data regarding microdeletions/microduplications within the context of NIPT.
    - [ ] Strongly disagree
    - [ ] Disagree
    - [ ] Neither agree nor disagree
    - [ ] Agree
    - [ ] Strongly agree
19. To what extent do you agree or disagree with the following statement: microdeletion/microduplication analysis should be offered in NIPT.
    - [ ] Strongly disagree
    - [ ] Disagree
    - [ ] Neither agree nor disagree
    - [ ] Agree
    - [ ] Strongly agree

20. Do you offer microdeletion/microduplication NIPT to <u>all</u> pregnancies?

[ ] Yes [ ] Only to high risk pregnancies

[ ] No [ ] Other _____

21. If not, do you plan to offer NIPT with microdeletion/microduplication in the next 12 months?

[ ] Yes

[ ] No

22. Which of the following criteria do you use to determine that a patient is at increased risk for having a fetus with a microdeletion/microduplication? (Check all that apply.)

[ ] Family with known microdeletion/microduplication disorder

[ ] Prior fetus affected with a microdeletion/microduplication disorder

[ ] Ultrasound finding

[ ] I don't know

[ ] Other _____

23. How is information about NIPT with microdeletion/microduplication analysis provided to your patients prior to testing? (Check all that apply.)

[ ] Patients speak face-to-face with a genetic counselor

[ ] Patients speak with a genetic counselor through telemedicine

[ ] I discuss NIPT with the patient

[ ] Patients speak to a health care provider other than a genetic counselor in my office

[ ] Patients speak to a health care provider other than a genetic counselor who is not part of my office

[ ] Patients read an information handout first

[ ] I do not order this test

[ ] Other _____

24. How confident are you interpreting patient microdeletion/microduplication NIPT results?

[ ] Not confident

[ ] Mostly not confident

[ ] Confident

[ ] Mostly confident

[ ] Very confident

[ ] N/A

25. How comfortable are you explaining microdeletion/microduplication NIPT results to your patients?

[ ] Not comfortable

[ ] Mostly not comfortable

[ ] Comfortable

[ ] Mostly comfortable

[ ] Very comfortable

[ ] N/A

26. If a patient has an abnormal microdeletion/microduplication NIPT result, do you offer invasive diagnostic testing (amniocentesis or chorionic villus sampling)?

[ ] Yes

[ ] No

[ ] Not enough information at the present time

27. What is your opinion about offering analysis of monogenic disorders in NIPT?

[ ] Highly disapprove

[ ] Mostly disapprove

[ ] Neither approve nor disapprove

[ ] Mostly approve

[ ] Highly approve

28. Which of the following criteria would you use to determine that a fetus is at an increased risk for monogenic disorders? (Check all that apply.)
    - [ ] Family history of a known monogenic disorder
    - [ ] Family where the mother or father is known to be a carrier for the disorder
    - [ ] Family where the mother or father is suspected to be a carrier for the disorder based on family history
    - [ ] Prior fetus affected with a monogenic disorder
    - [ ] Ultrasound finding
    - [ ] Would offer to all pregnancies
    - [ ] I don't know
    - [ ] Other _____
29. If a patient has an abnormal NIPT result regarding a monogenic disorder, would you offer invasive diagnostic testing (amniocentesis or chorionic villus sampling)?
    - [ ] Yes
    - [ ] No
    - [ ] It depends
30. In your opinion, would you want NIPT labs to offer screening only for the 3 categories (aneuploidy, microdeletion/duplications, and monogenic disorders) without option for fewer categories?
    - [ ] Yes, I would only want labs to offer all 3 categories every time for every patient
    - [ ] No, I would want to be able to choose the target category(ies) that are most pertinent to my patient; testing performed on a case by case basis with additional options (beyond aneuploidy testing) offered on an opt-in basis

Demographics

31. What is your field of specialty?
    - [ ] Certified Nurse Midwife
    - [ ] Certified Midwife
    - [ ] Genetic Counselor
    - [ ] Obstetrics & Gynecology
    - [ ] Other _____

32. Do you have a sub-specialty?

[ ] Yes _____

[ ] No

33. What is your primary work setting?

[ ] Academic

[ ] Private Practice

[ ] Hospital based

[ ] Lab

34. How many years have you been practicing?

[ ] 0-2 years

[ ] 2-5 years

[ ] 5-10 years

[ ] 10+ years

APPENDIX C: ADDITIONAL RESULTS FROM QUESTIONNAIRE

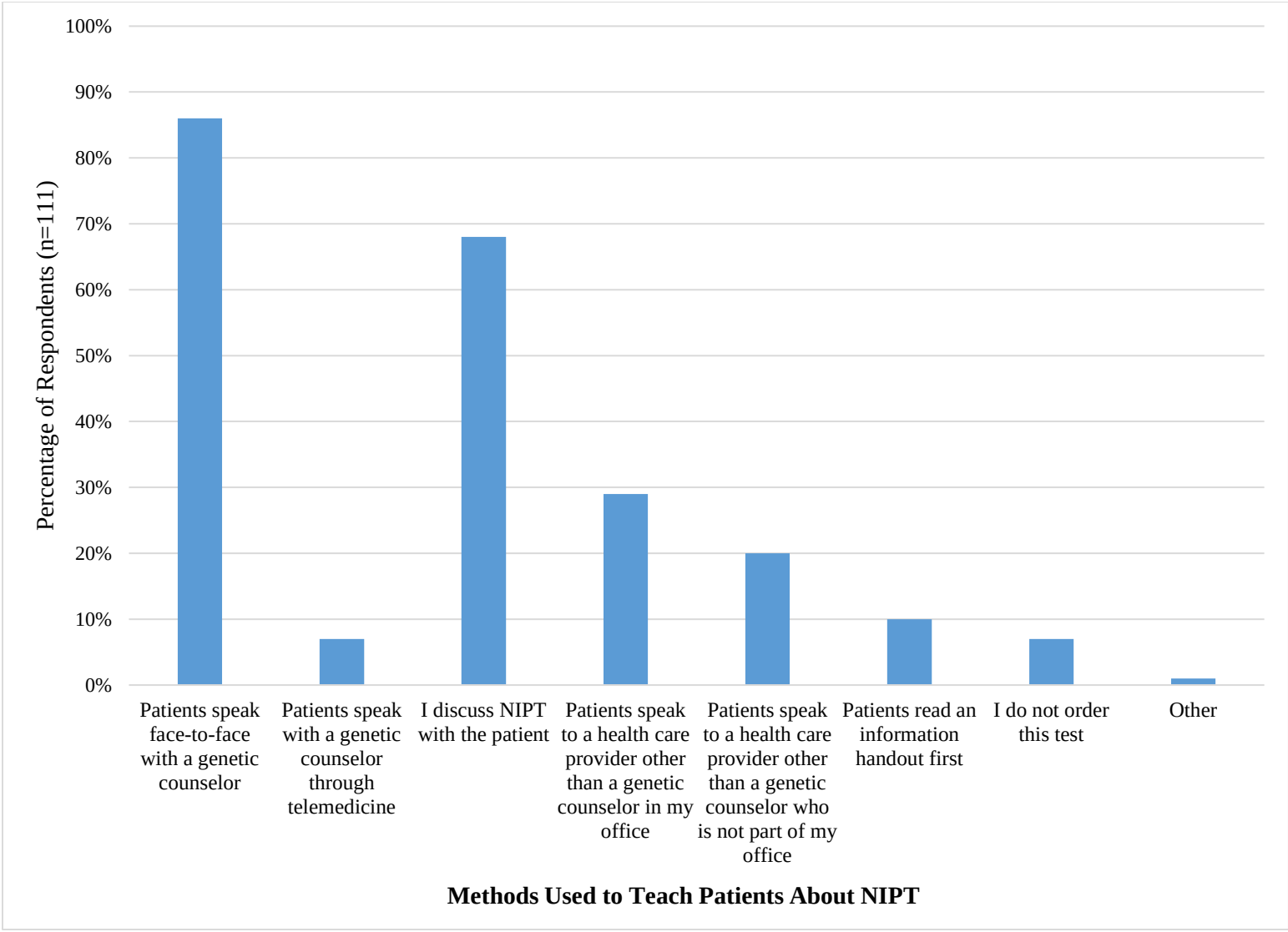
Many of the questions provided on the questionnaire were intended for analysis among multiple provider populations. This included when providers had patients speak to a genetic counselor, when they would find access to a genetic counselor most beneficial, and further information regarding how often they refer or offer NIPT to patients.

Genetic counselors were queried on their offering of NIPT. 95% of respondents indicated that they offer NIPT to high-risk patients. Of these participants, 87% indicated they offer NIPT to 90-100% of their patients. 8% indicated they offer NIPT to their high-risk patients 75-90% of the time, 5% of participants indicated they offer NIPT to their high-risk patients 50-75% of the time, and 1% of individuals offer NIPT to high-risk individuals 25-50% of the time. The 5% of patients who indicated they did not offer testing stated that they were non-prenatal genetic counselors. Genetic counselors were asked if a patient receives an abnormal test result, is the patient offered invasive diagnostic testing. 95% of participants indicated patients are offered invasive diagnostic testing, 5% stated that it depends and all indicated that they are not prenatal genetic counselors.

Genetic counselors were also queried regarding offering NIPT to average-risk patients. Of the 36% of participants who offer NIPT testing to average-risk patients, 53% indicated they offer NIPT 90-100% of the time, 5% offer NIPT 75-90% of the time, 15% offer NIPT 50-75% of the time, 8% offer NIPT 25-50% of the time, 5% offer NIPT 10-25% of the time, and 15% offer NIPT <10% of the time. 64% of counselors indicated they do not offer NIPT to average-risk patients. Of those, 81% indicated they do not plan to offer NIPT to high-risk patients in the next 12 months.

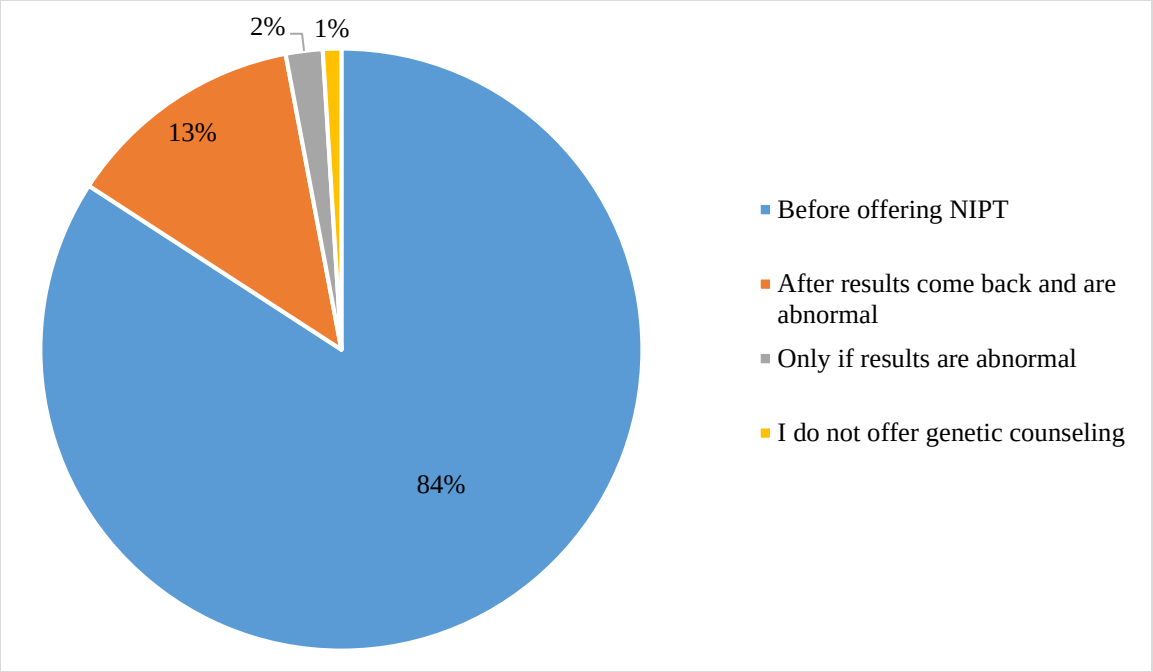
Genetic counselors were also asked how information regarding NIPT is provided to their patients prior to testing (**Supplemental Figure 1**). Genetic counselors indicated 86% of patients

speak face-to-face with a genetic counselor. 7% of patients speak with a genetic counselor through telemedicine, and 61% of respondents indicated they discuss NIPT with the patient. 26% of patients speak to a health care provider other than a genetic counselor within their clinical office and 18% of patients speak to a health care provider other than a genetic counselor who is not a part of the respondents clinical office. 9% of patients read an informational handout first, 6of respondents indicated they do not order this test, and 1% selected other. The individual who selected other indicated that their patients watch a video.



Supplemental Figure 1. Methods indicated to teach patients about Non-Invasive Prenatal Testing.

Participants were asked when they would find access to a genetic counselor helpful during the process of ordering NIPT (**Supplemental Figure 2**). 85% indicated before NIPT is offered, 13% indicated after results come back and are abnormal, 2% indicated only if results are abnormal, and 1% indicated that they do not offer genetic counseling.



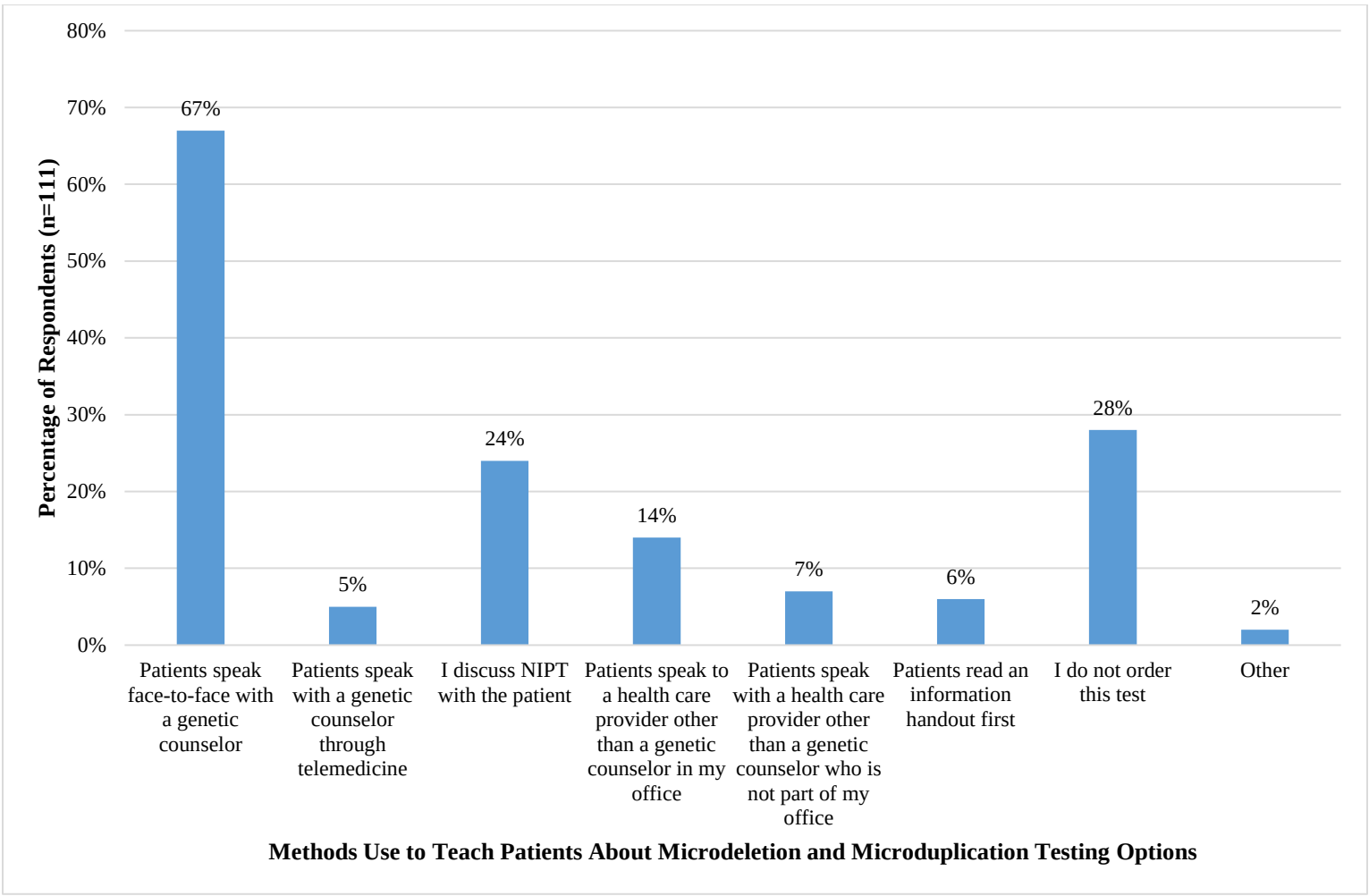
Supplemental Figure 2. When genetic counselors would prefer to see patients with regard to Non-Invasive Prenatal Testing counseling.

Further information regarding microdeletion and microduplication conditions was also assessed. Participants were queried regarding the criteria they used to determine if a patient was at increased risk for having a fetus with a microdeletion or microduplication anomaly. 88% of participants indicated when there is a family with a known microdeletion/microduplication disorder, 88% indicated when there was a prior fetus affected with a microdeletion/microduplication disorder, and 94% indicated when there was an ultrasound finding. 12% of participants indicated the option “other”. Within this category, participants indicated family history of birth defects/developmental delay (2), parental balanced translocation (2), abnormal serum screen (1), insurance coverage (2), and patient interest and comfort with uncertainty are criteria they use when offering microdeletion/microduplication analysis. 2 individuals indicated they would not offer NIPT for microdeletion/microduplication conditions,

but would instead offer invasive diagnostic testing, and 3 individuals indicated they do not offer NIPT with microdeletion/microduplication analysis.

In total 34% of providers indicated they offer microdeletion and microduplication analysis for high-risk pregnancies. Of the 66% who indicated they did not offer microdeletion and microduplication analysis to high-risk pregnancies, 76% stated they do not plan to offer this panel within the next 12 months, while 24% indicated they did plan to offer NIPT with microdeletion and microduplication analysis within the next 12 months.

Participants were queried as to how information regarding NIPT with microdeletion and microduplication analysis is provided to their patients prior to testing (**Supplemental Figure 3**). 67% indicated the patients speak face-to-face with a genetic counselor, 5% indicated patients speak to a genetic counselor via telemedicine, 42% indicated they discuss NIPT with the patient, 14% indicated the patients speak to a health care provider other than a genetic counselor within their clinical office, 7% indicated patients speak with a health care provider other than a genetic counselor who is not located within their clinical office, 6% indicated that patients read an information handout first, 28% indicated they do not order this testing, and 2% selected the "Other" category. Within the "other" category, participants indicated that they only offer 22q microdeletion analysis (1) or that both the patients and the providers are not aware the patient is having microdeletion analysis (1).

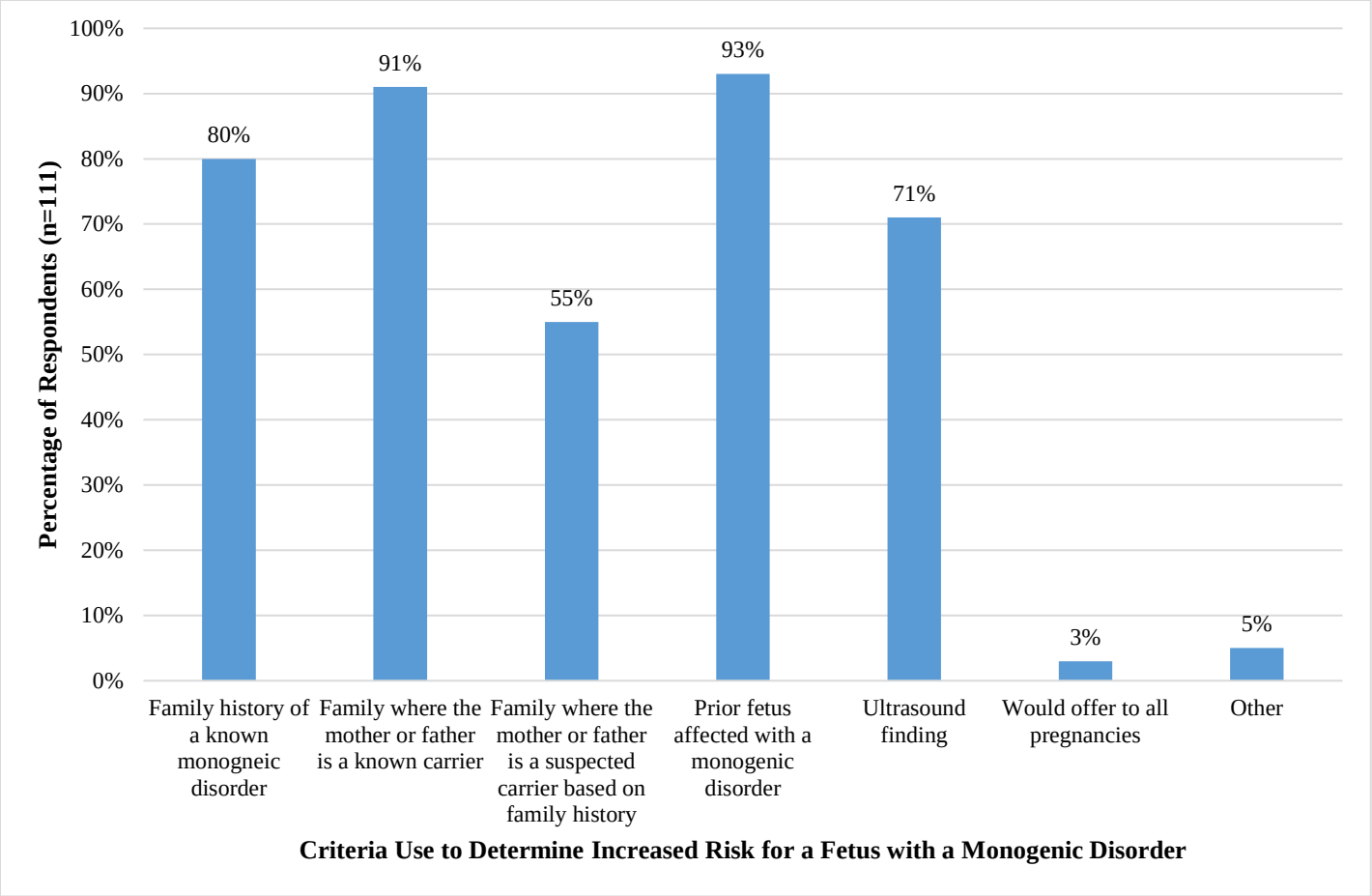


Supplemental Figure 3. Method used to teach patients about submicroscopic chromosomal changes and their testing options within Non-Invasive Prenatal Testing.

Participants were queried as to if they offer invasive diagnostic testing after microdeletion and microduplication results are reported abnormal. 92% of genetic counselors indicated they do offer invasive diagnostic testing, 1% indicated they do not, and 7% indicated there is not enough information at the present time.

The last set of questions enquired about opinions regarding the future inclusion of monogenic disorders within NIPT panels. Patients were queried regarding what criteria they would use to determine if a fetus is at an increased risk for monogenic disorders (**Supplemental Figure**

4). Genetic counselors indicated the following criteria: 80% for a family history of a known monogenic disorder, 91% for a family where the mother or father is a known carrier for a monogenic condition, 55% for a family where the mother or father is a suspected carrier for the disorder based on family history, 93% when a prior fetus is affected with a monogenic disorder, 71% indicated when there is an ultrasound finding, 3% indicated they would order monogenic disorder analysis for all pregnancies, and 5% selected “Other”. Within the “Other” category, 1 respondent stated when there is a known mutation(s), 1 respondent stated when the mother and father are known carriers of a recessive condition, 1 respondent indicated they would decide when the sensitivity and specificity of testing is released clinically, and 2 individuals stated not applicable.



Supplemental Figure 4. Genetic counselor's criteria to determine if a fetus has an increased risk for a monogenic disorder.

Participants were asked if they would offer invasive diagnostic testing for their patients who resulted with an abnormal monogenic disorder via NIPT analysis. 89% indicated they would offer invasive testing options, 1% would not offer invasive testing options, and 10% stated it would depend on clinically available data regarding monogenic disorder detection.

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