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## Evaluating Pregnancy Outcomes of Abnormal Non-Invasive Prenatal Screening Results in a High Risk Obstetrics Practice

Olivia Kesler

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EVALUATING PREGNANCY OUTCOMES OF ABNORMAL NON-INVASIVE  
PRENATAL SCREENING RESULTS IN A HIGH RISK OBSTETRICS PRACTICE

by

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Bachelor of Science  
Mississippi State University, 2017

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Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Genetic Counseling

School of Medicine

University of South Carolina

2020

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## DEDICATION

This project is dedicated to my late parents, Thomas and Lori Kesler. Thank you for providing me with every possible opportunity to succeed. I am forever proud to be your daughter.

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I would first like to thank my advisor, Jessica Fairey, for her wisdom and support throughout all phases of this project. You made yourself available and accessible even in the midst of new motherhood, and I am so grateful! Additionally, I would like to thank my committee members, Dr. Berry Campbell and Winn Surka, for their guidance and expertise that helped shaped this project. Thank you to USC Prenatal Genetic Counselors Janice Edwards, Vicki Vincent, and Debbie Zvejnieks for your help in identifying patients for this study.

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## ABSTRACT

Non-invasive prenatal screening (NIPS) has rapidly grown in uptake since its introduction to clinical practice in 2011. In contrast to more traditional methods of screening, NIPS is the first to utilize cell-free fetal DNA for risk assessment of chromosomal aneuploidy and other conditions. Clinical validity has been established for the most common autosomal aneuploidies (Trisomy 21, Trisomy 18, and Trisomy 13) and sex chromosome aneuploidies, though some laboratories screen for conditions beyond these. A screen positive does not always indicate a true positive, therefore professional guidelines recommend diagnostic testing for confirmation and informed decision making on pregnancy management. Furthermore, the methodology of NIPS means a positive result could be maternal or placental in origin and not necessarily represent the fetus. It is also possible to get a no call result that could suggest another genetic aberration, at which point patients and providers are left to follow up at their own discretion due to the lack of management guidelines. The goal of our study was to track pregnancy outcomes for patients receiving abnormal NIPS results, and use those outcomes to develop follow-up protocol for our practice. Additionally, we sought to make novel correlations for no call results. One hundred eighty one women were eligible for inclusion after medical record review. Consistent with other research, the greatest number of true positives were for autosomal aneuploidies. Patients' uptake of diagnostic testing was impacted by the individual result type, presence of ultrasound abnormalities, and laboratories' indications of a maternal or fetal abnormality. During the course of our

study, some laboratories began specifying reasons for no calls. This was helpful in guiding management, as certain types of no calls were more strongly associated with abnormalities and/or adverse fetal outcomes. Several no call results in our study led to the identification of genetic aberrations in both fetuses and mothers, suggesting the importance of follow-up and appropriate management. Overall, our study reiterates the importance of diagnostic testing as confirmation for screen positives, contributes outcome data to the growing incidence of abnormal NIPS results, and provides follow-up recommendations based on each result type.

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## LIST OF ABBREVIATIONS

ACMG.....	American College of Medical Genetics
ACOG.....	American College of Obstetrics & Gynecology
AFP.....	Alpha-fetoprotein
cfDNA.....	Cell-free DNA
CMA.....	Chromosomal Microarray
CNV.....	Copy Number Variant
CPC.....	Choroid Plexus Cyst
CPM.....	Confined Placental Mosaicism
CVS.....	Chorionic Villus Sampling
DNA.....	Deoxyribonucleic Acid
EIF.....	Echogenic Intracardiac Focus
EMR.....	Electronic Medical Record
FF.....	Fetal Fraction
FTS.....	First Trimester Screening
GA.....	Gestational Age
GC.....	Genetic Counselor
IUFD.....	Intrauterine Fetal Demise
IUGR.....	Intrauterine Growth Restriction
LFF.....	Low Fetal Fraction
MCC.....	Maternal-Cell Contamination

MFM	Maternal-Fetal Medicine
MPS	Massively Parallel Sequencing
MS-AFP	Maternal Serum Alpha-fetoprotein
MSS	Maternal Serum Screening
NIPS	Non-Invasive Prenatal Screening
NPV	Negative Predictive Value
NT	Nuchal Translucency
PH-G	Prisma Health-Greenville
PH-USC	Prisma Health-University of South Carolina
POC	Products of Conception
PPV	Positive Predictive Value
SAB	Spontaneous Abortion
SCA	Sex Chromosome Aneuploidy
SNP	Single Nucleotide Polymorphism
T13	Trisomy 13
T18	Trisomy 18
T21	Trisomy 21
TGA	Transposition of the Great Arteries
TOF	Tetralogy of Fallot
UDP	Uninformative DNA Pattern
VSD	Ventricular Septal Defect
VUS	Variant of Uncertain Significance
WES	Whole Exome Sequencing

## CHAPTER 1

### LITERATURE REVIEW

#### **1.1 What is NIPS?**

Non-invasive prenatal screening (NIPS) has rapidly grown in uptake since its introduction to clinical practice in 2011 (Palomaki et al., 2011). Arguably replacing more traditional methods of prenatal screening for chromosomal abnormalities such as the first trimester screen (FTS), NIPS analyzes cell-free fetal DNA (cfDNA) found circulating in maternal blood. This cfDNA originates from the placenta and presumably represents the fetus. Multiple clinical studies have deemed it valid for screening for the most common autosomal aneuploidies present at birth (Trisomy 21, Trisomy 18, and Trisomy 13) as well as sex chromosome aneuploidies (SCAs). While certain laboratories have begun including cfDNA screening for triploidy, copy number variants (microdeletions/microduplications), and forms of aneuploidy not viable in pregnancy (such as Trisomy 16 or Trisomy 22), inclusion of these conditions on NIPS is not recommended by the American College of Obstetricians and Gynecologists (ACOG) or the American College of Medical Genetics (ACMG) at this time (ACOG, 2016; Gregg et al., 2016).

#### **1.2 Methodologies**

There are two main methodologies used to conduct cfDNA screening. The first is colloquially known as the counting method, which can be broken down into subcategories of massively parallel sequencing (MPS) and targeted sequencing. MPS

amplifies and sequences maternal and placental DNA fragments from across the genome. While this allows greater depth of coverage, it also increases the number of false results (Avram, Shaffer, Sparks, Allen, & Caughey, 2019). Targeted sequencing reads only regions of interest and can therefore be considered more efficient. The second platform is single-nucleotide polymorphism (SNP) based, which also only sequences gene regions of interest. It determines copy number in each gene region, compares the allelic measurements, and then proceeds through an algorithm. A meta-analysis conducted by Yaron (2016) found that MPS had a lower failure rate (1.58%) than SNP-based platforms (6.39%). However, the SNP-based platform boasts the ability to identify triploidy, vanishing twins, and distinguish between monozygotic and dizygotic twins (Curnow et al., 2015; Mathieson & Roy, 2018; Norwitz et al., 2019).

### **1.3 Conditions screened**

Clinical validity has been established for the most common autosomal aneuploidies present at birth (Trisomy 21, Trisomy 18, and Trisomy 13) and SCAs, and some laboratories are offering copy number variants (CNVs), triploidy, and other forms of nonviable aneuploidies as well. The sensitivity of Down syndrome is the highest performing, with estimates consistently hovering around 99% (ACOG, 2016; Gil, Quezada, Revello, Akolekar, & Nicolaides, 2015; Mackie, Hemming, Allen, Morris, & Kilby, 2017). Other estimates include 96-98% for Trisomy 18 and 90-91% for Trisomy 13 (ACOG, 2016; Gil et al., 2015; Mackie et al., 2017). The sensitivity of SCAs does not seem to lag far behind, though data for these are more limited. Gil et al. (2015) found a 90.2% detection rate of Monosomy X (Turner syndrome), and a 93% pooled detection rate for other SCAs. The positive predictive value (PPV) for these conditions has been



reported in a range: 65-94% for Trisomy 21, 47-85% for Trisomy 18, and 12-62% for Trisomy 13 (Hu et al., 2019). Additionally, the PPV of SCAs has been reported to range from 25-75% (Fleddermann et al., 2019; Zhang et al., 2019).

Though some laboratories have begun screening for CNVs against the recommendation of professional guidelines, available data on performance detection are few. Interestingly, one study considered the cost-effectiveness of including these conditions on NIPS, and found that it was indeed financially practical (Avram et al., 2019). However, inclusion on NIPS would still lend itself to low PPVs due to the overall low prevalence of these conditions.

## **1.4 Possible results**

### **1.4.1 Screen positive**

As opposed to FTS generating an adjusted risk estimate such as 1 in 50, NIPS will indicate screen positive, screen negative, or no-call. Per ACOG and ACMG recommendations, screen positive results should be followed up with the offer of diagnostic testing and detailed ultrasonography to evaluate for fetal abnormalities (ACOG, 2016; Gregg et al., 2016). Occasionally, positive results may indicate maternal conditions, confined placental mosaicism, or vanishing twins and therefore not be representative of the pregnancy. This is a well-described limitation of NIPS that emphasizes the importance of diagnostic testing to confirm that the positive result represents fetal DNA.

### **1.4.2 Screen negative**

A screen negative result significantly reduces but does not eliminate the chance for a fetus to be affected by one of the conditions screened. Patients are generally given a residual risk, often less than 1 in 10,000.

### **1.4.3 No call**

A no call or failed result occurs in 0.5-3.0% of cfDNA screens, presenting a challenge for genetic counselors (GCs) and maternal-fetal medicine specialists (MFMs) (Qiao et al., 2019). The most common reason for a failed NIPS is insufficient fetal fraction (FF). Fetal fraction describes the proportion of DNA in maternal circulation that is of placental origin and thought to represent the pregnancy. Three to thirteen percent is generally regarded as the acceptable range for cfDNA analysis (ACOG, 2016; Qiao et al., 2019). If the amount of cfDNA falls below this threshold, NIPS will most likely be unsuccessful. Multiple studies have evaluated the success of a redraw in generating a screen positive or negative result, however, this is not a perfect solution to low FF cases, as many still do not receive a result after a second attempt.

A second reason NIPS may fail to produce a result is due to an uninformative DNA pattern. An uninformative DNA pattern describes the situation in which the DNA of the mother or fetus is unable to be analyzed. Multiple explanations as to why the DNA pattern may be uninformative have been put forward. These include the type of pregnancy (egg donor, surrogacy, or multiple gestations), vanishing twins, fetal or maternal mosaicism, maternal malignancy, increased stretches of homozygosity, sampling error, or fetal aneuploidy. Unlike cases of low fetal fraction, a redraw is

generally not requested by performing laboratories. Instead, clinicians are left to follow up at their own discretion.

NIPS may also fail due to processing errors by the performing laboratory or collection errors through the phlebotomy laboratory. In these circumstances, a redraw is recommended.

### **1.5 Integration into clinical practice**

The introduction of NIPS into clinical practice has decreased utilization of traditional maternal serum screening (MSS) methods. Providers and patients are drawn to the higher sensitivities of NIPS, as well as its advantage to predict gender as early as nine weeks. Providers still offering traditional screening options may value NIPS as a second-tier screen. It can serve as an optional next step in risk assessment following a positive serum screen; however, professional guidelines still recommend prenatal diagnosis for confirmation (ACOG, 2016; Gregg et al., 2016). Logistical considerations may also dictate what screening is ultimately chosen by the patient. A prime example of this is varying insurance coverage of NIPS, especially for individuals not considered high-risk (e.g. women below advanced maternal age) (Farrell, Agatista, Michie, Greene, & Ford, 2019).

Because NIPS has a higher sensitivity than MSS, uptake of diagnostic testing has decreased as well. While still offered, chorionic villus sampling and amniocentesis procedures are often declined given the associated risks. Providers and patients may view NIPS results as a reason not to proceed with diagnostic testing, especially in the presence of ultrasound abnormalities or other clues that the positive screen is indeed a true result. However, professional societies remain firm in their guidelines that

pregnancy management decisions should not be based on NIPS results. Diagnostic testing is still the standard recommended follow-up to any screen positive result or ultrasound finding; it serves to not only confirm the diagnosis, but also to distinguish aneuploidy resulting from a nondisjunction event or translocation, which influences counseling on recurrence risk (ACOG, 2016; Gregg et al., 2016).

## **1.6 Challenges of screen positive results**

### **1.6.1 Unknown etiology**

The foundational challenge of screen positive NIPS results is that the positive result could represent one of many variables: fetal DNA, maternal DNA, confined placental mosaicism, a vanished twin, or maternal malignancy. Confined placental mosaicism (CPM) is thought to impact 1-2% of all pregnancies. Hartwig, Ambye, Sorenson, and Jorgensen (2017) found that CPM could explain 39% of false positive NIPS results. Vanishing twins can also be a plausible explanation for screen positive results, as upwards of 70% of spontaneous abortions are due to chromosome abnormalities (Suzumori & Sugiura-Ogasawara, 2010). Additionally, Hartwig and colleagues (2017) found maternal mosaicism or maternal CNVs to be responsible for over half of false positive NIPS results. This suggests that while maternal chromosome analysis is a reasonable next step, diagnostic testing remains the standard follow-up for fetuses, and conditions cannot be confirmed or ruled out without it.

### **1.6.2 Varying severity of autosomal aneuploidies**

Beyond this foundational challenge, there are other considerations for screen positive results based on the type of condition indicated. The autosomal aneuploidies (Trisomies 21, 18, and 13) have higher PPVs and can sometimes be corroborated by

ultrasound findings, including soft markers (Ebrashy et al., 2016). Zhen, Li, Yang, and Li (2019) reported that 94.6% of confirmed Trisomy 18 cases and 100% of Trisomy 13 cases demonstrated ultrasound abnormalities prior to diagnostic testing; thus, their finding is that ultrasound is significant in adjusting the PPV for screen positive Trisomy 18 or Trisomy 13 results. Ultrasound for Trisomy 21 is less reliable, however; only about 50% of cases will have findings during a second trimester scan (ACOG, 2016).

Additionally, the conversation that GCs have with patients regarding a screen positive Trisomy 21 result can differ from the conversation had over a screen positive Trisomy 18 or Trisomy 13 result. Trisomy 21 (Down syndrome) is generally described as a condition in which individuals have variable medical complications and learning difficulties due to the presence of an extra chromosome, whereas Trisomy 18 and Trisomy 13 are generally described as life-limiting conditions. While thoughts on pregnancy management can be facilitated and discussed in the context of any screen positive result, Trisomy 18 and Trisomy 13 are conditions in which palliative care and/or surgical intervention options are particularly relevant to discuss.

### **1.6.3 Sex chromosome aneuploidies**

Screen positive results for SCAs are especially difficult to manage. There are no consistent guidelines for screen positive follow-up, and, compared to the autosomal aneuploidies, they have lower PPVs and usually no ultrasound findings to aid in screening interpretation. As SCAs tend to be associated with more social and developmental challenges, it is unusual to identify structural malformations; however, a known exception to this is Monosomy X (Turner syndrome) in which heart and renal differences can be identified prenatally.

A screen positive SCA can also be indicative of a maternal condition, which warrants further testing to aid in result interpretation. Current literature suggests that offering maternal karyotypes in the context of screen positive SCA results is done inconsistently, even though it has been reported that 8.6% of screen positives are attributable to maternal SCAs (Fleddermann et al., 2019; Wang et al., 2015). A separate study by Zhang et al. (2019) reported that the rate of maternal sex chromosome differences (full aneuploidy or CNVs) in screen positive SCAs was 21/86, or 24.42%.

#### **1.6.4 Microdeletion and microduplication syndromes**

Positive results indicating microdeletion or microduplication syndromes are challenging as well. The PPVs for these CNVs are described as low to moderate until further studies can better define their performance on NIPS (Liang et al., 2019). While reports of CNVs being detected on NIPS are few, Hu et al. (2019) released data indicating that the PPV of their screen positive CNVs on a genome-wide platform was 36.11%. Other research conducted on a genome-wide platform found that 26.7% of screen positive CNVs overlapped with the classic microdeletion/microduplication syndromes currently available on NIPS: 22q11.2 deletion/duplication, Prader-Willi/Angelman syndromes, Cri-du-chat, and 1p36 deletion syndrome (Liang et al., 2019). Lo, Shiau, Chen, Shaw, and Benn (2019) reported an amniocentesis-confirmed case of 22q11.2 deletion syndrome with discordant results on NIPS. NIPS via MPS rendered the fetus low risk, while NIPS via the SNP-based method indicated high risk with a 1/19 risk score. While helpful, studies like these are not enough to change current recommendations. There is continued work to be done to improve the sensitivity and PPV of these conditions to show that they are clinically validated for NIPS.

### **1.6.5 Twins or other multifetal gestations**

Data on NIPS in twin pregnancies are much more limited than in singleton pregnancies. Understandably, the risk of aneuploidy increases with the number of fetuses; however, no method of screening works as well for twin pregnancies as it does for singleton pregnancies. When NIPS is conducted in multifetal gestations, the laboratory report provides one result for the entire pregnancy, and therefore it is unclear which fetus(es) are indicating screen positive. Gil et al. (2015) found detection rates similar to that of singleton pregnancies, but much more data are needed. Until clinical validity can be demonstrated, screening multifetal gestations is not recommended by ACOG and ACMG at this time (ACOG, 2016; Gregg et al., 2016). In instances when laboratories offer NIPS for multifetal gestations and the result is screen positive, diagnostic testing is essential in determining which fetus(es) are affected. Not even SNP-based platforms can make this distinction, though they can report on zygosity (monozygotic vs. dizygotic).

### **1.7 Challenges of no call results**

As is the case with screen positive NIPS results, there is a foundational challenge of no call results: follow-up protocol. There are no consistent guidelines for managing this group of patients, leaving clinicians to make recommendations on a case-by-case basis. Though some laboratories have begun supplying reasons for no calls beyond low fetal fraction, such as suspected maternal abnormality or laboratory error, most reports do not include this information (Skotko et al., 2019).

### **1.7.1 Low fetal fraction**

Most commonly, however, NIPS fails to generate a result due to insufficient FF. Factors known to influence the FF include maternal weight, gestational age, maternal use of blood thinners, and aneuploidy. Maternal weight and FF are inversely related, with increasing maternal weight leading to a decrease of FF. Low FF can also occur if the gestational age at the time of the draw is earlier than the recommended 9-10 weeks of pregnancy, if the mother is taking blood thinners, or if the pregnancy is aneuploid. When faced with an insufficient FF result, most laboratories accept a redraw. The percentage of patients receiving a result after a second draw generally falls between 50-70% (Benn, Valenti, Shah, Martin & Demko, 2018; Galeva, Gil, Konstantinidou, Akolekar, & Nicolaides, 2019; Suzumori et al., 2019; White, Wang, Kunz, & Schmid, 2019).

Aneuploidy is the obvious area of interest for GCs considering low FF results, however. One study found that in over 1,000 pregnancies, 8% of cfDNA screenings failed due to low FF. Of those failures, 22% were determined to be aneuploid (Pergament et al., 2014). Currently, a select laboratory categorizes low FF into high risk versus no result in attempt to decrease the number of patients receiving an overall no call. The high risk category is assigned when the laboratory's internal algorithm suggests an increased risk for aneuploidy; this risk estimate is 1/17 for Triploidy, Trisomy 18, or Trisomy 13. This result is generated when the low FF cannot be attributed to maternal weight, maternal age, and gestational age in addition to FF. When a patient receives a high risk result based on this algorithm, prenatal diagnosis is the recommended follow-up as opposed to a redraw (Benn et al., 2019). Because the implementation of this algorithm is fairly recent, reports of pregnancy outcomes are scarce.



### **1.7.2 Uninformative DNA pattern**

A newer type of no-call result is attributed to an uninformative DNA pattern. Because there are many possible explanations for uninformative results and limited data on these pregnancy outcomes, redraws are not recommended.

### **1.7.3 Outcome data for no-calls**

Studies on pregnancy outcomes following no calls are limited. Suzumori et al. (2019) evaluated outcomes of pregnancies receiving multiple no calls. Of the 22 patients undergoing diagnostic testing after a second failure, 17 of those (77.2%) subsequently had a normal karyotype, while the remaining five (22.7%) were abnormal. Interestingly, six of the 22 (27.2%) were twin pregnancies that had a low FF. This is consistent with other literature that states twin gestations have a higher fail rate than singletons, with or without chromosome aneuploidy (Galeva et al., 2019).

### **1.7.4 Novel explanations for no calls**

Because many no calls go without explanation, research into other possible causes is ongoing. Putra et al. (2019) established a correlation between maternal hemoglobinopathies and no calls. They found that women with clinically significant hemoglobinopathies were more likely to have low FF and subsequent no calls. Additionally, Suzumori and colleagues (2019) described increasing maternal age and certain racial origins as correlations with test failure. Though these studies are helpful, it is reasonable to consider that there are other factors influencing the success of a NIPS draw outside of what has already been reported in the literature.

## **1.8 Differences in laboratories' reporting**

In addition to challenges unique to positive and no call results, there are also aspects of laboratories' reporting that can complicate interpretation of results. For instance, it is recommended by the ACMG that detection rate, specificity, PPV, negative predictive value (NPV), and FF be included on each report for autosomal aneuploidies, sex chromosome aneuploidies, and CNVs (Gregg et al., 2016). However, recent evaluation by Skotko et al. (2019) found that laboratories' adherence is variable. None of the ten laboratories analyzed fully met this requirement, and many did not distinguish PPV and NPV between conditions, especially the sex chromosome aneuploidies. PPV is the statistic that patients are generally most concerned with, as it is the number that informs them the chance that the positive result is indeed true. Counseling on a PPV that is nonspecific to the condition and is population-derived versus patient-specific is a significant hurdle in helping patients assess their actual risk; they may feel they are working with incomplete or conflicting information that is not specific to their pregnancies. Skotko and colleagues (2019) found that only one laboratory consistently reported patient-specific PPV, or population-derived or modeled PPV only when patient clinical information was unavailable for calculation.

Furthermore, it is challenging when the data source for laboratories' statistics is variable. For example, laboratories may be reporting based on population studies, clinical studies, their own internal data, or in the case of one particular laboratory, their self-designed algorithm. The lack of consistency indicates that a woman undergoing screenings with two laboratories at the same time could receive different results, and this is problematic for true risk assessment. On the positive, however, the recent analysis of

Skotko and colleagues (2019) found that laboratories are evolving in their reporting of no call results. Select laboratories are becoming more specific and supplying reasons for no calls beyond low fetal fraction and uninformative DNA pattern. Classifications recently observed include triploidy, vanishing twin, or unrecognized multiple gestation; suspected maternal abnormalities; and sample processing/laboratory error. A select laboratory is also distinguishing between maternal or fetal abnormalities in some of its reports, and this is very helpful for post-test counseling and management.

### **1.9 Importance of clear results in screening**

Prenatal screening is not a eugenics movement, though this perception is still held by many (Farrell et al., 2019). While some patients certainly use screening as a guide for pregnancy management, others simply wish to be prepared for the potential of having a child with complex medical and developmental needs. Nov-Klaiman, Raz, and Dolev (2019) identified parents of children with Trisomy 21 as being favorable toward NIPS, citing its accuracy, safety, and ability to help families prepare for a child with special needs. Similarly, 88.1% of parents of children with SCAs reported that early diagnosis via NIPS was positively impactful (Samango-Sprouse et al., 2019).

Other research has indicated that patients value actionability as a primary consideration of their personal utility for screening (Farrell et al., 2019). Though not equivalent to diagnostic testing, it is clear that many women view NIPS as a suitable alternative; they are reassured with low risk results, and certainly concerned with high risk or inconclusive ones. Therefore, it is extremely important that these screens are accurately reported and have clear guidelines for follow-up. Providers hope for the same things, as they are the ones sought for guidance and management. Richardson, Raine-

Fenning, Deb, Campbell, and Vedhara (2017) found that an uncertain diagnosis was more distressing to patients psychologically than a diagnosis with a poor outcome. Though this is always patient-dependent, there is enough research to show that uncertain results delay a diagnosis, complicate follow-up, and increase both patient and provider anxiety (Hancock et al., 2019).

### **1.10 Rationale**

Little research has been conducted to assess the pregnancy outcomes of those receiving an abnormal NIPS, particularly those resulting in a no call. Because the general uptake of NIPS is increasing, many abnormal results are generated. Our practice will benefit from any associations gleaned during the course of this study. The ultimate goal is to analyze patient data that will aid in guiding future patients who receive abnormal results.

### **1.11 Purpose**

#### *Hypothesis*

We predict that many pregnancy outcomes of low fetal fraction NIPS results will be normal, and they can likely be attributed to maternal weight or drawing blood at an early gestational age. Similarly, many pregnancy outcomes of uninformative DNA pattern results will likely also be normal. However, we do expect to observe novel correlations between uninformative DNA pattern results and pregnancy outcomes, since no call results outside of low fetal fraction are poorly understood.

#### *Objectives*

- 1) Observe positive predictive values of our patients' NIPS results, and compare with the positive predictive values given by the performing laboratory.

- 2) Compare next steps (such as the uptake of prenatal diagnostic or postnatal testing) based on the type of condition indicated on NIPS.
- 3) Establish novel correlations between no call results and pregnancy outcomes.
- 4) Confirm known correlations such as maternal weight and early gestational age with low fetal fraction results, and observe any factors that are not as strongly correlated.
- 5) Describe recommended management through our MFM clinic for no call results.

## CHAPTER 2

### EVALUATING PREGNANCY OUTCOMES OF ABNORMAL NON-INVASIVE PRENATAL SCREENING RESULTS IN A HIGH RISK OBSTETRICS PRACTICE<sup>1</sup>

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## **2.1 Abstract**

Non-invasive prenatal screening (NIPS) has rapidly grown in uptake since its introduction to clinical practice in 2011. In contrast to more traditional methods of screening, NIPS is the first to utilize cell-free fetal DNA for risk assessment of chromosomal aneuploidy and other conditions. Clinical validity has been established for the most common autosomal aneuploidies (Trisomy 21, Trisomy 18, and Trisomy 13) and sex chromosome aneuploidies, though some laboratories screen for conditions beyond these. A screen positive does not always indicate a true positive, therefore professional guidelines recommend diagnostic testing for confirmation and informed decision making on pregnancy management. Furthermore, the methodology of NIPS means a positive result could be maternal or placental in origin and not necessarily represent the fetus. It is also possible to get a no call result that could suggest another genetic aberration, at which point patients and providers are left to follow up at their own discretion due to the lack of management guidelines. The goal of our study was to track pregnancy outcomes for patients receiving abnormal NIPS results, and use those outcomes to develop follow-up protocol for our practice. Additionally, we sought to make novel correlations for no call results. One hundred eighty one women were eligible for inclusion after medical record review. Consistent with other research, the greatest number of true positives were for autosomal aneuploidies. Patients' uptake of diagnostic testing was impacted by the individual result type, presence of ultrasound abnormalities, and laboratories' indications of a maternal or fetal abnormality. During the course of our study, some laboratories began specifying reasons for no calls. This was helpful in guiding management, as certain types of no calls were more strongly associated with

abnormalities and/or adverse fetal outcomes. Several no call results in our study led to the identification of genetic aberrations in both fetuses and mothers, suggesting the importance of follow-up and appropriate management. Overall, our study reiterates the importance of diagnostic testing as confirmation for screen positives, contributes outcome data to the growing incidence of abnormal NIPS results, and provides follow-up recommendations based on each result type.

## **2.2 Introduction**

Though originally introduced as screening preferred for the high-risk population, NIPS has rapidly expanded in use and is now often the first choice over traditional screening methods. Because the uptake has dramatically increased, more women are faced with an abnormal result, either positive or no call. Current professional guidelines are not in agreement with recommendations for follow-up, and some results are not even addressed in these guidelines (ACOG, 2016; Gregg et al., 2016).

Because a screen positive result may not be representative of the fetus, diagnostic testing remains the standard recommended follow-up for all results. In some scenarios such as low fetal fraction (LFF), however, a redraw may be successful (Suzumori et al., 2019). Coverage of NIPS platforms has rapidly expanded, though professional guidelines have not been updated to reflect this. Currently, it is recommended to screen only for the three most common autosomal trisomies as well as sex chromosome aneuploidies (SCAs). Recommended follow-up for screen positive autosomal trisomies is always diagnostic testing and ultrasonography (ACOG, 2016; Gregg et al., 2016). In SCAs, however, follow-up guidelines are less consistent. While diagnostic testing is usually the most informative, providers have to also consider the chance that the positive



result represents a maternal sex chromosome difference, such as mosaic Monosomy X or XXX syndrome (Fleddermann et al., 2019). SCAs are also difficult to corroborate with ultrasound findings, which can often be done in the setting of a screen positive autosomal trisomy. As a result, these conditions approved by professional societies for inclusion on NIPS are without follow-up recommendations. For those conditions that professional societies consider invalid due to low prevalence and PPV, follow-up recommendations are not uniformly available; therefore, pregnancy management of a screen positive patient is left to the discretion of the provider.

In regard to no calls, the most common type is due to LFF. Sometimes, a LFF result can be correlated with risk factors such as high maternal weight, early gestational age, maternal use of blood thinners, and aneuploidy (Galeva, Gil, Konstantinidou, Akolekar, & Nicolaides, 2019). While redraws are often accepted, it is not a perfect solution. The percentage of patients receiving a result after a second draw generally falls between 50-70% (Benn, Valenti, Shah, Martin & Demko, 2018; Galeva et al., 2019; Suzumori et al., 2019; White, Wang, Kunz, & Schmid, 2019). In the setting of a failed redraw, it may not always be clear why screening has been unsuccessful. One laboratory is trying to address this with a new type of LFF result. When LFF cannot be attributed to maternal weight, maternal age, or gestational age, a 1/17 risk for Triploidy, Trisomy 13, or Trisomy 18 is suggested (Benn et al, 2019). For this type of result, the laboratory recommends diagnostic testing instead of a redraw. Similarly, several other types of no calls have recently been reported, such as maternal X abnormalities or atypical findings. When laboratories are able to make the distinction between a maternal or fetal abnormality, this allows genetic counselors (GCs) to recommend the most appropriate

follow-up to learn more about the abnormal result; however, it should be noted that learning of this distinction often requires a GC to call the laboratory directly for more information. Differences in maternal and fetal abnormalities are not always readily available on the laboratory report. Uninformative DNA pattern (UDP) results have also become more common, though the laboratory does not encourage sending a redraw. With many possible reasons for a UDP result and no guidelines for follow-up, next steps can look very different from patient to patient based on her own choice and discretion.

Many women rely on NIPS for accurate risk assessment of their pregnancies. They are reassured by low risk results, and certainly concerned by abnormal ones. Therefore, it is extremely important that these screens perform well, and equally important that laboratories and professional guidelines equip providers to recommend the most appropriate follow-up and management. Because each laboratory has different ways of reporting results and varying factors that contribute to their results, it is sometimes difficult for providers to decide how real or how worrisome an abnormal result should be. Therefore, we seek to provide valuable outcome data for both established and evolving types of results on NIPS platforms.

## **2.3 Materials and Methods**

### **2.3.1 Participants**

Participant selection was based on record review. Eligible participants were patients of Prisma Health-University of South Carolina Maternal Fetal Medicine (MFM) or Prisma Health-Greenville MFM that had an abnormal NIPS documented in their electronic medical record (EMR). Patients seen between January 2018 – March 2020 were eligible for inclusion. A total of 181 patients met these requirements. Demographic

characteristics of the participants are summarized in Table 2.1. The population consisted of mostly Caucasian (45.3%, n=82) and African American (43.6%, n=79) individuals.

All participants were female with a mean age of 31.4 years. The average gestational age at which NIPS was drawn was 13.6 weeks. Average maternal weight was 185.9 pounds.

**Table 2.1** Demographic characteristics of participants

<b>Characteristics</b>	<b>n</b>	<b>%</b>
<b>Age (n=181)</b>		
16-20	17	9.4
21-25	27	14.9
26-30	43	23.8
31-35	34	18.8
36-40	38	21.0
41-45	22	12.1
<b>Ethnicity (n=181)</b>		
Caucasian	82	45.3
African American	79	43.6
Hispanic/Latino	13	7.2
Asian	2	1.1
Multiethnic	5	2.8
<b>Gestational age (n=181)</b>		
9-13	126	69.6
14-18	34	18.2
19-23	12	6.6
24-28	8	4.4
29-33	1	0.55
34-38	1	0.55
<b>Gestation (n=181)</b>		
Singleton	173	95.6
Twin	8	4.4
<b>Gravidy (n=181)</b>		
Primigravida	35	19.3
Multigravida	146	80.7
<b>Weight (n=177)</b>		
100-179	94	53.1
180-259	64	36.2
260-339	17	9.6

340-419	1	0.55
420-499	1	0.55

### 2.3.2 Procedure

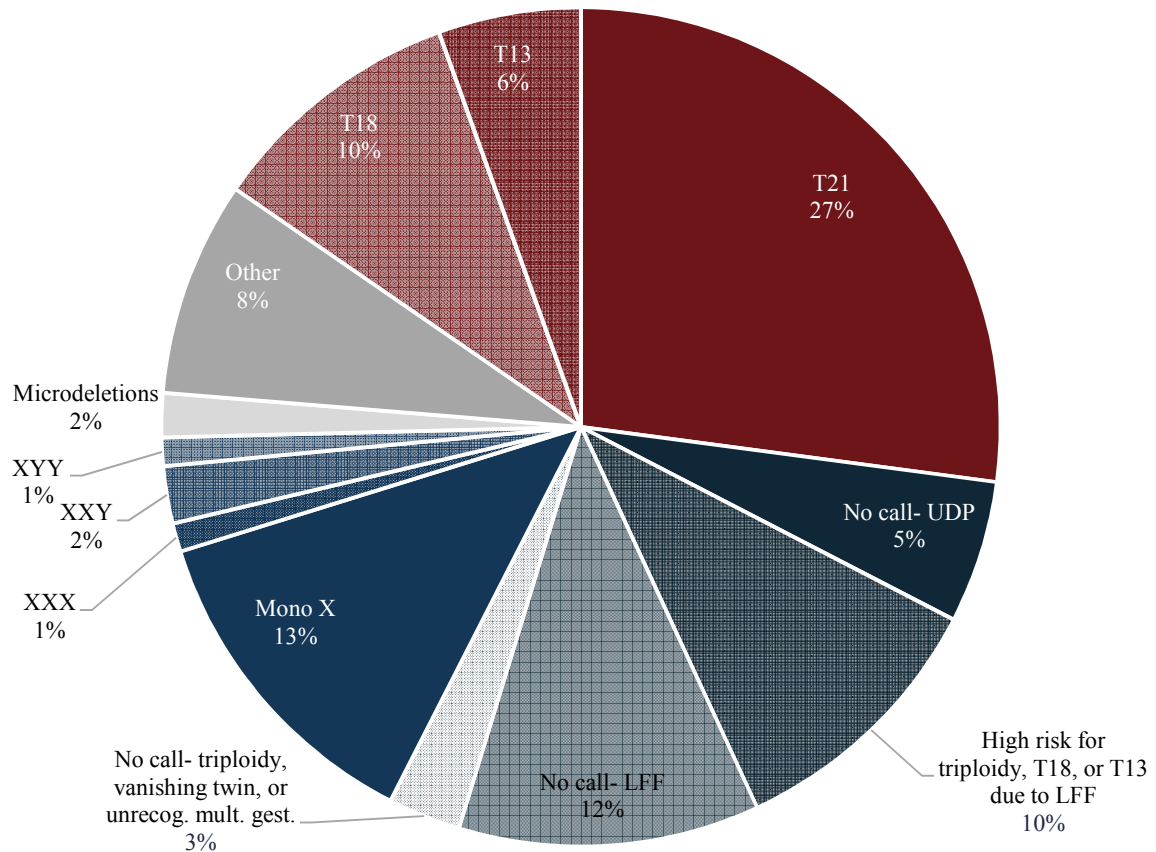
EMRs were reviewed to determine the eligibility of patients. Once eligibility was determined, a unique identifier was assigned to each patient based on where she was seen (PH-USC for Prisma Health University of South Carolina MFM or PH-G for Prisma Health Greenville MFM). A number of data points were extracted from each patient’s record: name; medical record number; address; phone number; age at delivery; weight; ethnicity; heparin/lovenox use (yes or no); gravidity and parity; gestational age; singleton or twin gestation; ultrasound findings; platform used for screening; was this repeat screening (yes or no); fetal fraction on laboratory report; the result- positive or no-call; if positive, what condition and the PPV; predicted fetal sex; follow-up plan (diagnostic testing or further ultrasounds); outcome (confirmed by diagnosis, clinical notes, or test results); and other (relevant maternal/placental conditions).

The goal was to document a pregnancy outcome for each abnormal result. This may have been accomplished through diagnostic testing or postnatal testing that was documented in the EMR. If patients did not have this information available in their record, they were sent a letter regarding a planned phone interview with the ability to opt out (Appendix A). When patients were called, they were only asked about their pregnancy outcomes. A total of 25 patients were sent a letter, and we were able to glean 12 outcomes from phone interviews. Another 12 patients could not be reached or did not return our phone call, and one patient declined to participate. None of the patients contacted for a phone interview were 18 years old or younger.

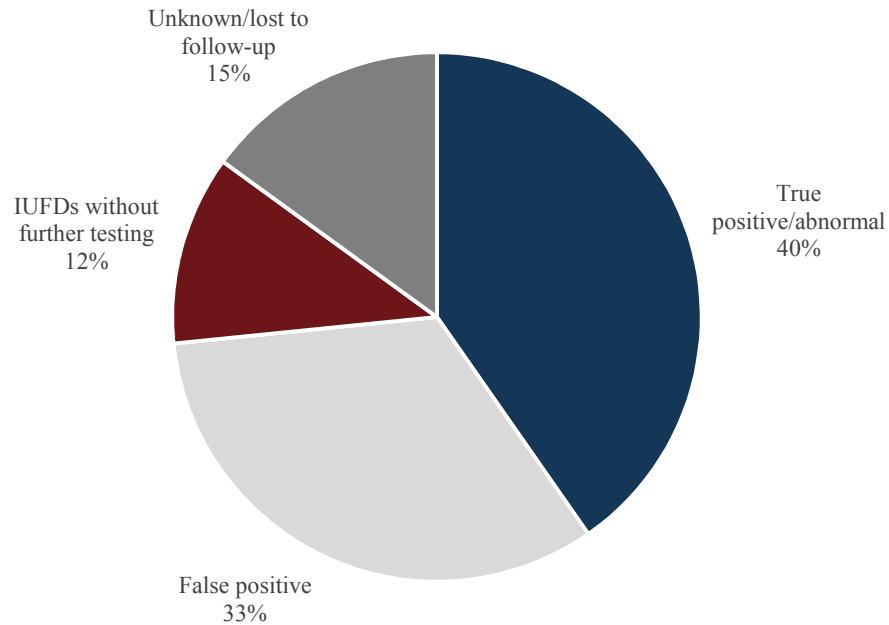
We utilized both qualitative and quantitative data analysis for our study. Analysis was performed from January 2020 to March 2020. Descriptive statistics were conducted for all 13 result types that were a part of our study. Quantitative data analysis was performed using SPSS statistical analysis software and Microsoft Excel.

## 2.4 Results

Information on all 181 patients was considered in reporting results and calculating statistics. A screen positive Trisomy 21 was the most common (27.1%, n=49), followed by Monosomy X (12.7%, n=23). Screen positives are outlined in Figure 2.1, and outcomes are classified in Figure 2.2. All results and outcomes are detailed in Table 2.2, and are further delineated by laboratory in Appendix B.



**Figure 2.1** Screen positive results



**Figure 2.2** Outcomes for all pregnancies

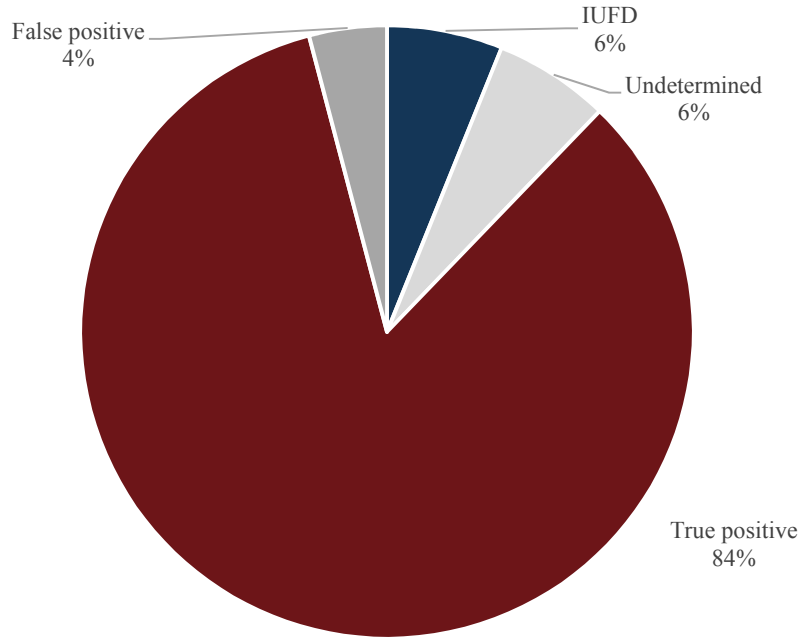
**Table 2.2** All results and outcomes

<b>NIPS result</b>	<b>True positive</b>	<b>False positive</b>	<b>IUFD</b>	<b>Unknown/lost to follow-up</b>	<b>Maternal diagnosis</b>	<b>Totals</b>
Trisomy 21	41	2	3	3	0	49
Trisomy 18	10	3	5	0	0	18
Trisomy 13	4	3	1	2	0	10
Monosomy X	3	6	8	6	0	23
XXY	1	2	1	0	0	4
XYY	0	0	0	2	0	2
XXX	0	0	0	2	0	2
Microdeletions	2	0	0	0	1	3
LFF (including Natera's high risk algorithm)	4	28	0	8	0	40
No call- UDP	2	6	1	0	1	10
No call- Triploidy, VT, or unrecog. mult. gestation	2	3	0	0	0	5
Other	0	5	2	5	3	15
<b>Total results</b>	<b>69</b>	<b>58</b>	<b>21</b>	<b>28</b>	<b>5</b>	<b>181</b>

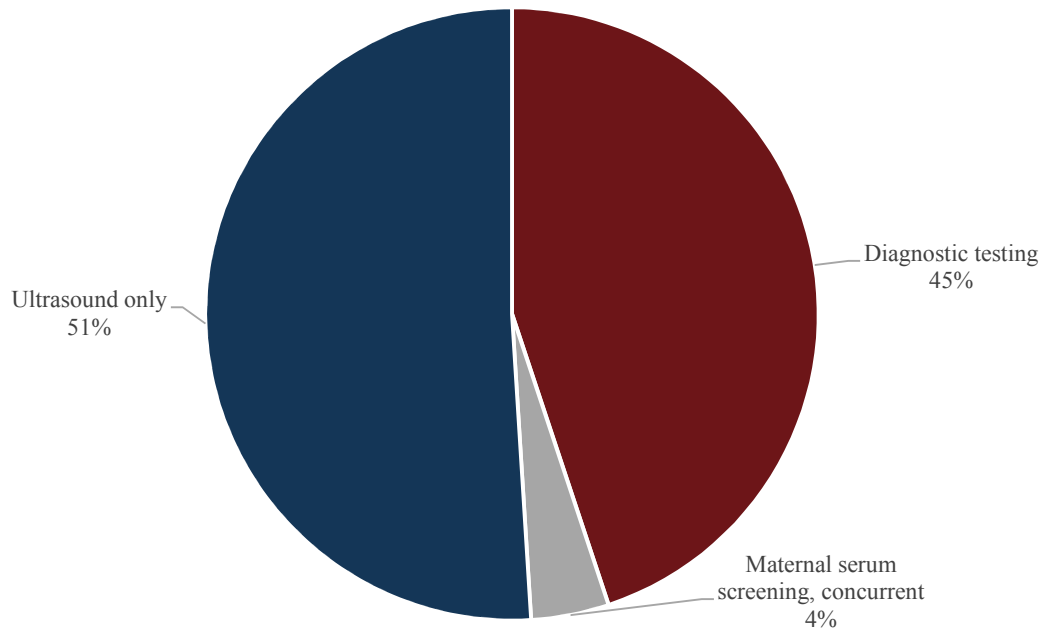
### **2.4.1 Trisomy 21**

A total of 49 patients were screen positive for Trisomy 21 (T21) (27.1%). Results were generated by eight different laboratories. The average maternal age of patients was 33.6 years, and the average gestational age was 13.0 weeks. Most were multigravida (86%, n=43) and advanced maternal age (AMA) (54%, n=27). The average PPV provided by laboratory reports was 81.1% (n=44). One screen positive occurred in a twin gestation (2%). Ultrasound abnormalities were detected in 65.3% (n=32). The majority were confirmed as true positives (83.7%, n=41). Outcomes for all screen positives are classified in Figure 2.3. Most patients declined diagnostic testing (55.1%, n=27). Decision-making for screen positives is outlined in Figure 2.4

Considering only true positives, the majority of women were AMA (58.5%, n=24). Most cases were diagnosed prenatally (51.2%, n=21), while the remaining 20 were postnatally confirmed (48.8%). One true positive was a partial duplication of chromosome 21, but the rest were full aneuploidy. One true positive was Twin A in a dichorionic/diamniotic gestation. Most affected pregnancies demonstrated ultrasound abnormalities, which are detailed in Appendix C (75.6%, n=31). Affected pregnancies were majority male (56.1%, n=23).



**Figure 2.3** Outcomes for T21 screen positives



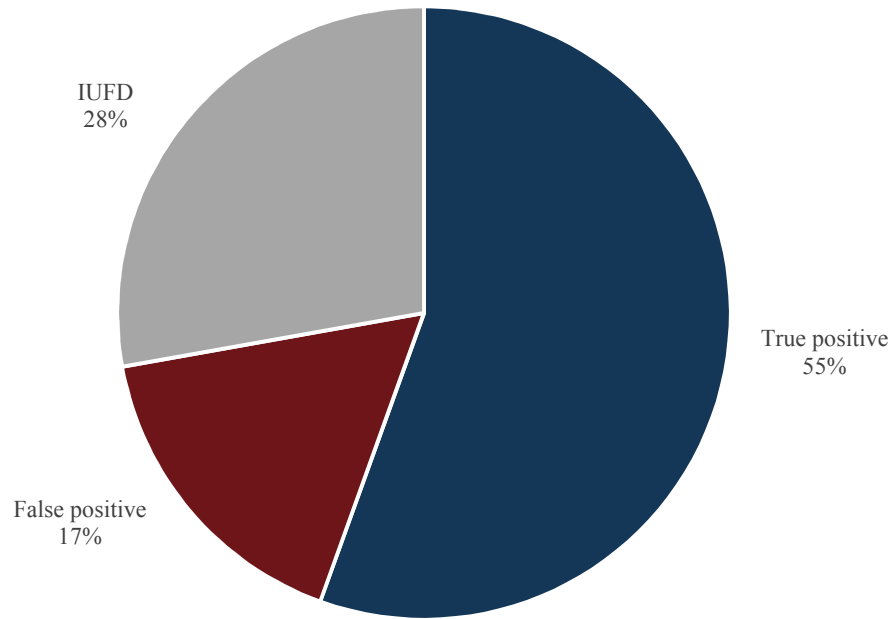
**Figure 2.4** Decision-making for T21 screen positives



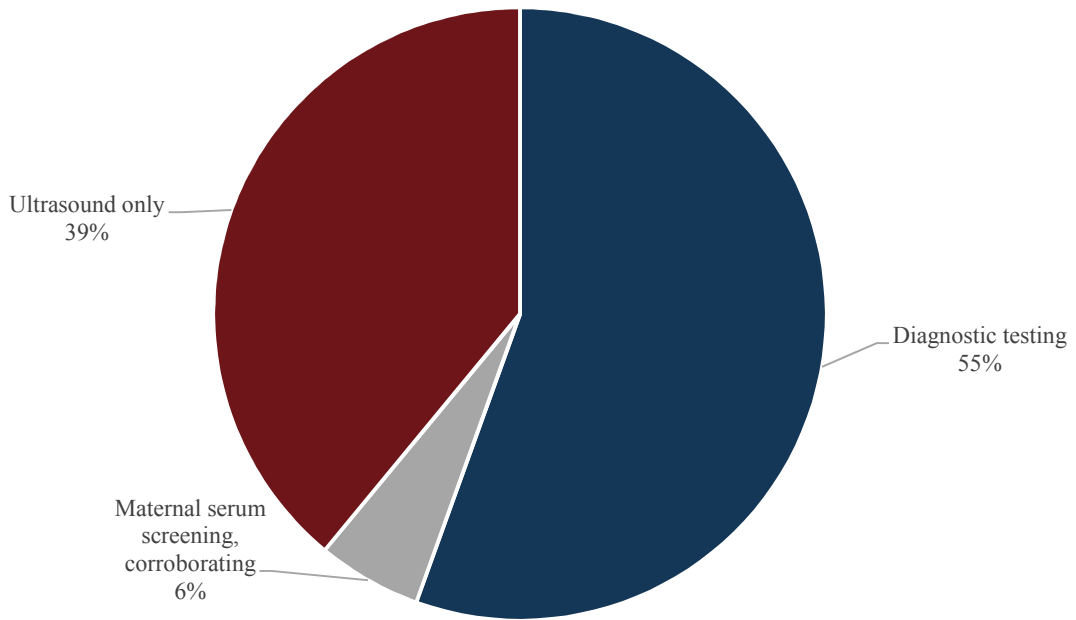
### **2.4.2 Trisomy 18**

A total of 18 patients were screen positive for Trisomy 18 (T18) (9.9%). The average maternal age of participants was 35.3 years, and the average gestational age was 12.2 weeks. Most were multigravida (77.8%, n=14) and AMA (66.7%, n=12). The average PPV provided by laboratory reports was 59.04% (n=16). The majority were confirmed as true positives (55.5%, n=10). Outcomes for all screen positives are classified in Figure 2.5. Most patients with a screen positive opted to proceed with diagnostic testing (55.5%, n=10), with the majority having ultrasound abnormalities (70%, n=7). Decision-making for screen positives is outlined in Figure 2.6.

Considering only true positives, the majority of women were AMA (80%, n=8). Most cases were diagnosed prenatally via amniocentesis (70%, n=7), while the rest were postnatally confirmed (30%, n=3). One case was mosaic T18, while the others were full aneuploidy. Most affected pregnancies demonstrated ultrasound abnormalities, which are detailed in Appendix D (90%, n=9). Affected pregnancies were majority male (70%, n=7).



**Figure 2.5** Outcomes for T18 screen positives

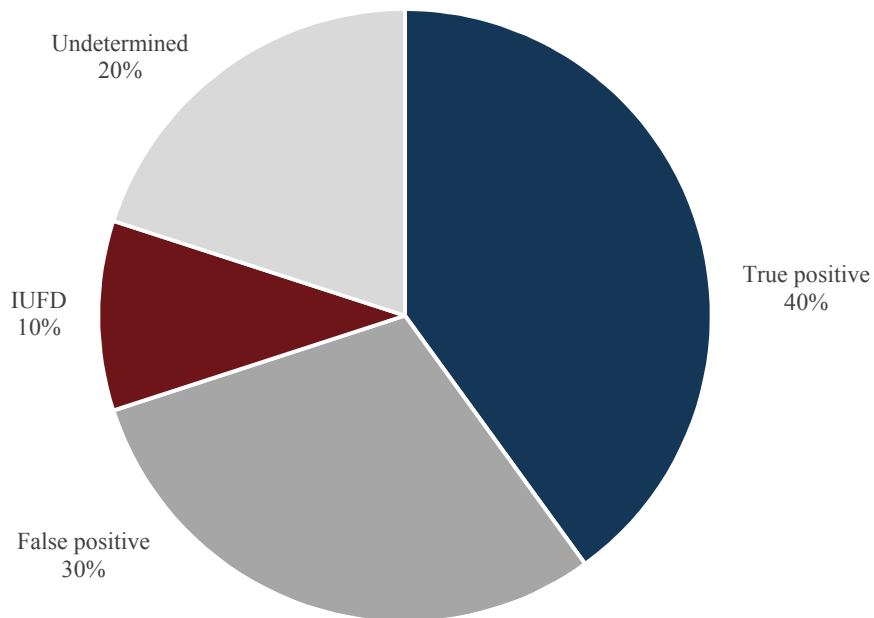


**Figure 2.6** Decision-making for T18 screen positives

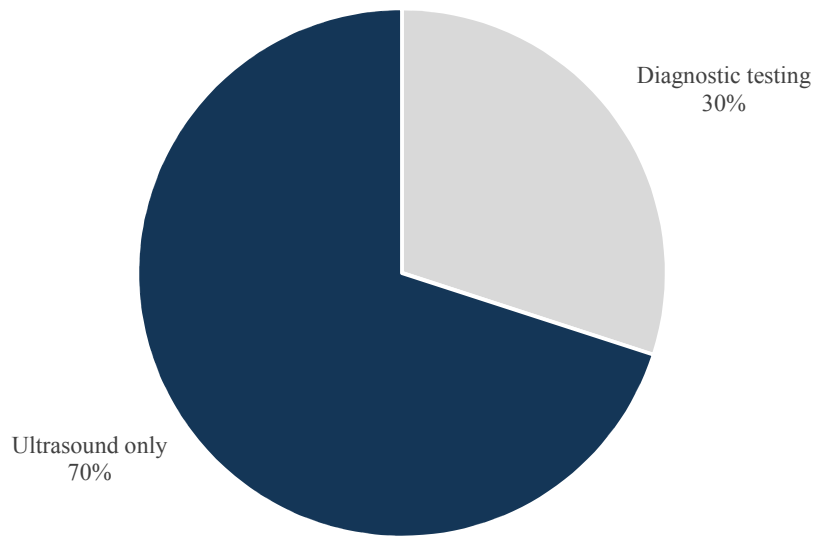
### 2.4.3 Trisomy 13

Ten patients were screen positive for Trisomy 13 (T13) (5.5%). The average maternal age of participants was 26.8 years, and the average gestational age was 15.3 weeks. Most were multigravida (80%, n=8), yet only one was AMA (10%). The average PPV provided by laboratory reports was 26.03% (n=7). Four were confirmed as true positives (40%). Outcomes for all screen positives are classified in Figure 2.7. Three patients opted to proceed with diagnostic testing (30%). Decision-making for screen positives is outlined in Figure 2.8.

Considering only true positives, the majority of women were not AMA (75%, n=3). Most cases were diagnosed prenatally (75%, n=3), while the remaining case was postnatally confirmed. One case was mosaic T13, while the others were full aneuploidy. Half of affected pregnancies demonstrated ultrasound abnormalities, which are detailed in Appendix E (50%, n=2). Affected pregnancies were majority male (75%, n=3).



**Figure 2.7** Outcomes for T13 screen positives

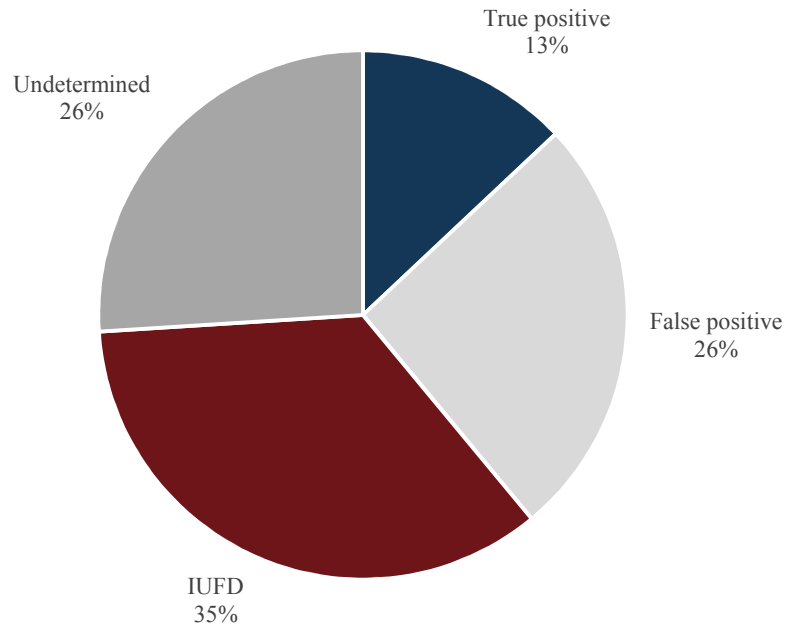


**Figure 2.8** Decision-making for T13 screen positives

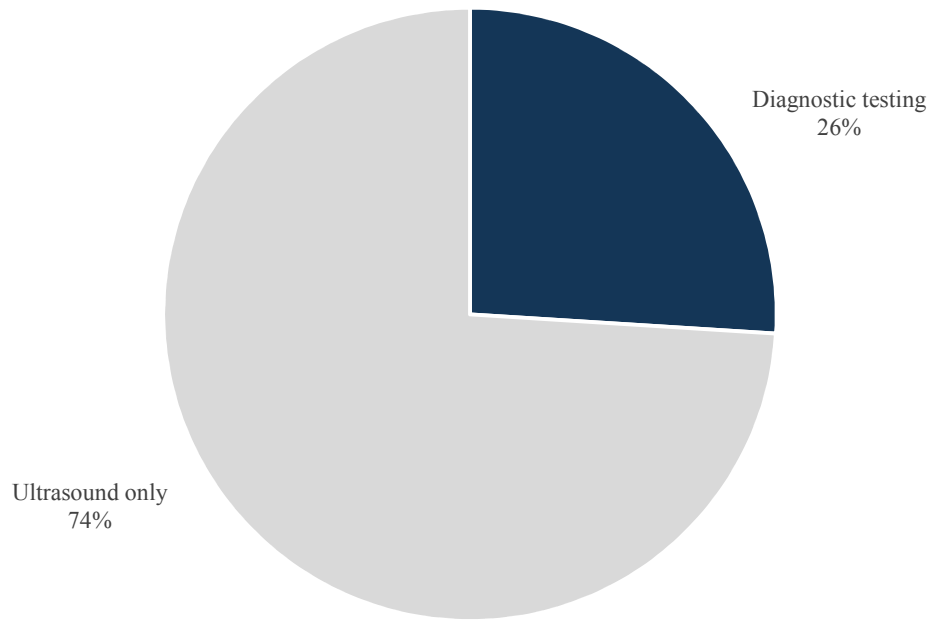
#### **2.4.4 Monosomy X**

A total of 23 patients were screen positive for Monosomy X (12.7%). The average maternal age of participants was 28.04 years, and the average gestational age was 11.7 weeks. Most were multigravida (73.9%, n=17) and below AMA (82.6%, n=19). The average PPV provided by laboratory reports was 24.9% (n=17). Three were confirmed as true positives (13%). Outcomes for all screen positives are classified in Figure 2.9. Six patients opted to proceed with diagnostic testing (26%). Decision-making for screen positives is outlined in Figure 2.10.

Considering only true positives, the average maternal age was 22.7 years. Two cases were diagnosed prenatally (66.7%, n=2), while the remaining case was confirmed via studies on products of conception. All affected pregnancies demonstrated ultrasound abnormalities, which are detailed in Appendix F (100%, n=3).



**Figure 2.9** Outcomes for Monosomy X screen positives

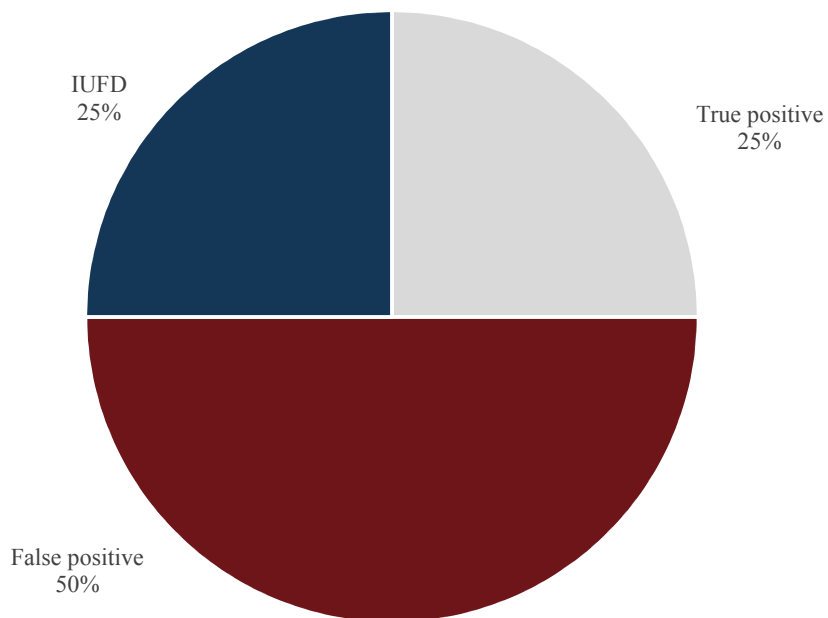


**Figure 2.10** Decision-making for Monosomy X screen positives

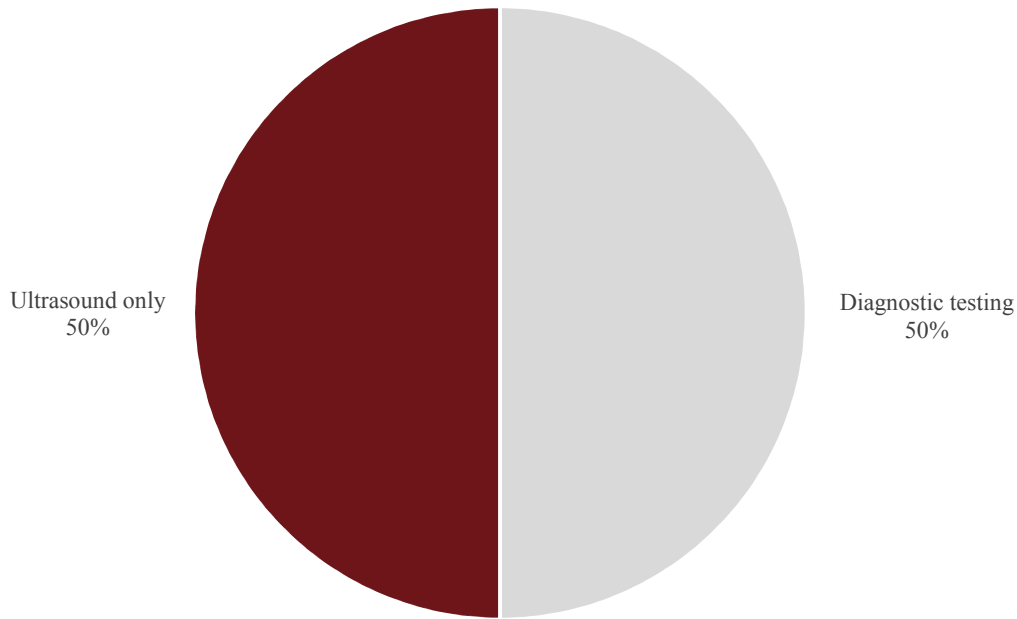
### 2.4.5 XXY

Four patients received a positive result for XXY, or Klinefelter syndrome (2.2%). The average maternal age of participants was 32.3 years, and the average gestational age was 11.0 weeks. Three of the four patients were multigravida (75%). The average PPV provided by laboratory reports was 64% (n=4). One case was a true positive (25%). Outcomes for all screen positives are classified in Figure 2.11. Half of patients opted for diagnostic testing (50%, n=2). Decision-making for screen positives is outlined in Figure 2.12.

Considering the only true positive case, the patient was 30 years old and she received the diagnosis via amniocentesis. The fetus demonstrated no abnormalities.



**Figure 2.11** Outcomes for XXY screen positives



**Figure 2.12** Decision-making for XXY screen positives

#### **2.4.6 XYY**

Two patients received a positive result for XYY (1.1%). The average maternal age of participants was 24.5 years, and the average gestational age was 12.0 weeks. Both patients were multigravida (100%). Results were generated by two different laboratories; one patient was given an 89% PPV while the other was not listed on the report. The patients had no ultrasound abnormalities, nor did they opt for diagnostic testing. One patient was lost to follow-up regarding postnatal testing, and the other had declined diagnostic testing and was still pregnant by the completion of our study.

#### **2.4.7 XXX**

We had two screen positive results for XXX syndrome (1.1%). The average maternal age was 39.0 years, and the average gestational age was 11.5 weeks. Both reports were generated by the same laboratory with a PPV of 38%. Both patients were multigravida with a history of spontaneous abortion (SAB). As a result, one patient opted

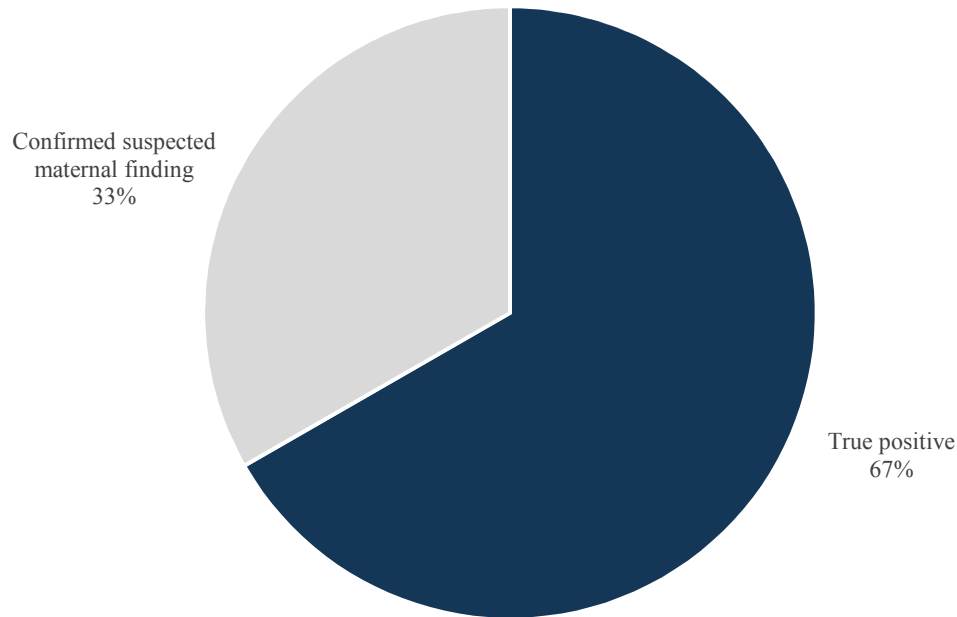
for maternal chromosome analysis, but it was ultimately normal. Neither patient demonstrated ultrasound abnormalities, nor did they opt for diagnostic testing. One patient could not be reached for follow-up, and another patient declined postnatal testing. As such, no outcome data are available.

#### **2.4.8 Microdeletions**

A total of three patients were screen positive for microdeletions, all 22q11.2 deletion syndrome (1.7%). One patient's report noted a suspected maternal finding. The average maternal age of patients was 22.6 years, and the average gestational age was 11.3 weeks. The average PPV provided by laboratory reports was 20% (n=2). Outcomes for screen positives are classified in Figure 2.13.

Considering only true positives, the average maternal age was 21.5 years (n=2). Both fetuses demonstrated Tetralogy of Fallot (TOF) on ultrasound. Both patients declined diagnostic testing and instead opted for postnatal confirmation. There was one male and one female affected (n=2). The patient whose report noted a suspected maternal finding underwent chromosomal microarray (CMA), which confirmed the presence of a pathogenic 22q11.2 deletion. She did not opt for prenatal diagnosis.





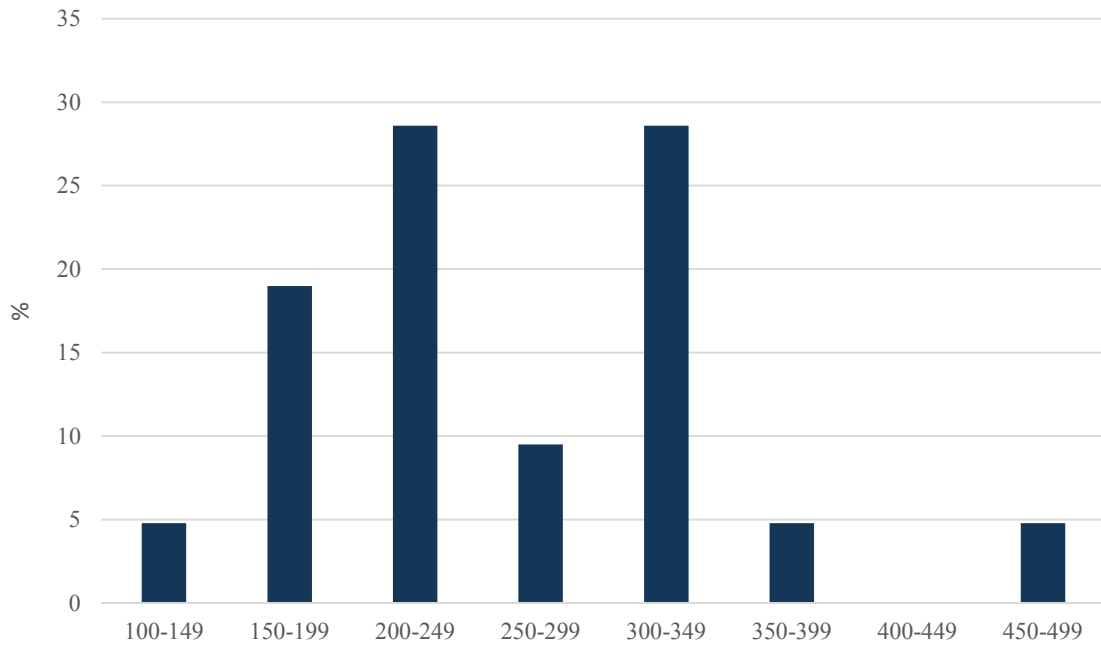
**Figure 2.13** Outcomes for microdeletions

#### **2.4.9 No call- low fetal fraction**

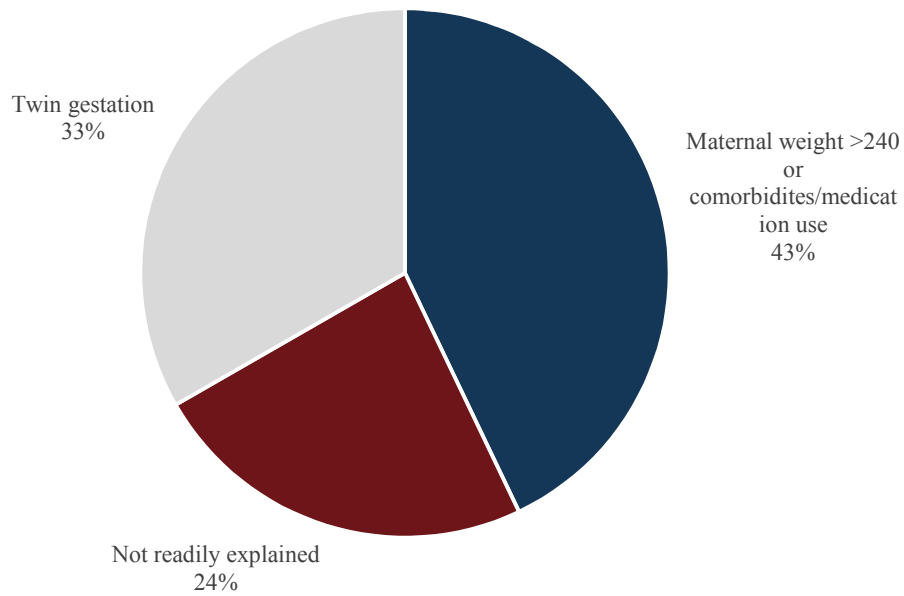
A total of 21 patients had a general no call- LFF result (11.6%). The average maternal age was 31.6 years, and the average gestational age was 14.5 weeks. Of provided fetal fractions, the average was 3.3 (n=12). The average maternal weight was 263.4 pounds, with the majority weighing over 240 pounds (57.1%, n=12). Maternal weights are graphed in Figure 2.14. Several associations of LFF results were noted in our patients, and these are outlined in Figure 2.15. A greater proportion of patients carrying singletons as opposed to twins were over 240 pounds (71.4%, n=10).

The majority of patients attempted a redraw (62%, n=13). Decision-making for the results are summarized in Figure 2.16. Patients receiving an informative redraw weighed slightly less (267.2 pounds) than patients receiving a second no call (275.1 pounds), however, this was not statistically significant,  $t(11) = 0.16, p = .88$ . They also had no reported comorbidities or medication use. All patients with documented

comorbidities had unsuccessful redraws. No genetic aberrations were confirmed among patients for whom outcome data were available (81%, n=17), though one patient with two LFF results and abnormalities was lost to follow-up, and another reported her that child was born with a heart defect. These cases are detailed in Table 2.3.



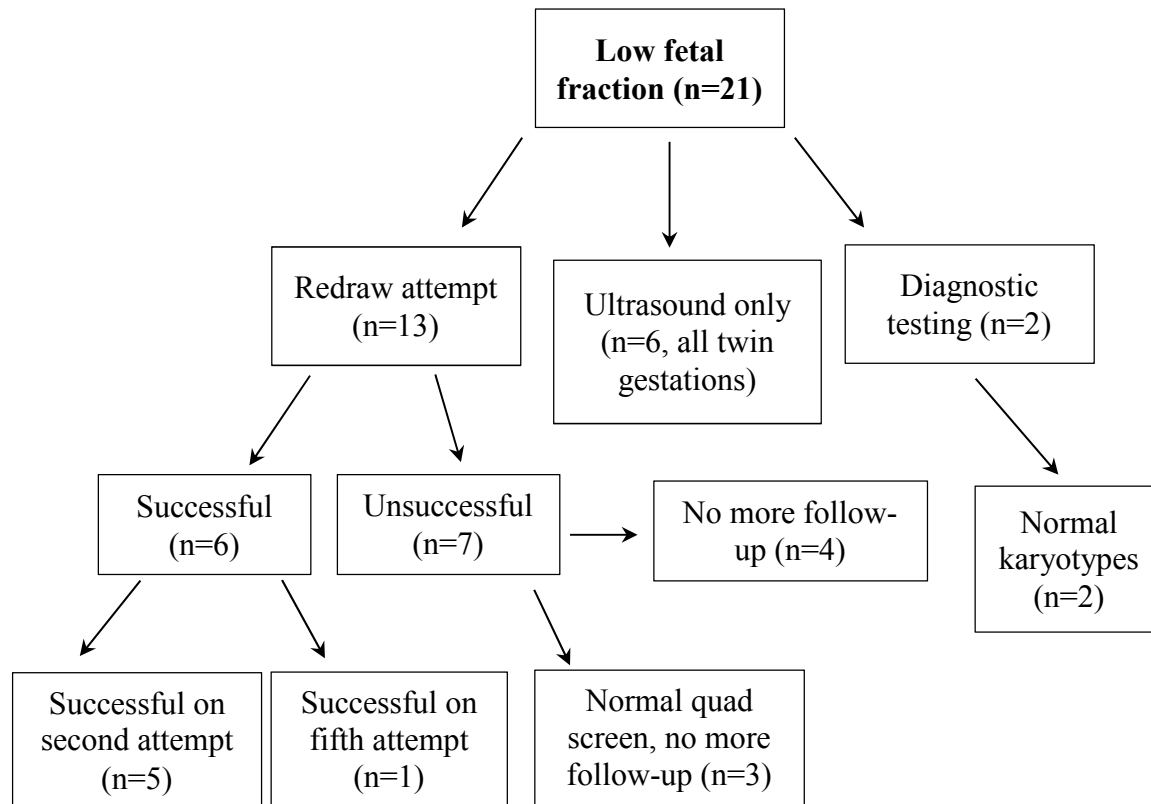
**Figure 2.14** Maternal weights for LFF results (in pounds)



**Figure 2.15** Attributes noted in patients with LFF results

**Table 2.3** LFF results with fetal abnormalities

<b>Patient</b>	<b>Age</b>	<b>Weight</b>	<b>GA</b>	<b>Comorbidities/ medication use</b>	<b>Laboratory</b>	<b>U/s findings</b>
<b>PH-USC 1</b>	30	304	24	None	LabCorp	Unilateral club foot, CPCs
<b>PH-USC 63</b>	43	280	14	Type 2 diabetes	Natera	TGA noted at birth

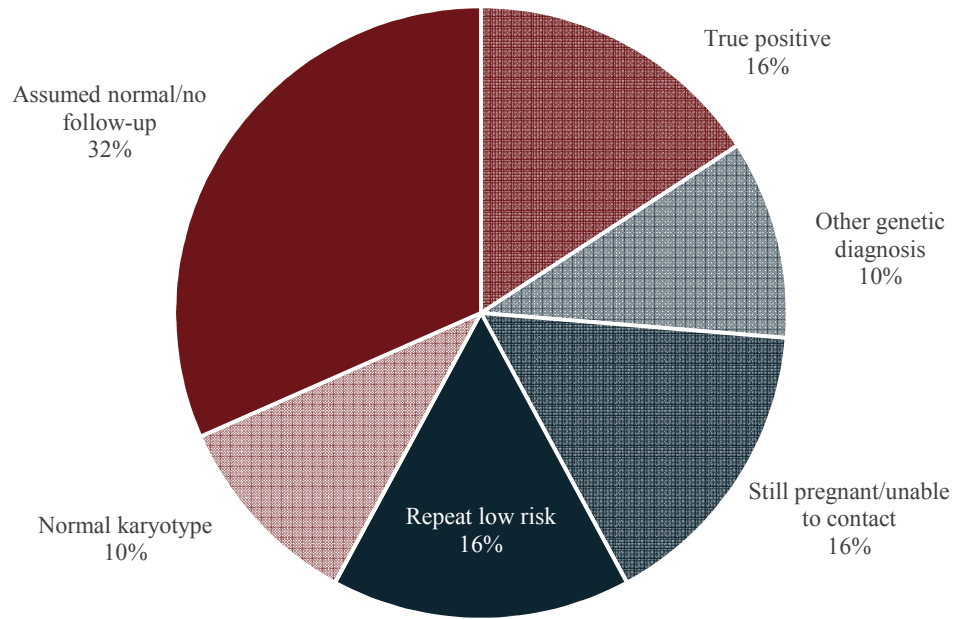


**Figure 2.16** Decision-making for LFF results

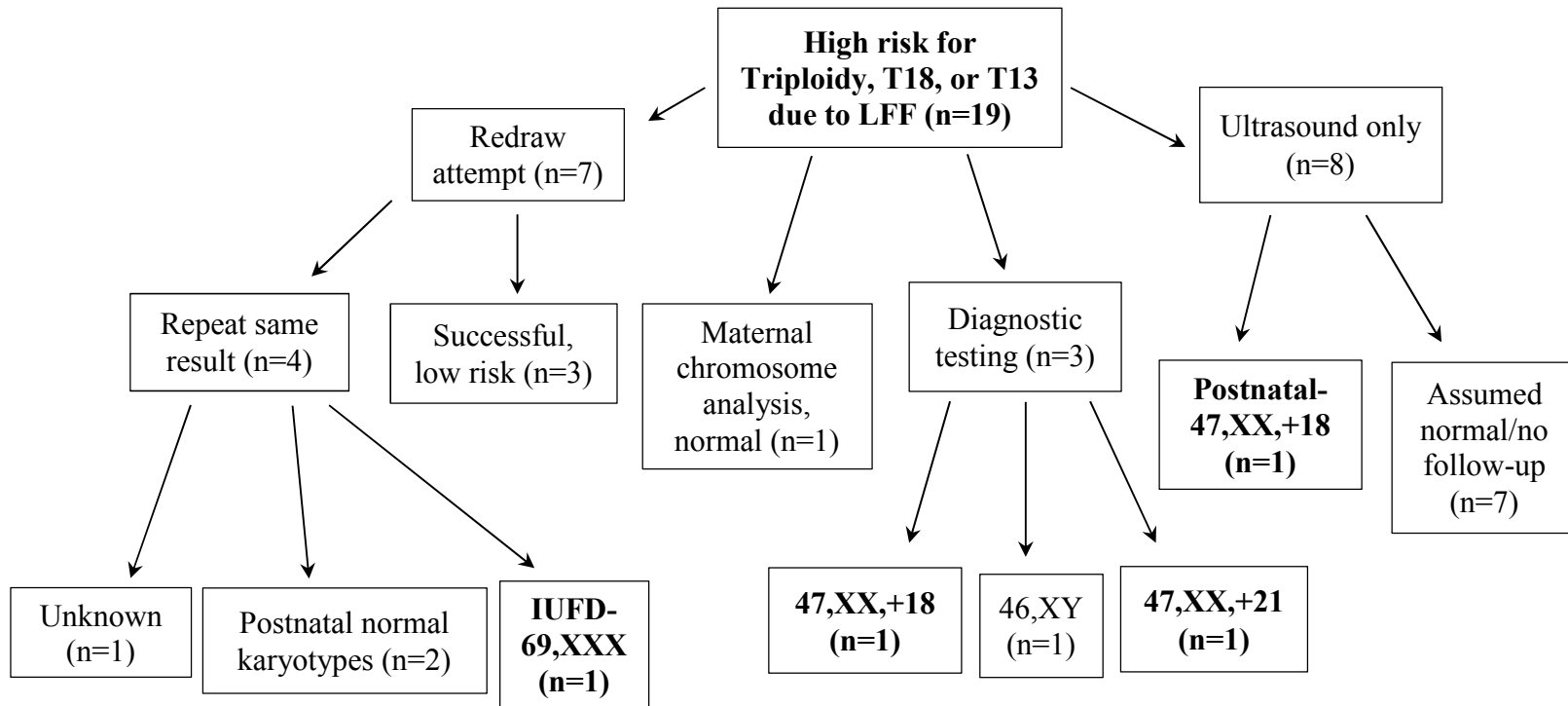
#### **2.4.10 High risk for triploidy, trisomy 18, or trisomy 13 due to LFF**

A total of 19 patients were high risk for Triploidy, T18, or T13 due to LFF (10.5%). This is a specific type of LFF result unique to Natera, and it is generated when LFF cannot be attributed to maternal age, gestational age, or maternal weight. Results are not given for other chromosomes, including the sex chromosomes. The average maternal age was 29.8 years, and the average gestational age was 14.3 weeks. Average maternal weight was 196.7 pounds. The difference in maternal weight from those with general LFF results was statistically significant,  $t(38) = 3.1, p = .004$ . Most were multigravida (84.2%,  $n=16$ ) but not AMA (78.9%,  $n=15$ ). The risk estimate for this result is 1/17 (5.9%), therefore all patients received the same PPV. Three were confirmed as true positives (15.8%). Outcomes for this result type are classified in Figure 2.17. Three patients opted for diagnostic testing (15.8%). Decision-making in this result type is outlined in Figure 2.18.

Considering only true positives, all three women were below AMA. Two cases were confirmed as T18 (66.7%), and the other was triploidy (33.3%). One case of T18 was diagnosed prenatally via amniocentesis (33.3%), while the other was postnatally confirmed. The case of triploidy was confirmed via postnatal studies after the patient had an IUFD at 17 weeks. All three affected pregnancies demonstrated ultrasound abnormalities and were female (100%). Abnormalities are detailed in Appendix G.



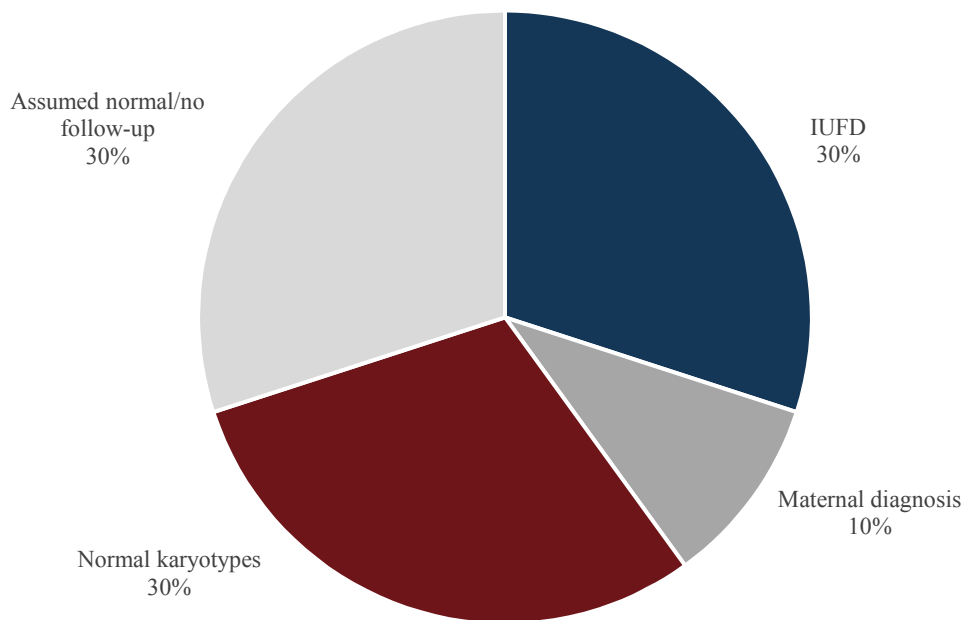
**Figure 2.17** Outcomes of high risk for Triploidy, T18, or T13 due to LFF results



**Figure 2.18** Decision-making for high risk for Triploidy, T18, or T13 due to LFF results

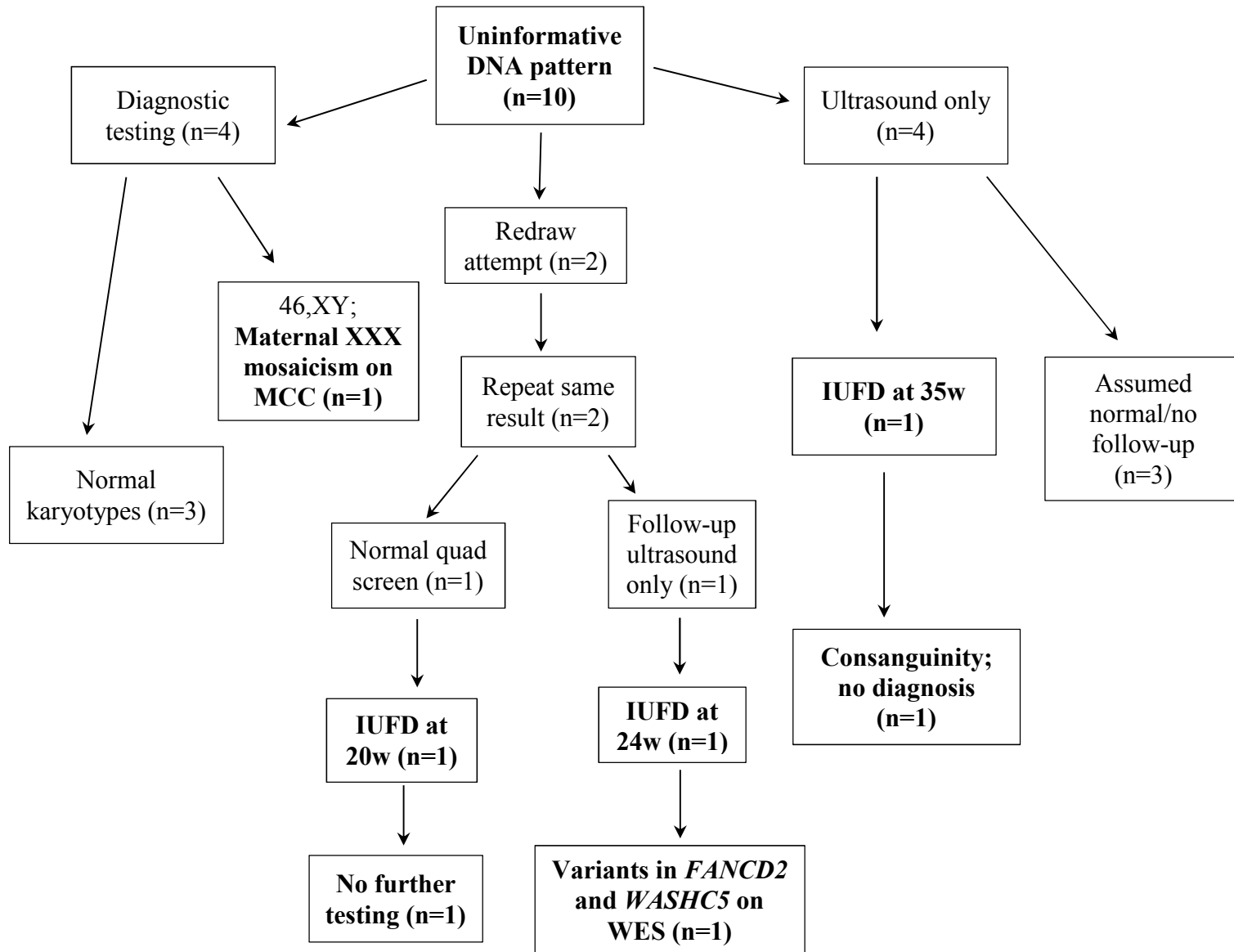
#### 2.4.11 No call- uninformative DNA pattern

A total of 10 patients received a no call UDP result (5.5%). This no call type is unique to Natera. Two of these patients (20%) received two UDP results. The average maternal age of participants was 30.1 years, and the average gestational age was 14.4 weeks. Three (30%) genetic findings across four abnormal outcomes were identified: maternal XXX mosaicism, consanguinity, and two variants of uncertain significance (VUS) in one patient. Outcomes for this result type are classified in Figure 2.19. Most patients with a screen positive declined diagnostic testing (60%, n=6). Decision-making for this result type is outlined in Figure 2.20.



**Figure 2.19** Outcomes of UDP results



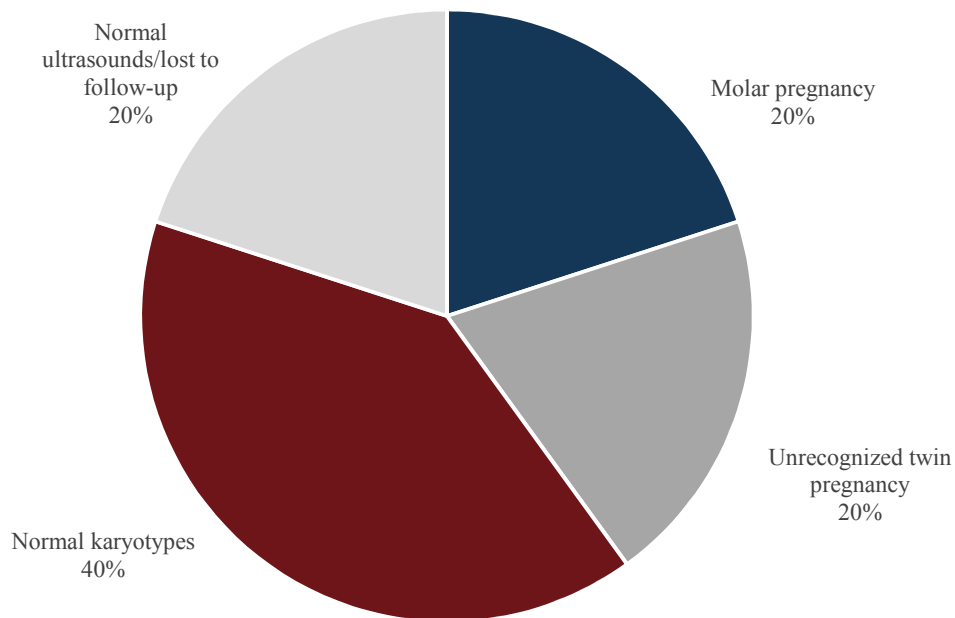


**Figure 2.20** Decision-making for UDP results

#### 2.4.12 No call- triploidy, vanishing twin, or unrecognized multiple gestation

We had a total of five patients that received a result for triploidy, vanishing twin, or an unrecognized multiple gestation (2.8%). This is a result unique to Natera, and it is generated when three DNA patterns are identified, but cannot be delineated based on origin. Typical risk assessment for aneuploidy cannot be run due to the unknown etiology of the third DNA contribution. The average maternal age was 27.4 years, and the average gestational age was 16.2 weeks. Two patients had identifiable outcomes consistent with this call, resulting in an overall PPV of 40%. Outcomes for this result type are classified in Figure 2.21.

Considering only true positives, the average maternal age was 30.5 years. Neither patient opted for diagnostic testing, as their ultrasounds revealed the likely reason for their abnormal screens: molar pregnancy and twin pregnancy.



**Figure 2.21** Outcomes of no call- triploidy, vanishing twin, or unrecognized multiple gestation results

### 2.4.13 Other results

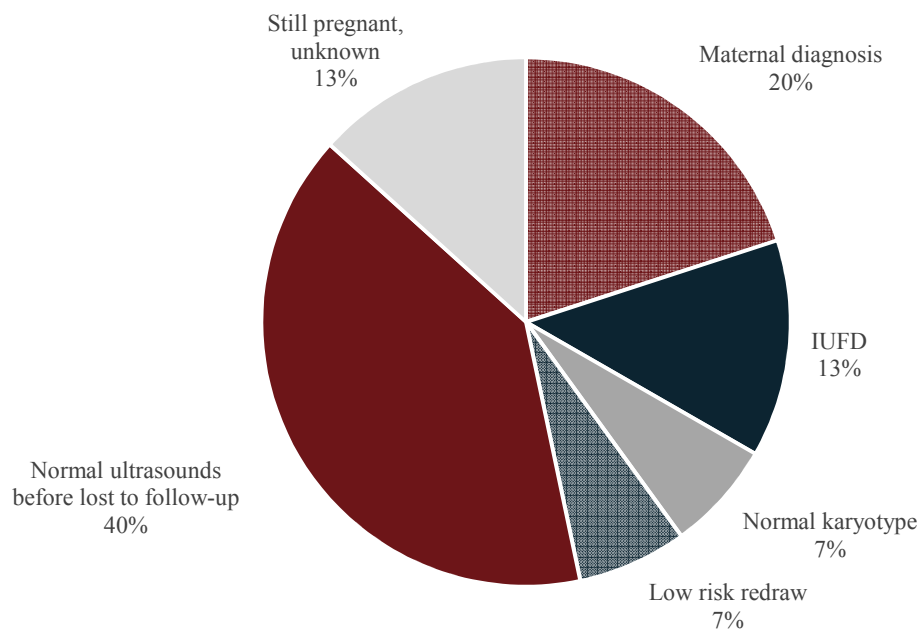
Fifteen patients received atypical findings including double screen positive results or another type of no call (8.3%). These results are summarized in Table 2.4. The average maternal age was 33.3 years, and the average gestational age was 12.7 weeks. Average maternal weight was 191.3 pounds. Most were multigravida (73.3%, n=11) and not AMA (66.7%, n=10). No fetal diagnoses were made, however, three maternal ones were confirmed: one mosaic Monosomy X, one 13q microdeletion, and one Xq;3q unbalanced translocation. Outcomes for these results are outlined in Figure 2.22. Only one patient with a screen positive opted for diagnostic testing of her fetus (6.7%), however, three patients chose chromosome analysis for themselves (20%). Decision-making for these results is highlighted in Figure 2.23.

Three patients had exactly the same abnormal results (20%). They each received a no call- LFF result from Natera followed by a high risk for Triploidy, T18, or T13 result on redraw before having assumed normal fetal outcomes. Knowing that FF is important for both of these result types, we calculated the means of factors known to be associated with LFF. Results are shown in Table 2.5. The average maternal weight falls between those of general LFF and high risk LFF results.

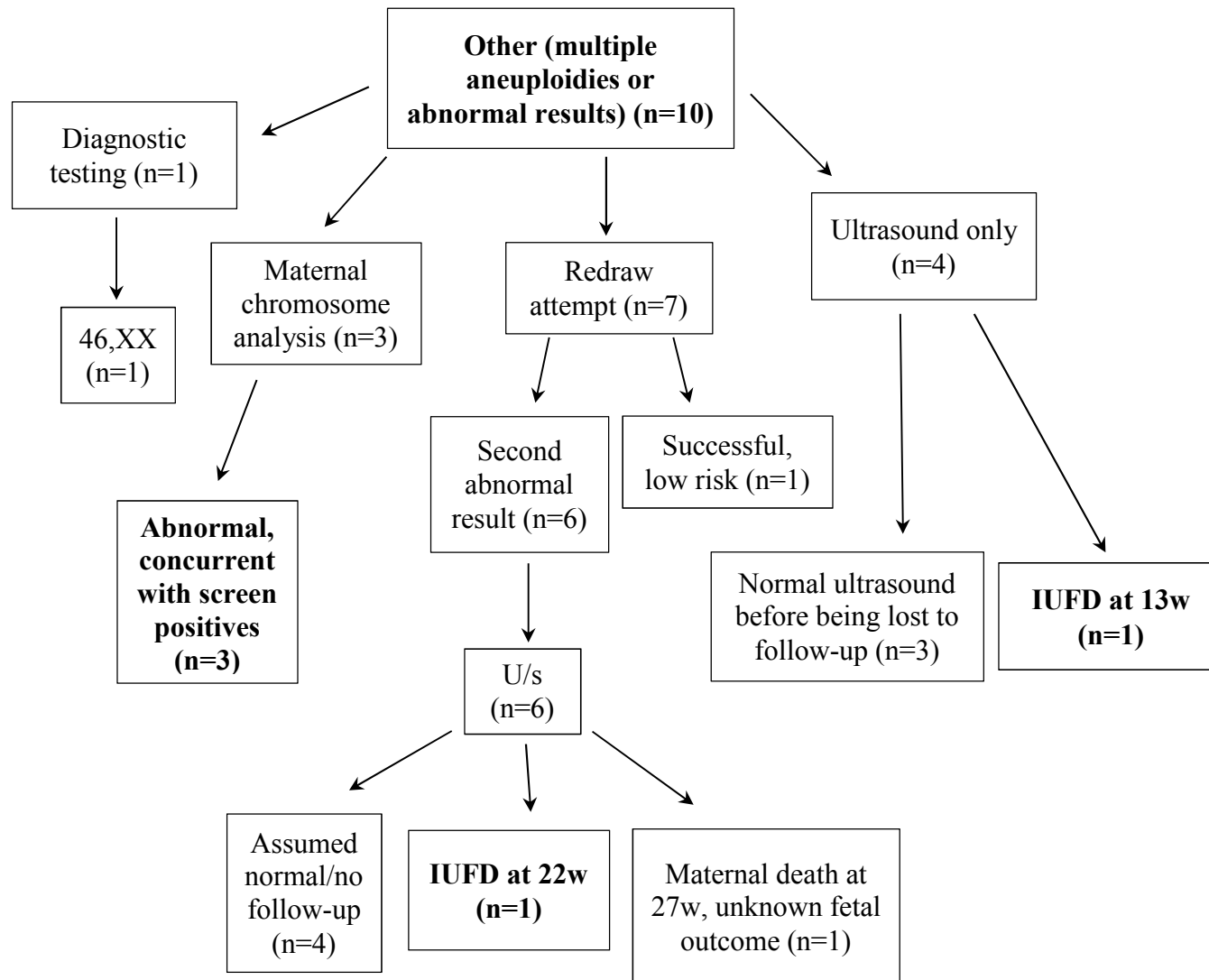
**Table 2.4** All results for other (multiple aneuploidies or abnormal results)

<b>Patient</b>	<b>Age</b>	<b>Weight</b>	<b>Laboratory</b>	<b>Result</b>	<b>U/s findings</b>	<b>Next steps/outcome</b>
<b>PH-USC 5</b>	39	201	Progenity	Monosomy X and Monosomy 13	None	Amnio-46,XX
<b>PH-USC 49</b>	38	119	Progenity	No call x2-multiple aneuploidy	None	U/s only; sudden maternal death at 27w
<b>PH-USC 58</b>	29	180	Natera	No call- LFF; high risk for Triploidy, T18, or T13 due to LFF	None	Lost to follow-up after normal u/s
<b>PH-USC 60</b>	30	236	Natera	No call- LFF; high risk for Triploidy, T18, or T13 due to LFF	None	Lost to follow-up after normal u/s
<b>PH-USC 61</b>	24	182	Natera	No call-maternal X abnormality	None	Maternal karyotype-mosaic 45,X
<b>PH-USC 140</b>	44	179	Progenity	T18 and Monosomy X	None	IUFD at 13w
<b>PH-USC 142</b>	34	230	Natera	No call-atypical finding	None	Maternal CMA-13q12.12 duplication (VUS)
<b>PH-USC 143</b>	28	201	Natera	No call- no result	None	Repeat low risk
<b>PH-USC 145</b>	28	122	Natera	No call-atypical sex chromosomes	None	Maternal karyotype-unbalanced 3q;Xq translocation
<b>PH-USC 146</b>	43	112	Natera	No call-maternal X abnormality	None	Declined further testing/lost to follow-up

<b>PH-USC 147</b>	25	274	Natera	No call- LFF; no call-atypical finding	None	Normal u/s; pregnancy still ongoing
<b>PH-G 24</b>	30	162	Counsyl	Trisomy 19	Renal pyelectasis	Viable at 20w; no further follow-up
<b>PH-G 28</b>	32	202	Counsyl	Monosomy 19	None	Viable at 38w; no further follow-up
<b>PH-G 29</b>	45	169	Natera	High risk for Triploidy, T18, or T13 due to LFF; T21	Single umbilical artery	IUFD at 22w
<b>PH-G 31</b>	30	301	Natera	No call- LFF; high risk for Triploidy, T18, or T13 due to LFF	None	Lost to follow-up after normal u/s



**Figure 2.22** Outcomes for other (multiple aneuploidies or abnormal results)



**Figure 2.23** Decision-making for other (multiple aneuploidies or abnormal results)

**Table 2.5** Average age, weight, and GA in those receiving both general LFF and high risk LFF results

	<b>PH-USC 58</b>	<b>PH-USC 60</b>	<b>PH-G 31</b>	<b>Means</b>
<b>Maternal age</b>	29	30	30	<b>29.7 years</b>
<b>Maternal weight</b>	180	236	301	<b>239 pounds</b>
<b>GA at first draw</b>	14	13	17	<b>14.7 weeks</b>



## 2.5 Discussion

Outcome data for pregnancies with abnormal NIPS are few, and most available literature has been generated by laboratories featuring their own data. Our study contributes outcome data for abnormal screening results across several laboratories and platforms. This is particularly important given that new result types are quickly evolving before professional guidelines can develop follow-up recommendations. Additionally, follow-up recommendations for conditions established on NIPS, such as SCAs, are inconsistent (Fleddermann et al., 2019). Importantly, we were able to develop management guidelines for our practice based on the various no call or atypical/uninformative result types, as seen in Figure 3.1. Recommended follow-up for any high risk result remains diagnostic testing, including karyotype and CMA. The importance of conducting both chromosome analyses was reinforced by two cases of mosaicism and one case of a partial chromosome duplication. Serial growth ultrasounds should be offered to those declining diagnostic testing in order to monitor for complications of intrauterine growth restriction (IUGR). Patients with unexplained LFF also benefit from serial growth ultrasounds, similar to how patients with unexplained elevations in AFP (alpha-fetoprotein) are managed. This is not only because the chance of aneuploidy remains without diagnostic testing to confirm or rule it out, but also that LFF could be due to a placental issue which would place a risk of growth restriction on the fetus. We also included a step to contact a GC at the performing laboratory for uninformative/atypical result types; in several cases, we were able to learn more about chromosomes of interest and whether or not the abnormality appeared to be maternal or fetal in origin when this information was not included on the report. Obtaining this

information from the laboratory GCs was valuable in guiding management, and it led to several patients pursuing their own chromosome studies when otherwise there would have likely been no follow-up.

We observed several patterns in our data that are in agreement with background research. Uptake of diagnostic testing was highest in the autosomal trisomies despite there not being full consistency between conditions (Figure 3.2) (Gil, Quezada, Revello, Akolekar, & Nicolaides, 2015.) Most patients with a screen positive T18 opted for diagnostic testing in the presence of ultrasound abnormalities, while most of those opting for diagnostic testing in T21 or T13 did so in the absence of abnormalities (Figure 3.3). There were cases that reinforced the importance of diagnostic testing to confirm or rule out an abnormal result, as several fetuses with ultrasound findings ultimately had normal karyotypes, therefore ruling out the screen positive (Table 3.1). Patients and providers may factor ultrasound findings into risk assessment when diagnostic testing is declined, however, it is extremely important to confirm a diagnosis for appropriate medical management and before irreversible pregnancy management decisions are made.

Unsurprisingly, we saw lower uptake of diagnostic testing in SCAs. This is likely due to the lack of medical complications and generally mild phenotypes associated with these conditions. There was a high rate of IUFD among those with screen positive Monosomy X, which is consistent with reports in the literature (Suzumori & Sugiura-Ogasawara, 2010). In combination with what is known about phenotypes of SCAs, patients likely declined diagnostic testing knowing the rate of miscarriage for true positives is high. Similarly, uptake of diagnostic testing was low for microdeletions and various types of no calls. One exception to this was for uninformative DNA pattern

results. We saw higher uptake of diagnostic testing compared to other no call results, but also an increased number of abnormalities, adverse fetal outcomes, and repeat screening failures. The specificity of this result suggesting an abnormality in the DNA may have impacted decision-making among patients. As a result, diagnostic testing (karyotype and CMA) was the most informative next step for this result type, including maternal karyotype and CMA, as we were able to make one maternal diagnosis after she received this result.

Interestingly, we saw reasonable success in redraws for result types that laboratories do not necessarily recommend, namely high risk for Triploidy, T18, or T13 due to LFF (Benn et al., 2019). Though Natera advises diagnostic testing as the next step for this result, the redraw success rate suggests that a second attempt may render the fetus low risk for conditions within the scope of the laboratory's screening platform (common trisomies and SCAs). On the contrary, we also observed several repeat failures in LFF results. For reasons unknown, several women with assumed normal fetal outcomes could not get a successful result even when they were of appropriate gestational age and weight with no other risk factors. It is possible that these low FF results were due to a genetic aberration outside the scope of the screening test, again underscoring the importance of diagnostic testing. Placental abnormalities could also be the explanation, further highlighting the importance of patients following-up with growth ultrasounds throughout the duration of their pregnancies.

In several cases, there was indication to offer maternal testing. As a result, we observed five maternal diagnoses (Table 3.2). In addition to 22q and 13q deletions, X chromosome abnormalities for three mothers were identified by three different Natera

result types. This suggests that while there may have been high accuracy in these calls, consistency between result types and what they are indicating needs to be further developed. Similarly, we saw the impact of laboratories specifically indicating maternal differences on their reports or providing this information to GCs when they called the laboratory. Uptake of maternal chromosome analysis was high when this distinction was made.

Several new types of no call results evolved over the course of our study. We observed several patients with double screen positive results, as well as results suggestive of maternal abnormalities. When a maternal distinction was made, patients were more likely to opt for their own chromosome analysis. With one abnormal result citing a general atypical finding, we were able to get more specific information by calling the laboratory. A laboratory GC is often able to provide information on the raw data that may be helpful in counseling patients in follow-up and management.

### **2.5.1 Trisomy 21**

T21 was our most frequent screen positive, which is not surprising given that it has the highest incidence of all conditions screened (27.1%, n=49). Diagnostic testing was mostly declined, likely because of the procedural risks and the fact that it would not impact pregnancy management. We also had a high frequency of abnormalities in this result type; interestingly, the ones that pursued diagnostic testing did so largely in the absence of abnormalities, suggesting that it may have been an important factor in patients choosing follow-up. Two patients chose maternal serum screening (MSS) as their next step, and both were abnormal for T21. As they did not follow-up with diagnostic testing after the second abnormal screen, it is likely they used this information as soft

confirmation until postnatal testing could confirm the diagnosis. This is consistent with previous literature that states patients may use abnormalities, concurrent MSS, or other factors as corroborating evidence for screen positive NIPS results, and therefore feel justified in not pursuing prenatal diagnosis (Zhen, Li, Yang, & Li, 2019).

The number of affected pregnancies showing ultrasound abnormalities was slightly higher than expected at 75.6%, given that most estimates hover around 50% (ACOG, 2016). This could be because our patients received targeted ultrasounds by high risk specialists. Additionally, many of them were scanned more than once which provided a larger timeframe for identification of abnormalities.

The unique circumstances of two cases further emphasized the importance of diagnostic testing and complete chromosome analysis. One screen positive was part of a dichorionic/diamniotic twin gestation. The laboratory could not identify which twin was indicating screen positive, however, multiple abnormalities in Twin A provided suspicion. Regardless of the presence of abnormalities, diagnostic testing was the only way to determine which, if any, twin had T21, as only one result is given for the entire pregnancy. Similarly, one true positive case was a partial chromosome 21 duplication. Partial aberrations versus full aneuploidy is an important distinction that can only be made by completing both karyotype and microarray.

The outcomes for three patients could not be classified. PH-USC 40 agreed to a follow-up phone interview after the outcome of her pregnancy was not documented in the EMR. She previously had a normal anatomy scan and declined diagnostic testing. At the time of the interview, she stated that her daughter did not have T21, but that two heart defects were identified upon her birth. She reported that genetic testing had not been

performed due to her daughter's lack of facial features typical of someone with T21. We could not classify her outcome without confirmatory testing, however, mosaic trisomy 21 could be a possible explanation. Perhaps the most interesting case, however, is PH-USC 89. Her history included one prior SAB at 12 weeks (G1), and a second pregnancy that was screen positive for T21 on second trimester MSS. That pregnancy (G2) ended in demise at 39 weeks with growth restriction and shortened femurs. No postnatal testing was carried out to determine a diagnosis. At the time of her positive NIPS in our practice (G3), she declined diagnostic testing but opted to have her chromosomes analyzed. She returned a normal karyotype. Likewise, her daughter (G3) returned a normal karyotype after delivery. Her partner and father of all three pregnancies declined chromosome analysis. It is possible that a maternal duplication on chromosome 21 too small for standard karyotype analysis is the explanation for this family. This possibility further supports offering both karyotype and microarray in the setting of a positive NIPS, as results may be flagged for partial chromosome aberrations and not necessarily full aneuploidy. The third patient, PH-USC 115, was lost to follow-up after normal imaging and 65% PPV.

### **2.5.2 Trisomy 18**

T18 made up 9.9% of our abnormal results (n=18). In contrast to those who were screen positive for T21, most opted for diagnostic testing, even though the majority did show ultrasound abnormalities. This may be due to patients' desire to discuss pregnancy management and/or surgical intervention options given the poor prognosis for this condition (Farrell, Agatisa, Michie, Greene, & Ford, 2019). One patient chose MSS as her next step, and it was concordant with her abnormal NIPS result. Similar to the T21

patients, she did not follow-up with diagnostic testing after the second abnormal screen, likely using that information as soft confirmation and justification for not pursuing prenatal diagnosis.

The unique circumstances of two cases further emphasized the importance of diagnostic testing and complete chromosome analysis. PH-USC 4 was a 38 year old who was screen positive with a 49% PPV. At her anatomy scan, CPCs and an EIF were identified. She opted for amniocentesis which subsequently revealed a normal female karyotype. Her case reinforces that while ultrasound abnormalities can be used in risk assessment for a screen positive, diagnostic testing is essential for confirming or ruling it out. Additionally, PH-USC 87 received a screen positive and had multiple ultrasound findings, however, karyotype revealed mosaicism as opposed to full aneuploidy. Mosaicism can complicate but also positively impact conversations surrounding prognosis and medical interventions; therefore, it is an important distinction to make.

### **2.5.3 Trisomy 13**

T13 made up 5.5% of our abnormal results (n=10). In contrast to those who were screen positive for T18, most declined diagnostic testing in the absence of ultrasound abnormalities. This was an interesting difference given that the two conditions have almost equally poor prognoses. A smaller proportion of affected pregnancies demonstrated abnormalities on ultrasound, though data may have been limited by only having first trimester imaging on two of the four patients. An overall lower average PPV for this condition compared to T18 likely contributed to less uptake of diagnostic testing. PPV is an important statistic for most patients when discussing abnormal results;

therefore, a lower PPV in combination with a normal ultrasound likely provided reassurance to patients.

The unique circumstances of two cases further emphasized the importance of diagnostic testing and complete chromosome analysis. Patient PH-USC 6 showed renal pyelectasis on anatomy scan, however, this was not an overly suspicious finding given her 8% PPV. Additionally, her anatomy scan was consistent with a male fetus, which was important to note given that pyelectasis is more common in males (Ebrashy et al., 2016). The fetal karyotype returned normal. PH-USC 27 had a normal ultrasound when she opted for amniocentesis, understanding that the lack of ultrasound findings could not serve as reassurance for a false positive. The karyotype subsequently returned mosaic for T13. In this case, the revelation of mosaicism likely complicated the conversation regarding prognosis and medical management, but it was still an important distinction to make.

#### **2.5.4 Monosomy X**

Monosomy X made up 12.7% of our abnormal results (n=23). Like those who were screen positive for T13, most declined diagnostic testing in the absence of ultrasound abnormalities. Ultrasound abnormalities in SCAs as a whole are less reliable factors for risk assessment, however, the one true positive patient that declined prenatal diagnosis had multiple abnormalities, suggesting this may have impacted her decision. Additionally, there is a high rate of miscarriage in Monosomy X. Knowing this statistic in combination with procedural risks for prenatal diagnosis may have deterred patients from pursuing this option. Roughly a third of the screen positive pregnancies ended in demise between 11-22 weeks and did not have further testing. All but one had either a



cystic hygroma or increased NT. Given the abnormal screening results, abnormalities, and demises, these are suspicious for true positives, however, without confirmatory testing they could not officially be classified.

While maternal mosaicism could be an explanation for at least a few of our false positive cases, none of the patients opted for maternal karyotype. Only one of these patients had a history of pregnancy loss, however, this same patient also had three healthy pregnancies.

### **2.5.5 XXY**

XXY, or Klinefelter syndrome, made up 2.2% of our abnormal results (n=4). Predictably, none of the four pregnancies demonstrated abnormalities. Half of the patients opted for diagnostic testing, which demonstrated their understanding that the lack of ultrasound findings could not serve as reassurance for a false positive (Fleddermann et al., 2019).

Two patients received PPVs from the same laboratory that were drastically higher than their risks on NSGC's PPV calculator, though one was ultimately a true positive. Natera reports factoring age-related risk into their calculation of PPV, however, this discrepancy is a great limitation in post-test counseling and guiding patients through management and decision-making.

Maternal mosaicism could be an explanation for our false positives, however, neither of the patients opted for maternal karyotype, nor did they have a history of pregnancy loss.

### **2.5.6 XYY**

XYY syndrome made up 1.1% of our abnormal results (n=2). Predictably, neither of the two pregnancies demonstrated abnormalities. They also declined diagnostic testing, possibly due to the reported mild phenotype associated with this condition.

Only one patient received a PPV (89%) on her laboratory report (Natera). As was the case in two XXY results, this number is drastically higher than her risk on NSGC's PPV calculator, 25%. It is difficult to counsel knowing this discrepancy exists. Similarly, it is difficult to counsel when no PPV is provided by the laboratory. This is a valuable statistic used by both providers and patients to answer the question, "How worried should I be?" Without other factors to consider in these types of conditions (i.e. ultrasound findings), it leaves patients limited to diagnostic testing as their option to further clarify the result. While always the most informative option, it is often selected against in our population.

### **2.5.7 XXX**

We had two screen positive results for XXX syndrome (1.1%, n=2). Similar to most other SCAs discussed thus far, neither pregnancy demonstrated abnormalities. Both mothers declined prenatal diagnosis, and one declined postnatal testing.

PH-USC 65 declined postnatal testing because she reported her daughter is meeting all developmental milestones. This suggests that parents may not feel it is necessary to test in the absence of typical features. This may especially be the case in SCAs, since the clinical symptoms include increased risks for social and developmental challenges as opposed to significant medical complications.

Maternal mosaicism could be an explanation for either abnormal result, however, PH-USC 65 had a normal karyotype secondary to personal history of pregnancy loss.

### **2.5.8 Microdeletions**

We had a small sample size of screen positive microdeletions, all for 22q11.2 deletion syndrome (1.7%, n=3). This is not surprising given the overall low prevalence of these conditions, as well as the fact that they are usually an opt-in when available on standard NIPS.

All three patients declined prenatal diagnosis, although one patient, PH-USC 96, received a result indicating a suspected maternal finding and opted for maternal microarray. The result confirmed the presence of a pathogenic 22q11.2 deletion. The other two patients (PH-USC 135 and PH-G 8) had pregnancies that each demonstrated TOF, a conotruncal defect known to be associated with 22q deletion syndrome. Postnatal testing confirmed the diagnosis for both infants.

The case of PH-USC 96 highlights the importance in distinguishing between a maternal and fetal result, as this information guides post-test counseling. As seen in this case and other maternally-indicated results, patients express greater comfort levels in having blood karyotypes rather than invasive prenatal testing.

### **2.5.9 No call- low fetal fraction**

No call- LFF made up 11.6% of our abnormal results (n=21). We identified several patterns consistent with other literature. First, most patients receiving this result were over 240 pounds, even after adjusting for singleton vs. twin gestations. Similarly, as maternal medications and/or comorbidities have been described in correlation with LFF results, nearly a quarter of our patients met this criteria. All patients with

documented medication use or other comorbidities had at least two LFF results, and only one of them eventually received a low risk result. One patient with a successful redraw was initially screened at nine weeks gestation. While nine weeks and beyond is an acceptable gestational age for NIPS, this early gestational age likely resulted in a LFF result. Fetal fraction continues to increase as the pregnancy progresses, which likely explains why this patient had a successful redraw (Benn, Valenti, Shah, Martin & Demko, 2018). Several of our results were produced by twin gestations, and it is known that multiple gestations have a higher fail rate than singletons due to a lower average FF per twin (Galeva, Gil, Konstantinidou, Akolekar, & Nicolaides, 2019; Gil, et al., 2015; Qiao et al., 2019). This likely explains why only one patient expecting twins opted for a redraw. Hemoglobinopathies are a newer area of interest when considering LFF results, however, these did not apply to any of our patients (Putra et al., 2019). Nevertheless, we were able to make attributions for the majority of our LFF results.

Considering redraws, our informative redraw rate of 46.2% was slightly below what has been described in the literature (Benn, Valenti, Shah, Martin & Demko, 2018; Galeva, Gil, Konstantinidou, Akolekar, & Nicolaides, 2019; Suzumori et al., 2019; White, Wang, Kunz, & Schmid, 2019). This could be due to higher maternal weights and more comorbidities in our population that work against the chance for a successful result. As those that had an informative redraw weighed slightly less than those with uninformative redraws and had no medication use or comorbidities, it seems these are important factors.

Uptake of diagnostic testing was low in this cohort, and three patients were lost to follow-up after normal MSS. Though used by these patients for reassurance, it should be

noted that MSS is not the most appropriate or informative follow-up. There is an increased risk for all aneuploidy with a LFF result, and in the case of these patients, they were only screened for T21 and T18 through quad screening.

Four patients, including one with ultrasound findings, were lost to follow-up. PH-USC 1 received two no calls beginning at 24 weeks gestation. Fetal fraction was not given on either report. Her fetus had unilateral club foot and CPCs identified on ultrasound, however, she declined all further testing and screening and could not be reached when contacted for our study.

PH-USC 63 was successfully contacted for a phone interview. She was 43 years old at delivery and weighed 280 pounds at the time of screening. She received two LFF results from Natera beginning at 14 weeks. She reported that her daughter was diagnosed with transposition of the great arteries (TGA) at birth but is otherwise normal. We were not able to make a classification based on the lack of postnatal genetic testing.

We could confirm that two of our patients with abnormalities ultimately had non-aneuploid outcomes. PH-USC 10 had four LFF results and a positive MSS for T21 before receiving a low risk NIPS result at 20 weeks. She weighed 240 pounds, was a lovenox user, and also suffered autoimmune disease. Her fetus demonstrated IUGR and oligohydramnios on ultrasound, but ultimately had a normal female karyotype. No CMA was performed. PH-USC 71, weighing 149 pounds, had one LFF result at 10 weeks and demonstrated an increased nuchal translucency (NT) during her late-trimester scan. She elected to pursue CVS which revealed a normal female karyotype and microarray. A second trimester MS-AFP also returned normal. These two patients are similar to the

T18/T13 patients with abnormalities but normal karyotypes, reiterating the significance of diagnostic testing for confirming or ruling out aneuploidy.

Two of the patients lost to follow-up did not have clear reasons for LFF. PH-USC 11 received two no calls beginning at 13 weeks. Fetal fraction was not given on either report. She weighed 188 pounds and had no comorbidities or medication use. Her second trimester ultrasound was unremarkable, and she also had normal MSS before being lost to follow-up. This was uninformative as MSS only screens for trisomies 21 and 18. PH-USC 74 had two no calls beginning at 12 weeks. Fetal fraction was not given on her report. She weighed 158 pounds and also had no comorbidities or medication use. An early second trimester scan was unremarkable before she was lost to follow-up.

#### **2.5.10 High risk for triploidy, trisomy 18, or trisomy 13 due to LFF**

High risk for Triploidy, Trisomy 18, or Trisomy 13 due to LFF made up 10.5% of our abnormal results (n=19). This result is generated after the laboratory cannot correlate low fetal fraction with maternal weight, maternal age, or gestational age. Indeed, all three means of these categories fell below what was observed in general LFF results, which is consistent with data produced by the performing laboratory (Benn et al., 2019). Similar to T13, most declined diagnostic testing in the absence of ultrasound abnormalities. This was surprising given the complexity of this result type as well as the poor prognoses for all three conditions. The relationship between ultrasound findings and uptake of diagnostic testing most resembled T18, as two of the three patients who elected amniocentesis had abnormalities.

The unique circumstances of two cases further emphasized the importance of diagnostic testing, as they ended up with findings outside the scope of their results. PH-USC 29 opted for MSS that indicated a 1/10 chance for T21, and subsequent amniocentesis confirmed a 47,XX,+21 karyotype. This was a surprising finding given the high sensitivity for T21 on NIPS, however, as part of this result type, she was not given a result for chromosome 21. PH-USC 92 received her abnormal result at 16 weeks, and ultrasound at that time revealed an EIF and a ventricular septal defect (VSD). Amniocentesis ruled out aneuploidy but incidentally found a maternally-inherited duplication of 20p13. Again, her case is an example of how ultrasound abnormalities can be used in risk assessment for an abnormal result, but ultimately diagnostic testing is essential for confirming or ruling it out.

Though Natera recommends against a redraw for this type of result, seven patients attempted. Nearly half of them were successful in getting a low risk result the second time, and that number is in agreement with the rate of successful LFF redraws. This scenario presents a couple of possibilities: 1) the pregnancy is truly low risk, or 2) there is another genetic aberration, potentially one not covered by the scope of the test. As we saw in the case of PH-USC 29, whose pregnancy with T21 was missed by Natera calling this result, diagnostic testing was the most informative next step in risk assessment. Patients opting to redraw should be cautioned on the limitations and potential for false reassurance in receiving a low risk result.

#### **2.5.11 No call- uninformative DNA pattern**

A no call- UDP result made up 5.5% of our results (n=10). Out of all no call results, uptake of diagnostic testing was highest in this category, and all who opted for

prenatal diagnosis did so in the absence of ultrasound abnormalities. As a result, three genetic aberrations across four abnormal outcomes were identified.

PH-USC 13 was a 28 year old G2P1001 who had two UDP results in the second trimester. Ultrasounds revealed a cystic hygroma (resolved), Dandy Walker malformation, overriding aorta, cleft lip, hand/foot syndactyly, hypertelorism, and a short philtrum. The pregnancy ended in demise at 24 weeks. Postnatal studies revealed a normal 46,XY karyotype. Reflex to whole exome sequencing (WES) revealed a maternal variant in *FANCD2* and a paternal variant in *WASHC5*. These changes were classified as VUS; therefore, conclusions related to the observed phenotype were not made.

PH-USC 47 was a 25 year old G2P0010 who received a UDP result at the end of her first trimester. She opted for amniocentesis that returned a 46,XY karyotype, however, maternal cell contamination studies revealed XXX mosaicism. She was counseled that this was the most likely explanation for her UDP result.

PH-USC 57 was a 31 year old G3P0020 that received two UDP results in the second trimester. In the presence of a normal ultrasound, she declined diagnostic testing and instead opted for MSS that was normal. The pregnancy ended in demise at 20 weeks and no further testing was performed. Similar to patients receiving general no call- LFF results, this patient opted for MSS as the next step in risk assessment, and likely used the normal results for reassurance. It should be noted that MSS was not the most appropriate or informative follow-up in this result type, either, as she was provided risk assessment for only two chromosome conditions. Interestingly, the patient has a diagnosis of focal segmental glomerulosclerosis and is status-post unilateral kidney transplant. Maternal kidney disease has been described in correlation with abnormal NIPS results, especially



in the setting of a transplant, but not necessarily fetal demise (Neufeld-Kaiser, Cheng, & Liu, 2015). Studies on the fetus would have been most informative, however, it may have been appropriate to also offer karyotype and microarray to the patient given her history of two prior losses.

PH-USC 85 was a 16 year old G1P0 who received a UDP result late in the second trimester after polyhydramnios and dilated bowel were identified on ultrasound. She declined diagnostic testing and opted for NIPS on a genome-wide platform as well as expanded carrier screening, both of which returned normal. The pregnancy ended in demise at 35 weeks. It was later determined that this patient's pregnancy was the result of sexual assault by a first-degree male relative. Consanguinity could be the explanation for the uninformative result as well as the poor outcome of the fetus.

Overall, three of our four cases of interest had outcomes concurrent with what has been put forth as explanations for UDP results. Maternal genetic aberrations, maternal comorbidities, and consanguinity have all been presented as explanations for this type of no call. Consideration of PH-USC 13's two abnormal results, remarkable ultrasounds, and poor fetal outcome suggests that an underlying genetic condition is likely responsible, however, testing was not able to definitively identify it.

Compared to patients receiving other types of no call results, patients with UDP results had pregnancies associated with more abnormalities and/or adverse outcomes. This suggests that diagnostic testing is the most informative follow-up in the setting of this result. This includes maternal testing, as one of our significant outcomes related to a maternal diagnosis. It is also telling that the two patients opting for redraw received a

second failure, suggesting that waiting for more advanced gestational age may not be the solution that can sometimes apply to LFF results.

#### **2.5.12 No call- triploidy, vanishing twin, or unrecognized multiple gestation**

In our study, 2.8% of our patients received this type of result, which is generated after the detection of three DNA patterns (n=5). We were able to identify two patients with outcomes related to this call. PH-USC 12 was a 25 year old G2P1001 who received her result after ultrasound revealed a placental mass. As she also had a normal-appearing male fetus, the mass was felt to be a molar pregnancy and the reason for the abnormal result.

PH-G 7 was a 36 year old G4P2012 who received an abnormal result at 22 weeks. A genetic counselor at the performing laboratory inquired with the clinician as to whether or not the patient was pregnant with twins. As she was late to prenatal care and had not yet had an ultrasound, this was unknown. Subsequent ultrasound revealed twins, providing the reason for the abnormal result.

Outcome performance for this result type is difficult to adequately assess due to the possibility of vanishing twins. At least from our data, however, there were no adverse fetal outcomes.

#### **2.5.13 Other results**

Other results categorized by multiple abnormalities or other types of no calls, including those on a genome-wide platform (8.3%, n=15). For this cohort, uptake of maternal chromosome analysis was higher than prenatal diagnosis, despite only two results specifically indicating a maternal abnormality. This is similar to the cohort of patients screen positive for a microdeletion, in which the distinction between a maternal

or fetal result was helpful in post-test counseling and ultimately, patients choosing their follow-up management. As a result, all of our diagnoses were made in mothers.

PH-USC 61 was a 24 year old G4P1021 who received a no call- abnormal maternal X result from Natera at 12 weeks gestation. She opted for chromosome analysis which returned a mosaic 45,X karyotype, a likely explanation for her history of pregnancy loss. At the time of our project's completion, she was still pregnant with an apparently normal male fetus.

PH-USC 142 was a 34 year old G2P1001 who received a no call- atypical finding result at 12 weeks gestation. This no call result generated by Natera did not specify suspected maternal or fetal. A phone call to a genetic counselor at Natera revealed chromosome 13 as the region of interest. In the absence of ultrasound abnormalities, the patient opted for maternal chromosome analysis which returned a 13q12.12 deletion (VUS). At the time of our study's completion, she was still pregnant and had declined diagnostic testing for her female fetus. This case reiterates the significance of calling the laboratory for more information. The patient was able to learn the reason for her abnormal result and make informed decisions on follow-up and management.

PH-USC 145 was a 28-year-old G2P1001 who received a no call- atypical sex chromosomes result from Natera at 13 weeks gestation. The laboratory report did not specify suspected maternal or fetal. Similar to PH-USC 142, this patient opted for maternal chromosome analysis in the absence of ultrasound abnormalities. Karyotype revealed an unbalanced translocation between chromosomes Xq and 3q. This rearrangement typically results in normal females due to X-inactivation, but features such as premature ovarian failure have been reported. It is lethal in males. At the time of our

project's completion, she was still pregnant and had declined diagnostic testing for her female fetus.

We also had several patients that received double screen positive results. In the cases of two patients, their pregnancies ended in IUFD without further testing. PH-USC 140 was a 44-year-old G3P2002 who received one result from Progenity indicating both T18 and Monosomy X. When she presented to our clinic at 13 weeks, it was discovered that the pregnancy had ended in demise. Given the screen positives and loss, it is likely that the fetus had one or both of these conditions. PH-G 29 was a 45 year old G1P0 who received separate abnormal results from Natera: first, a high risk for Triploidy, T18, or T13 due to LFF at 11 weeks gestation, and second, a screen positive T21 at 14 weeks. The fetus showed a single umbilical artery before miscarrying at 22 weeks. If the fetus indeed had T21, it would be the second in our data set that was initially called high risk for Triploidy, T18, or T13 due to LFF.

We also had three patients who all received both a no call- LFF and high risk for Triploidy, T18, or T13 due to LFF result from Natera. In each case, the general LFF call resulted first. None of the patients were AMA, and their screening was performed within the specified timeframe. The mean maternal weight was 239 pounds, suggesting increased body habitus as a contributing factor. LFF may have been called initially, and then the high risk for Triploidy, T18, or T13 algorithm was triggered as the result of all patients being below AMA and attempting their redraws well into their second trimesters. This theory is based on the observation that waiting for more advanced gestational age can sometimes “correct” issues from borderline maternal weights that initially prohibit a result (Benn et al., 2018).

Within this same cohort of two different LFF results, the case of PH-USC 60 was interesting. She was a 30 year old G2P1001 that was born with bilateral syndactyly of her hands and feet in addition to shortened and absent long bones. She had also been newly diagnosed with hypertrophic cardiomyopathy. She reported that a genetic evaluation in childhood attributed her features to amniotic band syndrome. In our practice, she was counseled that this was unlikely and was recommended to have a second evaluation, however, she declined. Given her unique features it is possible that she has an underlying condition that contributed to her abnormal results. At the close of our study, she had just given birth to a normal male infant.

Several patient outcomes were unavailable at the close of our study. Two of these results, PH-G 24 and PH-G 28, were generated by Counsyl's genome-wide platform and involved chromosome 19 aneuploidy. Both patients were followed with normal ultrasounds until 20 and 38 weeks, respectively, before they were lost to follow-up. It is possible these results were placental in origin, as full chromosome 19 aneuploidy would not be compatible with life. Another patient, PH-USC 49, received two no call- multiple aneuploidy results from Progenity in her second trimester. The laboratory report did not specify chromosomes of interest. Tragically, this patient died of cardiac arrest and her daughter was delivered via emergency cesarean at 27 weeks gestation. We were unable to determine if the infant had complications beyond that of prematurity.

## **2.6 Limitations and future research**

### **2.6.1 Limitations**

Our patient population was primarily composed of Caucasian and African American individuals and our data originates from two high risk obstetric clinics in South

Carolina. This uniformity in demographics may mean our performance data could not extend to other populations.

Overall, outcome data was limited by a significant portion of our patients being lost to follow-up or having IUFDs without testing to confirm a diagnosis. Though many of the pregnancies ending in demise had abnormalities related to their abnormal results and were likely true positives, we were unable to classify them as such due to the lack of confirmatory testing.

We were unable to find commonalities in patients that might serve as novel correlations for LFF results, though we observed many well-described associations: increased maternal weight; comorbidities such as diabetes, autoimmune disease, and hypertension; maternal use of blood thinners; and early gestational age.

Considering genetic testing and management, we identified cases that would have benefitted from both karyotype and microarray analysis. This came to attention when we discovered that a screen positive T21 was actually a partial duplication of chromosome 21. We also had a non-AMA patient with two pregnancies screen positive for T21, and she only had karyotype. As many of our false positives were classified after karyotype-only, underlying copy number variants cannot be ruled out.

Frequency of ultrasound abnormalities was an important statistic for each of our result types. As several patients were not followed in our clinic throughout the duration of their pregnancies, we were often reviewing only one or two ultrasound reports that may not have represented the best window for visualizing abnormalities. It is possible that ultrasound findings were detected after patients' last visits to our clinic.

We also had a small sample for certain conditions, namely XXY, XYY, XXX, and microdeletions. This was not surprising given that the detection rates for these conditions are not well established, and many patients and providers opt out of microdeletion screening. Additionally, several of our no calls were newer types that began resulting during the course of our study, which meant we had a small sampling of each. Completing this study over a longer period of time would have produced a larger sample of these underrepresented result types.

Finally, while most of our outcomes were classified by data in the EMR, several were by patient report. We did not require reports from patients to confirm or rule-out a diagnosis; therefore, it is possible that we unintentionally factored in false information to our data.

### **2.6.2 Future research**

Other clinics are encouraged to track their own abnormal screening outcome data in order to evolve the definition and outcomes of no call results. Future research could also target conditions we had low representation of, such as SCAs and microdeletions. Though included on NIPS platforms for some time, they are still underperforming compared to autosomal aneuploidies. More research could add to the continuing conversation on appropriate management and follow-up.

It has been long recognized that presumed false positive serum analyte screening via normal ultrasound or declined diagnostic testing is associated with an increased risk for a poor perinatal and/or maternal outcome. The same association has been suggested with abnormal NIPS (both presumed true and false positives), LFF, and other no call results for other reasons. While the numbers in the current study are small, the data does

suggest an increased risk for poor perinatal outcomes (growth abnormalities, structural abnormalities, and fetal demise). Given these results, several clinical recommendations can be considered as part of routine obstetrical care in these patients. These include serial ultrasound examinations for growth, assessment of anatomy and fetal viability, and consideration of antenatal testing for fetal wellbeing in the third trimester.

As data become available from future studies, these management recommendations can be further refined.

Though not the primary goal of the study, our data in large part was an evaluation of patients' decision-making and handling of abnormal results. Generalizations are difficult to make knowing each patient's decisions are highly situational and specific to their own wants, needs, and values. As a result, future research could also include their perspectives on receiving abnormal results, and what factors are important in their decision-making process moving forward.

## **2.7 Conclusion**

As coverage of NIPS platforms continues to evolve, research on performance and outcomes will need to stay active as professional guidelines seek to establish clear follow-up and management recommendations. Data from clinics contribute to outcome statistics that are usually supplied by performing laboratories. As such, data from individual clinics limits bias in what laboratories publish about their work, and it challenges them to continue improving NIPS so that it is the most accurate and specific it can be. Clinics also benefit from considering their outcome data and using that information to develop management guidelines for results that will undoubtedly occur again, as we did in our study. While research should continue both in frequency and

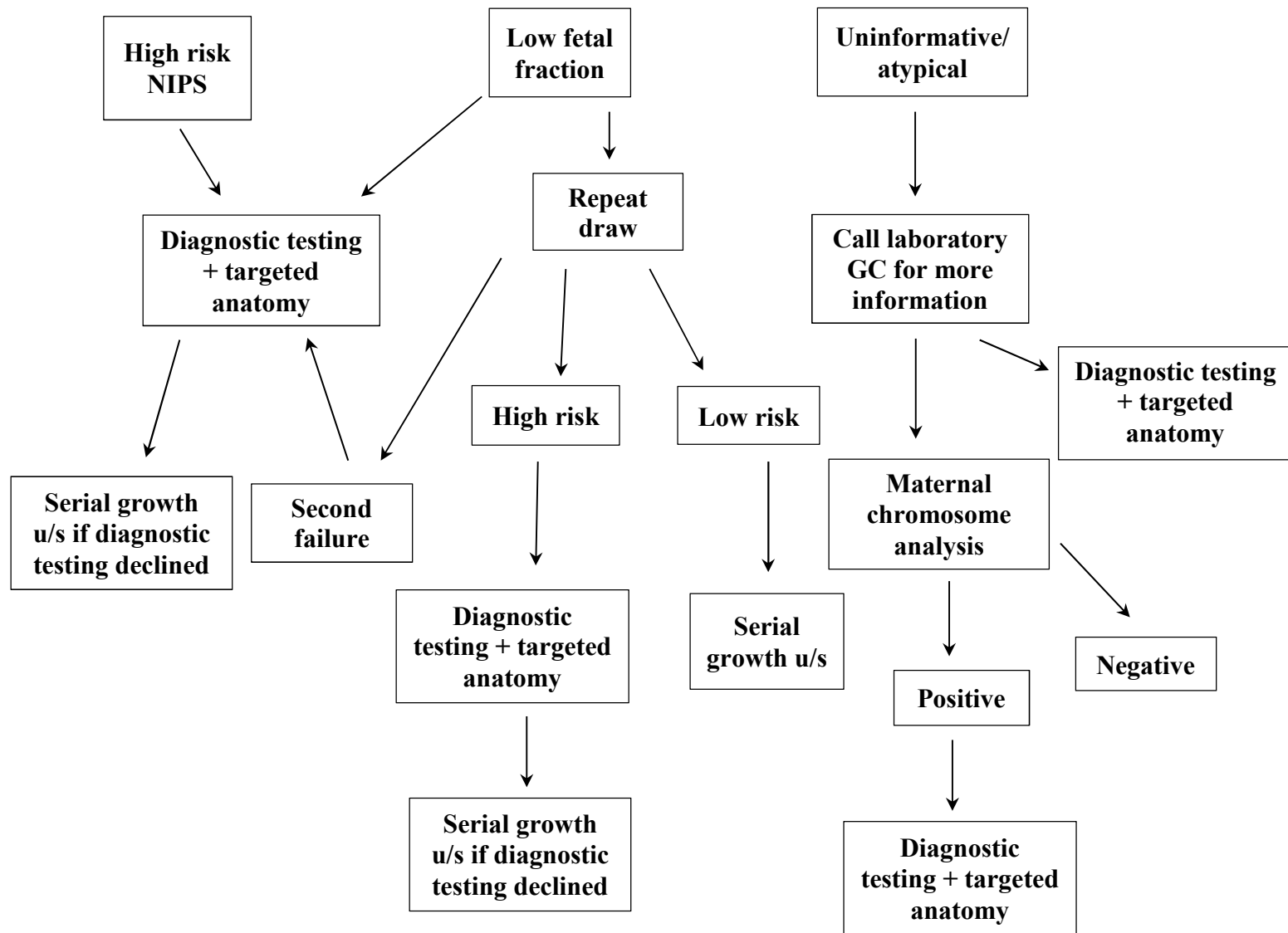


expansion, this outcome data is valuable not only for our own practice but also other genetic counselors and MFMs guiding patients through abnormal results.

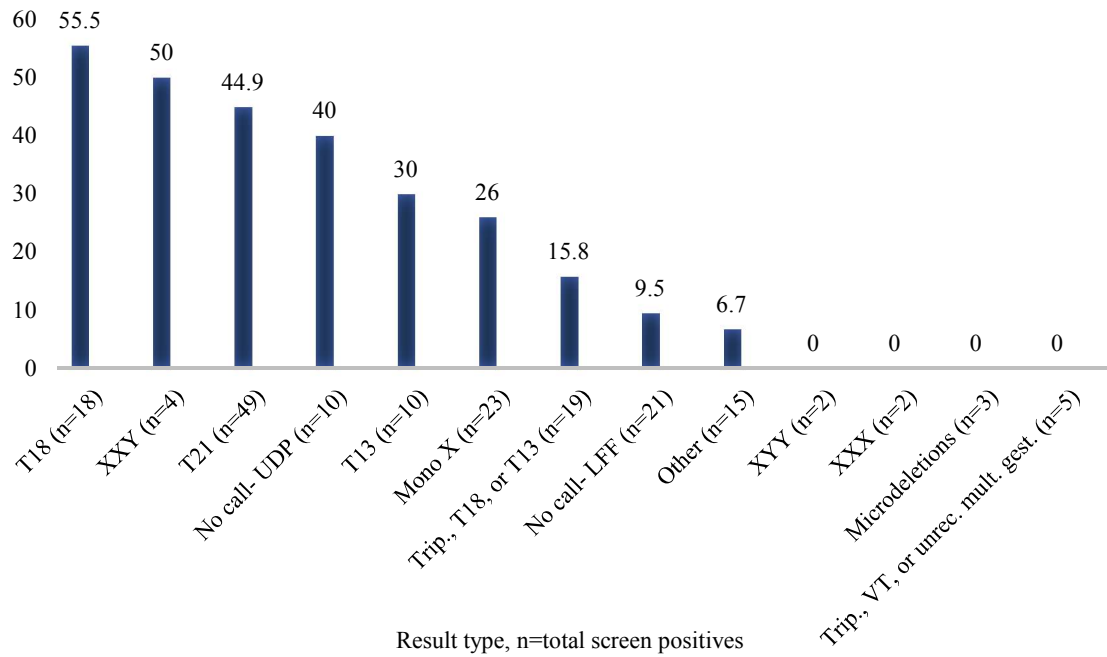
## CHAPTER 3

### CONCLUSION

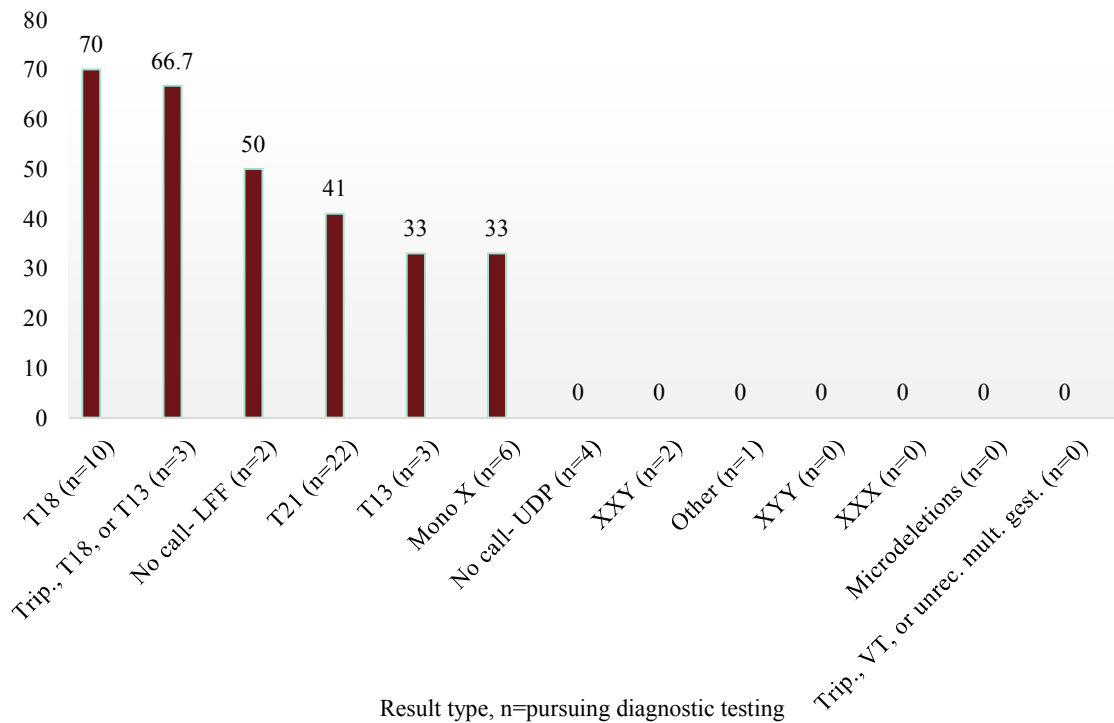
As coverage of NIPS platforms continues to evolve, research on performance and outcomes will need to stay active as professional guidelines seek to establish clear follow-up and management recommendations. Data from clinics contribute to outcome statistics that are usually supplied by performing laboratories. As such, data from individual clinics limits bias in what laboratories publish about their work, and it challenges them to continue improving NIPS so that it is the most accurate and specific it can be. Clinics also benefit from considering their outcome data and using that information to develop management guidelines for results that will undoubtedly occur again, as we did in our study. While research should continue both in frequency and expansion, this outcome data is valuable not only for our own practice but also other genetic counselors and MFMs guiding patients through abnormal results.



**Figure 3.1** Follow-up recommendations by result type



**Figure 3.2** Uptake of fetal diagnostic testing (%)



**Figure 3.3** Frequency of ultrasound abnormalities in those pursuing fetal diagnostic testing (%)

**Table 3.1** Patients with abnormal results and ultrasound findings, but normal karyotypes

<b>Patient</b>	<b>Age</b>	<b>Laboratory</b>	<b>Result/PPV</b>	<b>U/s findings</b>	<b>Next steps/outcome</b>
<b>PH-USC 4</b>	38	Progenity	T18, 49%	CPCs, EIF	Amnio- 46,XX
<b>PH-USC 6</b>	28	Progenity	T13, 8%	Renal pyelectasis	Postnatal karyotype- 46,XY
<b>PH-USC 10</b>	41	Natera	No call- LFF	IUGR, oligohydramnios	Postnatal karyotype- 46,XX
<b>PH-USC 71</b>	34	Progenity	No call- LFF	Increased NT	CVS- 46,XX
<b>PH-USC 92</b>	33	Natera	High risk for Triploidy, T18, or T13 due to LFF	EIF, VSD	Amnio- 46,XY 20p13 duplication (VUS-maternal)
<b>PH-USC 125</b>	19	Natera	No call- triploidy, VT, or unrecog. mult. gest.	EIF	Amnio- 46,XX

**Table 3.2** Maternal diagnoses after abnormal NIPS results

<b>Patient</b>	<b>Laboratory</b>	<b>Result</b>	<b>Follow-up</b>	<b>Outcome</b>
<b>PH-USC 47</b>	Natera	No call- UDP	Amnio	MCC studies= XXX mosaicism
<b>PH-USC 61</b>	Natera	No call- maternal X abnormality	Karyotype	Mosaic 45,X
<b>PH-USC 96</b>	Natera	22q11.2 deletion (suspected maternal)	CMA	Confirmed 22q11.2 deletion (pathogenic)
<b>PH-USC 142</b>	Natera	No call- atypical finding	Karyotype/CMA	13q12.12 deletion (VUS)
<b>PH-USC 145</b>	Natera	No call- atypical sex chromosomes	Karyotype	Unbalanced 3q;Xq translocation

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## APPENDIX A

### LETTER INVITATION TO PARTICIPANTS

Dear Ms. X:

My name is Olivia Kesler, and I am a senior genetic counseling student at the University of South Carolina. I am conducting a research study as part of the requirements of my Master's degree, and I would like to include your participation.

I am studying the pregnancy outcomes of abnormal NIPS, or non-invasive prenatal screening results. My training program is in the same office you met with a genetic counselor about your abnormal results sometime during 2018 or 2019. This screening seeks to inform women if they have a higher risk of having a child with certain genetic conditions such as Down syndrome. Occasionally, the screening may also fail to give a result.

In particular, you will be asked questions about the outcome of your pregnancy. You may have had further testing that was done to confirm or rule out what the screening said could be a possibility for your pregnancy. You do not have to answer any questions that you do not wish to answer. It is expected that answering these questions would not take longer than 5-10 minutes of your time.

I plan to contact you by phone on Thursday, November 21, 2019, between 12 PM – 5 PM. If you do not wish to participate, please contact the genetic counseling office by phone at 803-545-5775 to opt out.

Participation is confidential. Study information will be kept in a secure location at the University of South Carolina. The results of the study may be published or presented at professional meetings, but your identity will not be revealed.

We will be happy to answer any questions you have about the study. You may contact me by phone at 803-545-5775, by email at [olivia.kesler@uscmed.sc.edu](mailto:olivia.kesler@uscmed.sc.edu), or my faculty advisor, Jessica Fairey, by phone at 803-545-5746, or by email at [jessica.fairey@uscmed.sc.edu](mailto:jessica.fairey@uscmed.sc.edu).

With kind regards,  
Olivia Kesler

APPENDIX B

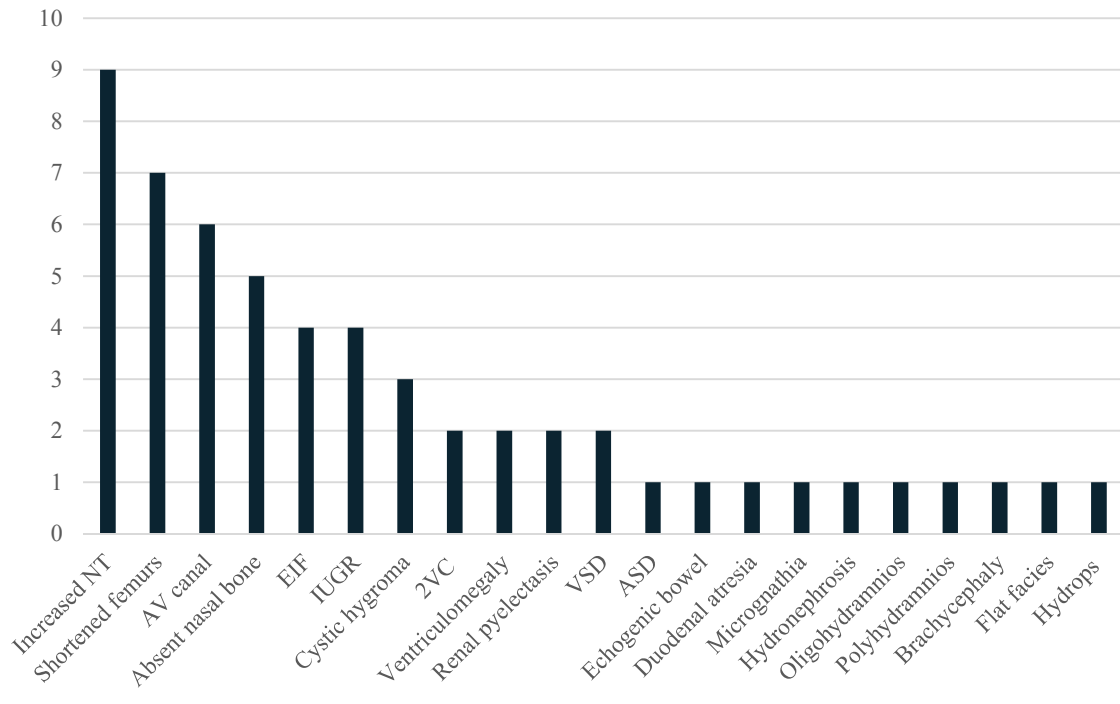
ALL RESULTS AND OUTCOMES FURTHER DELINEATED BY LABORATORY

Results	True positive	False positive	IUFD	Unknown/ lost to follow-up	Maternal diagnosis	Total
<b>Counsyl</b>						
Autosomal aneuploidy (n=6)	4	0	2	0	0	6
SCA (n=1)	0	0	0	1	0	1
LFF (n=0)	0	0	0	0	0	0
No call/other (n=2)	0	0	0	2	0	2
<b>Integrated Genetics</b>						
Autosomal aneuploidy (n=16)	12	0	4	0	0	16
SCA (n=3)	0	0	2	1	0	3
LFF (n=1)	0	1	0	0	0	1
No call/other (n=0)	0	0	0	0	0	0
<b>Invitae</b>						
Autosomal aneuploidy (n=1)	1	0	0	0	0	1
SCA (n=1)	0	0	0	1	0	1
LFF (n=0)	0	0	0	0	0	0
No call/other (n=0)	0	0	0	0	0	0
<b>LabCorp</b>						
Autosomal aneuploidy (n=3)	1	1	1	0	0	3
SCA (n=1)	1	0	0	0	0	1
LFF (n=2)	0	1	0	1	0	2
No call/other (n=0)	0	0	0	0	0	0
<b>Myriad</b>						
Autosomal aneuploidy (n=2)	2	0	0	0	0	2
SCA (n=0)	0	0	0	0	0	0

LFF (n=0)	0	0	0	0	0	0
No call/other (n=0)	0	0	0	0	0	0
<b>Natera</b>						
Autosomal aneuploidy (n=21)	15	1	2	3	0	21
SCA (n=8)	1	1	4	2	0	8
LFF (including high risk algorithm) (n=32)	4	23	0	5	0	32
Microdeletions (n=3)	2	0	0	0	1	3
No call/other (n=25)	3	14	2	2	4	25
<b>Progenity</b>						
Autosomal aneuploidy (n=23)	15	6	0	2	0	23
SCA (n=17)	2	7	3	5	0	17
LFF (n=3)	0	3	0	0	0	3
No call/other (n=3)	1	0	1	1	0	3
<b>Quest</b>						
Autosomal aneuploidy (n=1)	1	0	0	0	0	1
SCA (n=0)	0	0	0	0	0	0
LFF (n=0)	0	0	0	0	0	0
No call/other (n=0)	0	0	0	0	0	0
<b>Roche</b>						
Autosomal aneuploidy (n=4)	4	0	0	0	0	4
SCA (n=0)	0	0	0	0	0	0
LFF (n=2)	0	0	0	2	0	2
No call/other (n=0)	0	0	0	0	0	0
<b>Total results</b>	<b>69</b>	<b>58</b>	<b>21</b>	<b>28</b>	<b>5</b>	<b>181</b>

## APPENDIX C

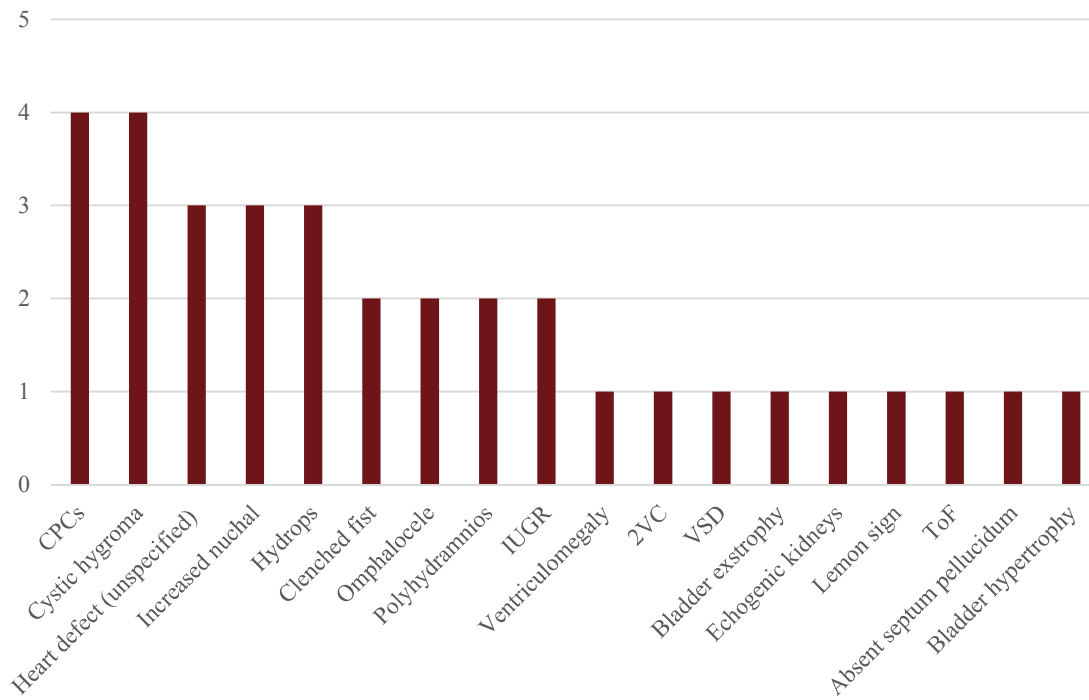
### ABNORMALITIES FOR T21 TRUE POSITIVES





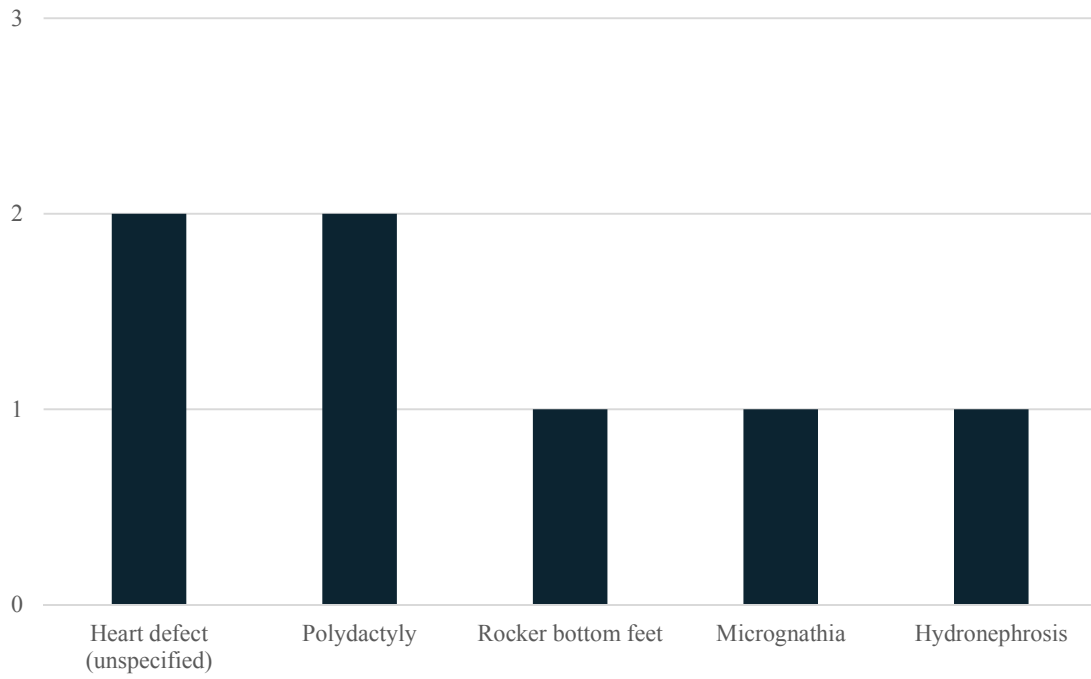
## APPENDIX D

### ABNORMALITIES FOR T18 TRUE POSITIVES



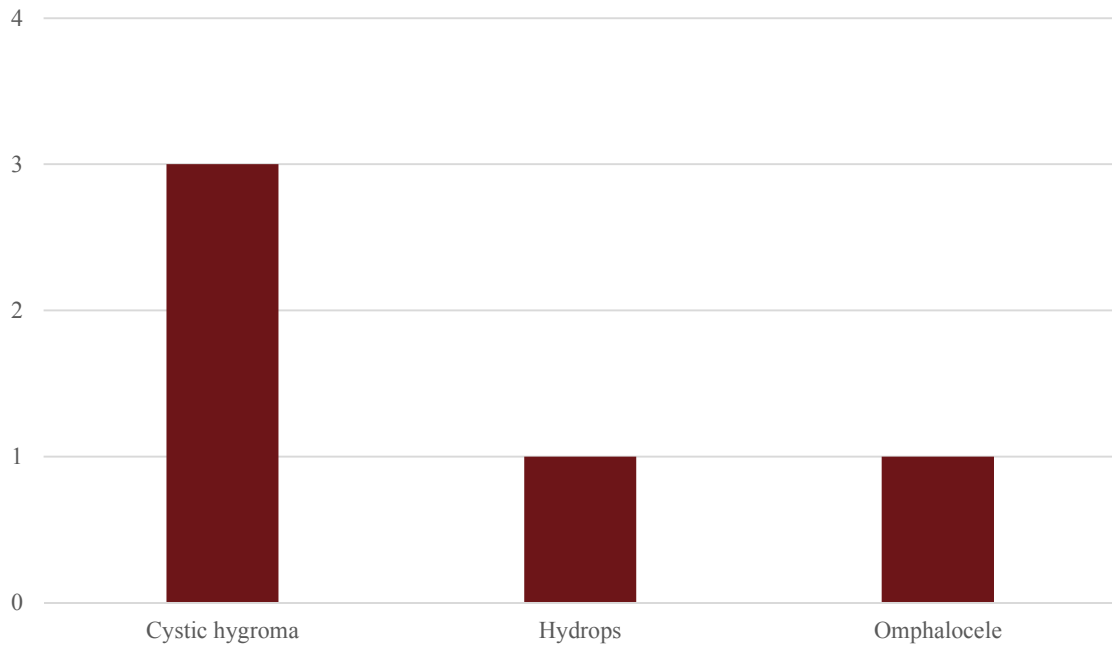
APPENDIX E

ABNORMALITIES FOR T13 TRUE POSITIVES



APPENDIX F

ABNORMALITIES FOR MONOSOMY X TRUE POSITIVES



## APPENDIX G

### ABNORMALITIES FOR HIGH RISK LFF TRUE POSITIVES

