5-2018

CURRENT GENETIC COUNSELING PRACTICE FOLLOWING POSITIVE NON-INVASIVE PRENATAL TESTING FOR SEX CHROMOSOME ABNORMALITIES

Lauren Fleddermann

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by

Lauren Elizabeth Fleddermann, B.A.

APPROVED:

_____
Claire Singletary, MS, CGC
Thesis Chair

_____
Lara Friel, MD

_____
Lauren Murphy, MS, CGC

_____
David Rodriguez, MD

_____
Blair Stevens, MS, CGC

APPROVED:

_____
Dean, The University of Texas
MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences

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A

THESIS

Presented to the Faculty of

The University of Texas

MD Anderson Cancer Center UTHealth

Graduate School of Biomedical Sciences

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

by

Lauren Elizabeth Fleddermann, B.A.
Houston, Texas

May, 2018

CURRENT GENETIC COUNSELING PRACTICE FOLLOWING POSITIVE NON-INVASIVE PRENATAL TESTING FOR SEX CHROMOSOME ABNORMALITIES

Lauren Elizabeth Fleddermann, B.S

Thesis Chair: Claire Singletary, MS, CGC

The purpose of this study was to describe current prenatal and pediatric genetic counseling practice following a non-invasive prenatal testing (NIPT) result positive for a sex chromosome abnormality (SCA). The positive predictive value for SCA with NIPT is lower than seen for Trisomy 21 due to natural loss of the X chromosome from maternal cells during aging, confined placental mosaicism, and undiagnosed maternal sex chromosome abnormality. Except for 45,X, individuals with SCA usually have no ultrasound or postnatal findings. This makes follow-up for unresolved positive NIPT necessary; however, there are currently no clinical guidelines. This study used a prospective anonymous questionnaire to survey 176 prenatal and pediatric genetic counselors. Greater than 70% of pediatric respondents and >80% of prenatal respondents were somewhat or extremely comfortable counseling patients about SCAs. However, prenatal respondents in the field for <5 years were significantly less comfortable counseling about every condition except 45,X ($p<0.02$). A majority of prenatal respondents always offered diagnostic testing (>88%) and anatomy ultrasound (~90%), but the percent consistently offering maternal karyotype (22-52%) and postnatal evaluation (28-87%) varied. Maternal karyotype was offered more often when NIPT was positive for 45,X or 47,XXX and patients had normal diagnostic testing ($p<0.023$) or declined testing ($p<0.019$). Offer of postnatal evaluation was more likely when diagnostic testing was declined ($p<0.01$). A majority of pediatric providers always offered

the child a karyotype postnatally (>72%) but the percent offering maternal karyotype (6-46%) varied widely. With the current inconsistencies, many newborns with undiagnosed SCAs who could benefit from growth hormone therapy, early intervention, and/or targeted surveillance may be missed. There is a need for professional guidelines to help improve clinical care for patients with NIPT results positive for SCAs.

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INTRODUCTION

Research has been ongoing for decades to create a highly sensitive non-invasive screening method for fetal chromosome abnormalities during pregnancy. Following the identification of fetal leukocytes in the maternal blood stream during pregnancy in the late 1960s, there was hope that these cells could be used for prenatal screening, and potentially diagnosis, of chromosome abnormalities (1). However, further research discovered that these fetal cells persist in the maternal blood stream for years following the birth of a baby. This finding decreased the clinical utility of fetal leukocytes since it is technically difficult to determine which cells came from which pregnancy in a multiparous woman (2). Therefore, scientists continued to look for other ways to use the maternal bloodstream to learn about fetal genetic characteristics.

In the late 1990s, it was determined that cell-free DNA of placental origin is present in the maternal bloodstream during pregnancy at an average concentration of 10% (3, 4). This discovery of cell free DNA (cfDNA) led to a flurry of experimentation to determine whether this could be used to provide meaningful information about genetic characteristics of a pregnancy in a non-invasive manner.

It has since been determined that cfDNA can be reliably used to screen for fetal aneuploidy using several techniques (5). Testing using cfDNA is referred to by several names including cell free DNA, non-invasive prenatal screening (NIPS), and non-invasive prenatal testing (NIPT). CfDNA is largely placental in origin, and therefore may not be representative of the complete genetic composition of the fetus in some cases (6). For this reason, NIPT is still considered a screening test and diagnostic testing is recommended following a positive result. When laboratories first introduced NIPT for high risk women in

2011, the test only screened for trisomy 21. Screening for trisomies 18, 13 and the presence or absence of Y chromosome was introduced in 2012.

While labs offering NIPT often quote their own statistics for sensitivity and specificity, a 2015 meta-analysis by Gil et al. determined a detection rate of 99.2% for Trisomy 21, 96.3% for Trisomy 18, and 91% for Trisomy 13. The false positive rates from this analysis were <0.1%, 0.13%, and 0.13% respectively (7). Therefore, NIPT has a higher detection rate and lower false positive rate compared with other screening tests for aneuploidy, specifically first trimester screening and quadruple marker screening (8). This makes it an alluring test for soon to be parents who desire the most accurate information without the risk of miscarriage associated with invasive testing.

Since 2013, NIPT has included sex chromosome abnormalities as well as the three common autosomal aneuploidies (9). Sex chromosome abnormalities (SCA) are genetic conditions in which an individual has an abnormal number of sex chromosomes. As a group, SCAs are the most common chromosome abnormalities present at birth, occurring in every 1 in 300-400 live births (10). The most common are 45,X (Turner Syndrome) and other karyotypes, and 47,XXY (Klinefelter Syndrome). Other abnormal sex chromosome complements include 47,XXX and 47,XYY. The risk of having an affected child with 47,XXX and 47,XXY increases as maternal age increases like the maternal age effect seen for the common autosomal aneuploidies. However, 45,X and 47,XYY are thought to occur with a similar frequency throughout the population. While not associated with increased paternal age, 100% of 47,XYY cases and about 50-66% of 45,X cases derive from paternal origin (11).

Screening for SCAs by NIPT is challenging for several reasons, including fetal and maternal factors. Approximately 1-2% of pregnancies have confined placental mosaicism,

when a chromosome complement in all or part of the placenta is discordant from that of the fetus. Therefore, when evaluating cfDNA from the placenta, there is a risk to obtain a false positive or false negative result due to confined placental mosaicism (12). A 2015 study by Malvestiti et al. found that of pregnancies with an abnormal CVS for a SCA, 33% had fetal involvement on amniocentesis while 67% were not confirmed in amniocytes (13). Discrepancies have been found to be true for other chromosome conditions as well.

Additional challenges unique to SCAs include maternal factors, such as the loss of an X chromosome from maternal cells as a natural part of aging, and maternal SCA mosaicism or full maternal SCA. Since both maternal and placental DNA are examined by NIPT, any differences in maternal DNA could lead to an abnormal result. Maternal cells have been shown to naturally lose an X chromosome as a woman ages, which could skew the amount of X chromosome available for quantification (14). A 2007 study looking at the frequency of X chromosome loss in women from birth to age 80 found a frequency of X chromosome loss of approximately 1% for women under age 30, 2% for women at age 40, and 3% for women at the age of 50 (15). In addition, there is evidence to support that most, if not all, women with 45,X have some level of mosaicism (16). About 2% of women with 45,X spontaneously achieve pregnancy and may not know that they have a 45,X cell line. There are also women who have 45,X, 47,XXX or a double aneuploidy (ex. 45,X/47,XXX) but have never had genetic testing and are therefore not aware that they have an extra or missing chromosome. In 2013, Wang et al. found that 8.6% of pregnancies in their sample with NIPT positive for a SCA were due to maternal abnormalities in X chromosome number. Research into these phenomena illustrates how maternal and placental abnormalities can confound NIPT results.

These biologic phenomena increase the false positive rate of the test thereby decreasing the positive predictive value (PPV) of NIPT for SCAs. The sex chromosome abnormalities are quoted to have a PPV of approximately 20-50%, making it difficult to counsel patients with a positive result, many of whom may think that the chances of an affected pregnancy are much higher based on detection rate alone (17). As reported by Gil et al. in their 2015 meta-analysis, 45,X has a detection rate of 90.3% and a false positive rate of 0.23%, while all other sex chromosome aneuploidies had a combined detection rate of 93% and a false positive rate of 0.14%. It is important to note that in the study the confidence intervals for SCA conditions had a wider range than the autosomal trisomies (7).

Following positive NIPT for one of the autosomal aneuploidies, a high-resolution ultrasound is often used to adjust the likelihood of having an affected pregnancy. This is more complicated with SCAs. In 45,X, ultrasound findings may include increased nuchal translucency, cystic hygroma, hydrops, heart defects, and kidney defects. It has been reported that 68% of fetuses with 45,X have some type of sonographic finding (18). While these ultrasound findings increase the likelihood of the fetus having 45,X, the exact diagnosis is based on karyotype results and phenotype and can only be appropriately ascertained postnatally with a physical examination and pelvic ultrasound making it necessary for patients to have appropriate follow up. Other sex chromosome abnormalities often have no ultrasound findings with which to guide the counseling and testing path, but a karyotype is sufficient to diagnose or rule out the condition. If a patient has normal diagnostic testing or declines diagnostic testing, there is ambiguity regarding the reason for a positive NIPT result and the healthcare professional is left in a dilemma as to next steps. Based on the known reasons for a false positive result, further investigation into the

positive result might include offering maternal karyotype or a genetics evaluation after birth with a karyotype on the neonate.

Difficulty in knowing how to counsel patients about a positive NIPT for SCAs is a potential problem currently facing the genetic counseling profession. A 2015 study by Geeter examined genetic counselors' views on the issues surrounding the use of NIPT to screen for SCAs. There was consensus among genetic counselors concerning what was necessary to discuss with patients both in pre- and post-test counseling, including the phenotypic variability seen in these conditions, why a false positive result might occur, and the PPV (19). However, there is a lack of information on what genetic counselors offer patients following a positive NIPT for a SCA, in the absence of diagnostic confirmation, to help determine the likelihood of a true positive or undiscovered mosaicism in a child or woman.

Individuals with SCAs benefit from diagnosis at a young age so that appropriate management via early intervention, hormone treatment, and specialized surveillance can take place leading to better outcomes. Even though not ideal to be uncovered in adulthood, discovering maternal SCA can have important implications for future health, as women with 45,X have increased lifelong risks for hearing loss, aortic root dilation, and fractures, and women with 47,XXX may be at risk of primary ovarian insufficiency (20, 21). As well, individuals with 45,X and 47,XXY are at an increased risk for a pregnancy with a SCA if they are able to achieve pregnancy (22). Having a diagnosis is therefore important for future management and treatment in patients.

As the use of NIPT continues among pregnant women, receiving NIPT results positive for SCAs and dealing with unresolved prenatal testing will continue to be a daily part of prenatal genetic counseling practice, and the number of referrals to medical

genetics postnatally for these cases will increase. Therefore, this study was designed to evaluate current genetic counseling practices in regards to follow up testing and management for patients with positive NIPT results for a SCA.

METHODS

Participants

Eligible study participants included currently practicing genetic counselors in prenatal and pediatric genetics who see patients as part or all of their current position. Genetic counselors who do not see patients in prenatal or pediatrics, or who work in a non-clinical setting, were excluded from the study. Genetic counselors were recruited using the National Society of Genetic Counselors (NSGC) student research eblast which targeted the overall membership, as well as the Pediatric and Clinical Genetics Special Interest Group and Prenatal Special Interest Group. Overall, the survey was sent to 3,535 individuals through the full membership eblast. Using the number of NSGC members listing pediatric or prenatal as their specialty on their NSGC profile, it is estimated that the survey was sent to approximately 1,290 prenatal counselors and 749 pediatric counselors. Overall, there were 176 eligible respondents to the survey giving an estimated response rate of 8.6%. Of the total respondents, 122 were by counselors who exclusively see prenatal patients, 28 were by counselors who exclusively see pediatric patients, and 13 were by counselors who indicated they see both prenatal and pediatric patients.

Instrumentation

The study used a prospective anonymous questionnaire that was created by the authors using the survey software Qualtrics (Appendix A). The survey was made up of three sections. The first section included nine questions about demographic information such as number of years practiced, job setting, specialty, and state of practice. All participants completed the demographic section and were then directed to the appropriate section based on current specialty. Section two was for pediatric genetic counselors and

was made up of 21 questions. Information gathered included number and type of SCA cases seen, comfort level with counseling patients about various SCA indications, and four clinic scenarios which evaluated current practice for different SCA NIPT results. The third section was for prenatal genetic counselors and was made up of 34 questions. Information gathered was similar to section two, including number and type of SCA cases seen, comfort level in counseling patients about SCA conditions, and four scenarios to evaluate current practice for different SCA results. Respondents were not required to answer all questions. For sections two and three, the four scenarios asked about the same SCA conditions in context of the practice area. Scenarios 1 and 2 asked about NIPT positive for 45,X with or without ultrasound or physical findings, scenario 3 asked about NIPT positive for 47,XXX, and scenario 4 asked about NIPT positive for 47,XYY. The 13 respondents who indicated working in both the pediatric and prenatal fields were presented with sections two (pediatric) and three (prenatal) in a randomized fashion. Six respondents received the pediatric section first and seven received the prenatal section first. Participants who completed the survey were given the opportunity to provide their email address in a separate unlinked window for consideration in the drawing of two $50 Visa gift cards.

Procedures

A proposal of the study was approved by the University of Texas Institutional Review Board, which is governed by the Committee for the Protection of Human Subjects (HSC # 17-0586). Following IRB approval, an email containing a description of the study, invitation to participate, and link to the survey was sent to all eligible participants through the NSGC student research eblast. The online survey tool Qualtrics was used to administer the survey via email and collect data. The survey was sent to the full NSGC

membership as well as the Pediatric and Clinical Genetics Special Interest Group and Prenatal Special Interest Group in September and October 2017.

Anonymous survey responses were gathered in password protected Qualtrics software and stored on a secure UT Health computer. Only the principal investigator and co-investigator had access to the stored data. Data did not contain any PHI or other personally identifying information. However, participants who completed the full survey had the option of submitting an email address through a separate unlinked window to be considered for the drawing of one of two $50 Visa gift cards. In total, 114 respondents provided an email address for the gift card drawing. Email addresses were used only for the incentive and will not be used for future contact.

Data Analysis

Data was described using frequencies and percentages. Following initial data collection, scenario-based questions were grouped for comparison. Answers to the scenario questions ranged from 0-100%. The raw data was recategorized into the following five categories: never (0%), rarely (1-33%), sometimes (34-66%), often (67-99%), and always (100%). This recategorization was done to aid in meaningful data interpretation due to the non-normal distribution of the data. Differences in responses by individuals between scenarios was analyzed using the Wilcoxon Signed-Rank Test. Differences in responses for each scenario between respondents in the field for <5 years and those in the field for ≥5 years was analyzed using a Mann-Whitney test. Data was analyzed by Stata (v. 14.1, College Station, TX) using a level of significance at $p<0.05$.

RESULTS

Demographics

In total, 3,535 genetic counselors were invited to take the survey of which approximately 2,039 were eligible to participate. There were 204 responses. Of those responses, 14 were excluded because they were not clinical genetic counselors and 14 more were excluded because they did not see prenatal or pediatric patients. Thus, 176 respondents were eligible to participate in the survey. Of the 176 eligible respondents, 135 indicated that they exclusively see prenatal patients, 28 indicated that they exclusively see pediatric patients, and 13 indicated that they see both prenatal and pediatric patients. Most prenatal counselors worked in a university medical center (42%), had worked in the field for more than 5 years (53%), and saw 11-20 patients per week (56%) (Table 1). Most pediatric counselors worked in a university medical center (51%), had worked in the field for less than 5 years (88%), and saw 1-10 patients per week (74%) (Table 1). Years in a prenatal or pediatric specialty were classified based on whether a respondent had started practicing prior to the introduction of NIPT (≥5 years) or since its introduction (<5 years). Among prenatal counselors, 47% had been in the field for less than 5 years, and 88% of pediatric counselors had been in the field for less than 5 years. Of the 13 respondents who saw both prenatal and pediatric patients, 58% had been seeing prenatal patients for less than 5 years and 50% had been seeing pediatric patients for less than 5 years (Table 1). There were no significant differences between respondents seeing both prenatal and pediatric patients and those seeing patients in only one specialty. Survey respondents were from 41 states.

Table 1: Participant Demographics			
Variable		**n**	**%**
Work Setting (n=176)			
	University medical center	79	44.9
	Private hospital/medical facility	44	25.0
	Public hospital/medical facility	39	22.2
	Physician's private practice	14	8.0
Prenatal Respondents (n=148)			
Years as a prenatal genetic counselor only (n=126)*			
	<5	59	46.8
	>=5	67	53.2
Years as a prenatal genetic counselor for respondents doing both specialties (n=12)*			
	<5	7	58.3
	>=5	5	41.7
Patients seen per week (n=146)*			
	1-10	36	24.6
	11-20	82	56.2
	21-30	22	15.1
	31+	6	4.1
Clinic services offered			
	CVS	123	88.5
	Amniocentesis	137	99.3
	NIPT	141	98.6
Pediatric Respondents (n=41)			
Years as a pediatric genetic counselor only (n=25)*			
	<5	22	88.0
	>=5	3	12.0
Years as a pediatric genetic counselor for respondents doing both specialties (n=12)*			
	<5	6	50.0
	>=5	6	50.0
Patients seen per week (n=39)*			
	1-10	29	74.4
	11-20	10	25.6

* No response: years as a prenatal GC only (n=9), years as a prenatal genetic counselor for respondents doing both specialties (n=1), prenatal patients seen per week (n=2), years as a pediatric genetic counselor only (n=3), years as a pediatric counselor for respondents doing both specialties (n=1), pediatric patients seen per week (n=2)

Table 2: Sex Chromosome Abnormality Cases Seen			
Prenatal		**n**	**%**
SCA case seen? (n=136)	yes	128	94.1
	no	8	5.9
SCA cases seen	45,X	115	89.8
	47,XXY	78	60.9
	47,XXX	67	52.3
	47,XYY	40	31.3
	Other	10	7.8
Pediatric		**n**	**%**
SCA case seen? (n=40)	yes	33	82.5
	no	7	17.5
SCA cases seen	45,X	24	72.7
	47,XXY	21	63.6
	47,XXX	15	45.5
	47,XYY	8	24.2
	Other	2	6.1

Prenatal section

A total of 148 individuals were eligible to take the prenatal portion of the survey. Of these respondents, 94.1% indicated they had seen at least one patient with NIPT positive for a SCA (Table 2). When asked about type of SCA cases seen in the last year, 45,X was the most common answer (89.8%) followed by 47,XXY (60.9%), 47,XXX (52.3%), 47,XYY (31.3%), and "other" SCA results (7.8%), including suspected maternal 45,X mosaicism, non-reportable X, XXY, and reported sex discrepant with ultrasound (Table 2). When asked what their clinic offers to patients, the majority of respondents indicated their clinic offers CVS, amniocentesis, and NIPT to patients (Table 1). NIPT that includes analysis of chromosomes 13, 18, 21, and SCAs was the most commonly used NIPT among respondents, with 22% indicating they offer this option all the time. When seeing a patient with a positive NIPT, 83% of respondents said they always discuss the positive predictive value (PPV). When asked about comfort level in counseling prenatal patients for a positive NIPT for a SCA, greater than 90% of respondents indicated they were somewhat or

extremely comfortable counseling patients about the more common SCAs (45,X, 47,XXX, 47,XXY, and 47,XYY). However, only 80% of respondents indicated being comfortable when counseling patients for "other" SCA results (Figure 1; Table 3). While overall respondents self-reported being comfortable counseling about these indications, respondents in the field for less than 5 years were significantly less comfortable than those in the field for 5 or more years counseling about 47,XXY (p=0.02), 47,XXX (p=0.003), 47,XYY (p=0.001), and "other" SCA results (p=0.0001).

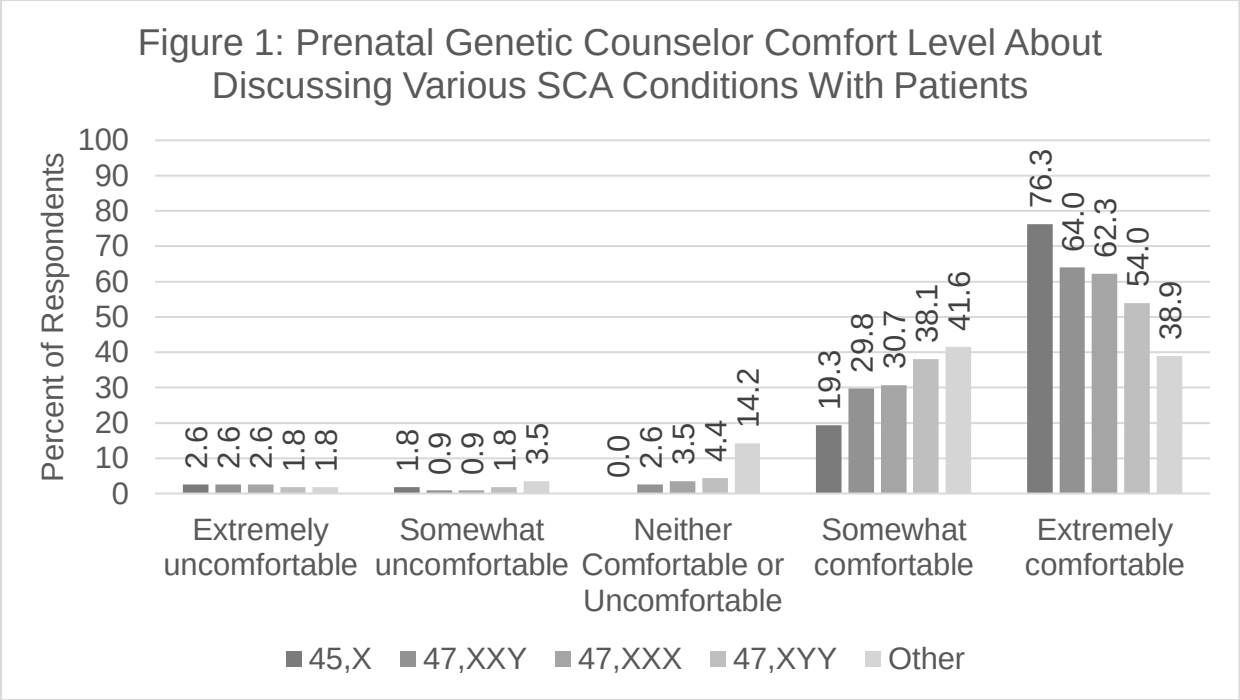

Table 3: Prenatal Respondents Self-Reported Comfort Level Counseling About SCA Conditions					
Prenatal Comfort	**45, X**	**47, XXY**	**47, XXX**	**47, XYY**	**Other**
Extremely Uncomfortable	2.6% (n=3)	2.6% (n=3)	2.6% (n=3)	1.8% (n=2)	1.8% (n=2)
Somewhat Uncomfortable	1.8% (n=2)	0.9% (n-1)	0.9% (n=1)	1.8% (n=2)	3.5% (n=4)
Neither Comfortable nor Uncomfortable	0% (n=0)	2.6% (n=3)	3.5% (n=4)	4.4% (n=5)	14.2% (n=16)
Somewhat Comfortable	19.3% (n=22)	29.8% (n=34)	30.7% (n=35)	38.1% (n=43)	41.6% (n=47)
Extremely Comfortable	76.3% (n=87)	64.0% (n=73)	62.3% (n=71)	54.0% (n=61)	38.9% (n=44)

In the scenarios, when asked what they would offer at an initial appointment for a positive NIPT for 45,X with or without ultrasound findings (scenarios 1 and 2), 47,XXX

(scenario 3), or 47,XYY (scenario 4) the vast majority of prenatal genetic counselors always offered diagnostic testing (either CVS [>88%] and or amniocentesis [>90%]), and prenatal ultrasound (>90%) to patients (Table 4). Other options including chromosome microarray (CMA), single gene testing, maternal testing through either karyotype or X,Y FISH, and postnatal evaluation, exhibited variability in the frequency with which the option was being offered between and within scenarios. These patterns held true across scenarios with a few notable exceptions. With NIPT positive for 45,X and a cystic hygroma on ultrasound (scenario 2), respondents were significantly more likely to offer their patient a CMA and or single gene testing if the patient had had a normal karyotype through CVS or amniocentesis ($p<0.01$). As well, respondents were significantly more likely to offer a CMA at the initial appointment of a patient with NIPT positive for 45,X and ultrasound findings (scenario 2; $p<0.01$) and/or if a patient had NIPT positive for 47,XXX (Scenario 3; $p=0.016$) than they were for a patient with NIPT positive for 45,X and no ultrasound findings (scenario 1). Across scenarios, respondents were significantly less likely to offer a postnatal evaluation to a patient who had a normal karyotype with diagnostic testing compared to a patient at the initial appointment ($p<0.01$). With the exception of a patient with NIPT positive for 47,XYY (scenario 4), respondents were significantly more likely to offer a postnatal evaluation to a patient who declined diagnostic testing during pregnancy than to a patient who had a normal karyotype during the pregnancy ($p<0.01$). Looking at the frequency with which respondents offered maternal karyotype, respondents were significantly more likely to offer a maternal karyotype to patients with NIPT positive for 45,X with or without ultrasound findings (scenarios 1 and 2) and to patients with NIPT positive for 47,XXX (scenario 3) following a normal karyotype result ($p=0.023$, $p=0.022$, and $p<0.01$ respectively) or if the patient declined diagnostic testing ($p=0.014$, $p<0.01$, and $p=0.019$ respectively) than they were to offer it at the initial appointment. For maternal X,Y FISH,

respondents were significantly more like to offer it to a patient with NIPT positive for 45,X with or without ultrasound findings (scenarios 1 and 2) following normal karyotype on diagnostic testing than at the initial appointment ($p=0.028$, and $p=0.014$). However, respondents only offered maternal X,Y FISH testing more frequently to patients who declined diagnostic testing than to those at the initial appointment if there was an NIPT result positive for 45,X and ultrasound finding of cystic hygroma (scenario 2; $p=0.015$).

Table 4: Percent Of Prenatal Respondents Offering A Service By Scenario

Scenario	Frequency	Frequency Services Offered (% of Respondents)								
S1 Prenatal		**CVS**	**Amniocentesis**	**CMA**	**Single Gene Testing**	**Anatomy U/S**	**Maternal Karyotype**	**Maternal X,Y FISH**	**Postnatal Evaluation**	**Other**
Initial Appointment	Always	88.2	94.6	34.6		92.3	24.8	6.4	48.7	64.7
	Often	7.1	4.6	17.8		6.2	19.1	8.5	18.3	11.8
	Sometimes	0.0	0.8	22.4		1.6	26.7	12.8	17.4	0.0
	Rarely	3.2	0.0	19.6		0.0	23.8	29.8	13.9	0.0
	Never	1.6	0.0	5.6		0.0	5.7	42.6	1.7	23.5
Normal 46, XX CVS	Always		27.2	51.4		89.4	35.1	15.2	32.0	50.0
	Often		12.0	12.5		5.7	21.3	15.2	14.7	0.0
	Sometimes		20.7	12.5		2.4	24.5	21.2	21.3	14.3
	Rarely		31.5	13.9		1.6	14.9	33.3	16.0	7.1
	Never		8.7	9.7		0.8	4.3	15.2	16.0	28.6
Normal 46, XX Amniocentesis	Always			57.6		90.0	35.2	9.7	28.1	33.3
	Often			12.1		3.3	23.1	12.9	8.8	0.0
	Sometimes			13.6		4.2	23.1	29.0	24.6	11.1
	Rarely			13.6		2.5	17.6	25.8	22.8	22.2
	Never			3.0		0.0	1.1	22.6	15.8	33.3
No Diagnostic Testing	Always						36.7	6.7	71.4	83.3
	Often						15.6	10.0	17.0	8.3
	Sometimes						24.4	30.0	6.3	0.0
	Rarely						23.3	33.3	4.5	0.0
	Never						0.0	20.0	0.9	8.3
S2 Prenatal		**CVS**	**Amniocentesis**	**CMA**	**Single Gene Testing**	**Anatomy U/S**	**Maternal Karyotype**	**Maternal X,Y FISH**	**Postnatal Evaluation**	**Other**
Initial Appointment	Always	94.3	95.`6	65.4	40.0	92.7	23.3	0.0	68.8	82.4
	Often	4.1	4.0	14.0	15.2	4.0	6.7	0.0	13.5	11.8
	Sometimes	0.8	0.8	11.2	21.0	3.2	15.0	15.4	12.5	0.0
	Rarely	0.8	0.0	8.4	21.0	0.0	40.0	50.0	4.2	0.0
	Never	0.0	0.0	0.9	2.9	0.0	15.0	34.6	1.0	5.9
Normal 46, XX CVS	Always		41.7	80.9	61.4	93.9	27.8	0.0	70.8	83.3
	Often		15.5	10.9	16.7	4.4	11.1	3.6	12.4	5.6
	Sometimes		17.9	5.5	14.9	1.7	22.2	25.0	14.6	5.6
	Rarely		13.1	2.7	7.0	0.0	34.7	42.9	2.3	0.0
	Never		11.9	0.0	0.0	0.0	4.2	28.6	0.0	5.6
Normal 46, XX Amniocentesis	Always			82.6	60.9	93.4	25.0	0.0	74.7	79.0
	Often			10.1	18.3	3.3	17.7	7.4	11.0	0.0
	Sometimes			5.5	16.5	3.3	17.7	29.6	13.2	5.3
	Rarely			0.9	4.4	0.0	33.8	40.7	0.0	0.0
	Never			0.9	0.0	0.0	5.9	22.2	1.1	15.8
No Diagnostic Testing	Always					96.7	33.3	4.8	85.6	90.0
	Often					1.6	11.1	4.8	7.6	0.0
	Sometimes					1.6	30.6	38.1	5.1	0.0
	Rarely					0.0	20.8	33.3	1.7	0.0
	Never					0.0	4.2	10.1	0.0	10.0
S3 Prenatal		**CVS**	**Amniocentesis**	**CMA**	**Single Gene Testing**	**Anatomy U/S**	**Maternal Karyotype**	**Maternal X,Y FISH**	**Postnatal Evaluation**	**Other**
Initial Appointment	Always		96.6	60.7			45.6	8.7	75.3	66.7
	Often		3.4	13.1			15.2	4.4	13.5	0.0
	Sometimes		0.0	10.7			21.5	39.1	5.6	0.0
	Rarely		0.0	9.5			12.7	21.7	4.5	0.0
	Never		0.0	6.0			5.1	26.1	1.1	33.3
Normal 46, XX Amniocentesis	Always			66.7			52.4	8.7	51.5	50.0
	Often			8.0			19.1	13.0	9.1	0.0
	Sometimes			9.3			13.1	30.4	12.1	0.0
	Rarely			9.3			14.3	30.4	12.1	0.0
	Never			6.7			1.2	17.4	15.2	50.0
No Diagnostic Testing	Always						51.3	6.7	85.1	50.0
	Often						16.3	6.7	9.4	0.0
	Sometimes						16.3	34.8	3.7	25.0
	Rarely						12.5	26.1	1.9	0.0
	Never						3.8	21.7	0.0	25.0
S4 Prenatal		**CVS**	**Amniocentesis**	**CMA**	**Single Gene Testing**	**Anatomy U/S**	**Maternal Karyotype**	**Maternal X,Y FISH**	**Postnatal Evaluation**	**Other**
Initial Appointment	Always		97.4	62.8			22.2	0.0	82.6	40.0
	Often		1.7	11.5			8.3	10.5	9.3	0.0
	Sometimes		0.0	16.7			13.9	36.8	5.8	0.0
	Rarely		0.0	7.7			25.0	31.6	1.2	0.0
	Never		0.9	1.3			30.6	21.1	1.2	60.0
Normal 46, XY Amniocentesis	Always			64.3			27.0	5.3	54.7	33.3
	Often			10.0			2.7	0.0	14.1	0.0
	Sometimes			7.1			24.3	26.3	10.9	0.0
	Rarely			12.9			27.0	42.1	9.4	0.0
	Never			5.7			18.9	26.3	10.9	66.7
No Diagnostic Testing	Always						33.3	0.0	87.5	33.3
	Often						11.1	10.5	5.8	0.0
	Sometimes						8.3	21.1	1.9	0.0
	Rarely						33.3	42.1	3.9	0.0
	Never						13.9	26.3	1.0	66.7

Pediatric section

A total of 41 individuals were eligible to take the pediatric specific portion of the survey. Of these respondents 82.5% indicated that they have seen at least one patient referred for a prenatal NIPT positive for a sex chromosome abnormality (Table 2). The most common SCA seen by counselors in the last year was 45,X (72.7%) followed by 47,XXY (63.6%), 47,XXX (45.5%), 47,XYY (24.2%), and "other" SCA results (6.1%), (Table 2). When asked about their comfort in counseling patients for the various SCA conditions, greater than 70% of respondents indicated that they were somewhat or extremely comfortable counseling patients about any of the potential SCAs (Figure 2; Table 5). Compared to 45,X, pediatric counselors were significantly less comfortable counseling patients about 47,XXX (p=0.033), 47,XYY (p=0.033), and "other" SCA results (p=0.016). No other significant differences were observed when comparing comfort between the other conditions.

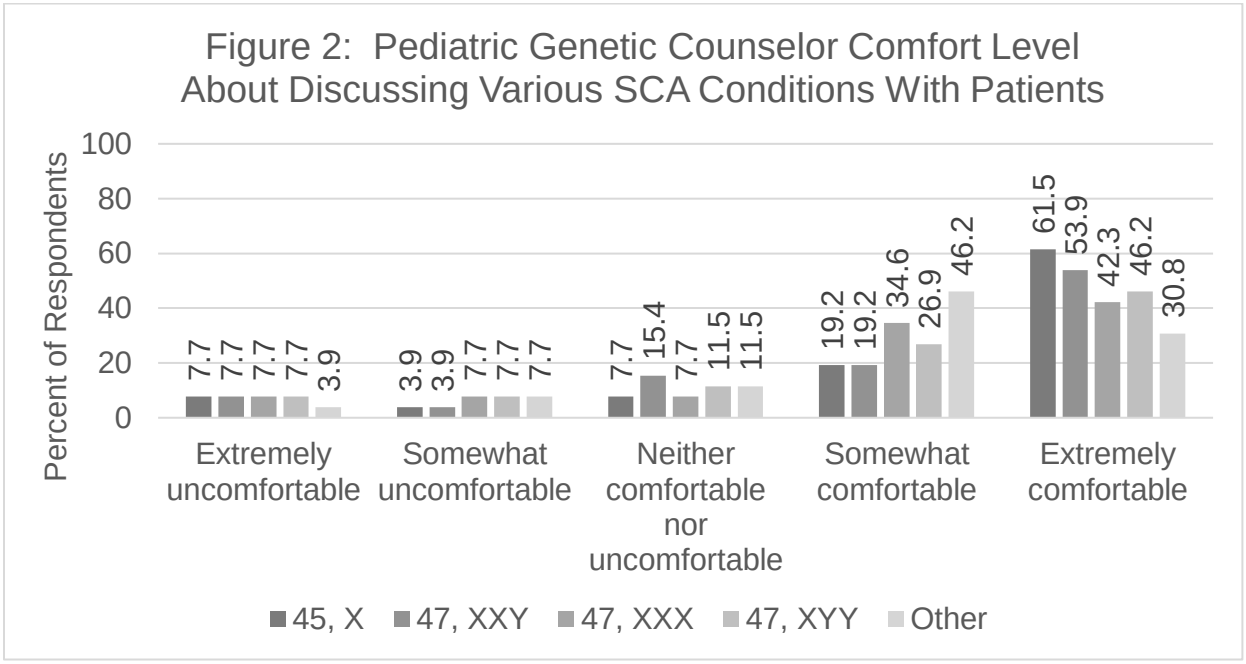

Table 5: Pediatric Respondents Self-Reported Comfort Level Counseling About SCA Conditions					
Pediatric Comfort	**45, X**	**47, XXY**	**47, XXX**	**47, XYY**	**Other**
Extremely Uncomfortable	7.7% (n=2)	7.7% (n=2)	7.7% (n=2)	7.7% (n=2)	3.9% (n=1)
Somewhat Uncomfortable	3.9% (n=1)	3.9% (n=1)	7.7% (n=2)	7.7% (n=2)	7.7% (n=2)
Neither Comfortable nor Uncomfortable	7.7% (n=2)	15.4% (n=4)	7.7% (n=2)	11.5% (n=3)	11.5% (n=3)
Somewhat Comfortable	19.2% (n=5)	19.2% (n=5)	34.6% (n=9)	26.9% (n=7)	46.2% (n=12)
Extremely Comfortable	61.5% (n=16)	53.9% (n=14)	42.3% (n=11)	46.2% (n-12)	30.8% (n=8)

When asked what they would offer at an initial appointment for a newborn with prenatal NIPT positive for 45,X, with or without physical features indicative of 45,X (scenarios 1 and 2), 47,XXX (scenario 3), or 47,XYY (scenario 4), the majority of pediatric genetic counselors (>70%) indicated they always offer a karyotype to patients (Table 6). The remaining options, including X,Y FISH, maternal karyotype, CMA, ultrasound, and echocardiogram were variable in the frequency with which they were offered within and between scenarios (Table 6). More than 50% of respondents indicated they always offer X,Y FISH at the initial appointment, but that number decreased if the baby had a normal karyotype for all scenarios and was not statistically significant (p=0.333). Compared to the initial appointment, respondents were significantly more likely to offer a maternal karyotype following normal diagnostic testing for newborns with NIPT positive for 45,X with physical findings indicative of 45,X (scenario 1; p=0.048), and newborns with NIPT positive for 47,XXX (scenario 3; p=0.047). When considering imaging options, echocardiogram was offered significantly more frequently for a newborn with prenatal NIPT positive for 45,X and webbed neck and puffy hands (scenario 1) at the initial appointment than following a normal karyotype (p=0.018). Looking between scenarios, echocardiogram was offered significantly more frequently if a newborn with a prenatal NIPT positive for 45,X had physical findings indicative of 45,X (scenario 1) than if they did not have physical findings (scenario 2; p=0.026). When asked about ultrasound (pelvic/renal/abdominal), respondents were significantly more likely to offer an ultrasound at the initial appointment

for a baby with prenatal NIPT positive for 45,X who has a webbed neck and puffy hands (scenario 1) than they were if the baby had normal karyotype results (p= 0.027).

Table 6: Percent of Pediatric Respondents Offering A Service By Scenario								
S1 Pediatric		**X, Y FISH**	**Karyotype**	**CMA**	**U/S (Pelvic/Renal/Abdominal)**	**Maternal Karyotype**	**Echocardiogram**	**Other**
Initial Appointment	Always	52.2	84.9	32.0	50.0	5.9	70.0	50.0
	Often	13.0	6.1	16.0	10.0	11.8	16.7	0.0
	Sometimes	17.4	6.1	20.0	5.0	23.5	10.0	25.0
	Rarely	17.4	0.0	28.0	35.0	52.9	3.3	0.0
	Never	0.0	3.0	4.0	0.0	5.9	0.0	25.0
Normal 46, XX Karyotype	Always	16.7		28.6	37.5	28.6	52.4	33.3
	Often	41.7		38.1	12.5	21.4	23.8	16.7
	Sometimes	8.3		23.8	25.0	21.4	19.1	16.7
	Rarely	16.7		9.5	12.5	21.4	4.8	16.7
	Never	16.7		0.0	12.5	7.1	0.0	16.7
S2 Pediatric		**X, Y FISH**	**Karyotype**	**CMA**	**U/S (Pelvic/Renal/Abdominal)**	**Maternal Karyotype**	**Echocardiogram**	**Other**
Initial Appointment	Always	64.3	73.1	28.6	36.4	28.6	52.9	100.0
	Often	0.0	11.5	14.3	9.1	28.6	17.7	0.0
	Sometimes	7.1	7.7	14.3	18.2	14.3	11.8	0.0
	Rarely	14.3	7.7	42.9	27.3	28.6	17.7	0.0
	Never	14.3	0.0	0.0	9.1	0.0	0.0	0.0
S3 Pediatric		**X, Y FISH**	**Karyotype**	**CMA**	**U/S (Pelvic/Renal/Abdominal)**	**Maternal Karyotype**	**Echocardiogram**	**Other**
Initial Appointment	Always	77.8	85.2	41.7		30.0		0.0
	Often	0.0	3.7	33.3		20.0		0.0
	Sometimes	11.1	7.4	0.0		30.0		100.0
	Rarely	0.0	3.7	25.0		20.0		0.0
	Never	11.1	0.0	0.0		0.0		0.0
Normal 46, XX Karyotype	Always	14.3		27.3		46.2		No responses
	Often	14.3		18.2		30.8		No responses
	Sometimes	0.0		9.1		15.4		No responses
	Rarely	57.1		36.4		7.7		No responses
	Never	14.3		9.1		0.0		No responses
S4 Pediatric		**X, Y FISH**	**Karyotype**	**CMA**	**U/S (Pelvic/Renal/Abdominal)**	**Maternal Karyotype**	**Echocardiogram**	**Other**
Initial Appointment	Always	63.6	84.6	50.0		33.3		No responses
	Often	18.2	3.8	25.0		0.0		No responses
	Sometimes	0.0	0.0	8.3		33.3		No responses
	Rarely	18.2	11.5	16.7		33.3		No responses
	Never	0.0	0.0	0.0		0.0		No responses
Normal 46, XY Karyotype	Always	60.0		30.8		33.3		No responses
	Often	20.0		30.8		16.7		No responses
	Sometimes	0.0		7.7		50.0		No responses
	Rarely	20.0		15.4		0.0		No responses
	Never	0.0		15.4		0.0		No responses

DISCUSSION

The purpose of this study was to describe current prenatal and pediatric genetic counseling practice following NIPT results positive for a sex chromosome abnormality. One hundred and seventy-six clinical prenatal and pediatric genetic counselors completed a scenario-based survey about their current practice following positive NIPT for SCA.

Comfort Level

On both the prenatal and pediatric side, the majority of counselors (94% of prenatal and 80% of pediatric) indicated they have seen at least one case of NIPT positive for an SCA in the past year with respondents reporting seeing an average of 1.4-3.2 cases per year. Monosomy X (45,X) was the most commonly seen indication with an average of 3.2 cases for prenatal counselors and 2.5 cases for pediatric counselors.

While the average number of SCA cases respondents are seeing in a year is relatively low, the majority of counselors self-report being somewhat or extremely comfortable counseling patients about the various SCA conditions. Among prenatal counselors, greater than 80% indicated being somewhat or extremely comfortable counseling patients about any of the SCA conditions. Among pediatric counselors, greater than 70% indicated being somewhat or extremely comfortable counseling patients about SCA conditions. For counselors in both specialties, comfort decreased as the condition became less common (45,X= 47,XXY> 47,XXX> 47,XYY> other), (Tables 3 and 5). While this can be attributed to the lower frequency of the more rare sex chromosome conditions, it may also reflect that there is a lack of attention given to less common SCAs during training. The textbook, *Genetics In Medicine*, is used by many genetics training programs. While all four common sex chromosome abnormalities are mentioned, only Klinefelter

syndrome and Turner syndrome are discussed in detail (23). The American College of Medical Genetics has a Genetics and Genomics Review Course that includes a list of 100 syndromes every geneticist should know. Turner syndrome and Klinefelter syndrome are the only sex chromosome abnormalities on the list (24). With the rapid uptake of NIPT testing, the likelihood of seeing families with other SCA conditions has increased, thus training programs may need to incorporate additional lectures about sex chromosome abnormalities beyond Turner syndrome and Klinefelter syndrome.

The vast majority of prenatal counselors indicated they are comfortable counseling patients for NIPT positive for SCAs; however, counselors who had been in the field for less than 5 years were significantly less comfortable counseling about all SCA conditions except 45,X ($p<0.02$). This result was not found among pediatric counselors. This finding could be indicative of several things. First, there is the potential that prenatal counselors in the field for ≥5 years have seen an overall greater number of SCA cases through patients undergoing CVS and amniocentesis procedures. This may have provided the foundational knowledge about these conditions prior to the introduction of NIPT. Second, this may simply be a reflection of a new graduate's overall lack of comfort or confidence, and as time goes on they will become more comfortable with the indications (25).

Regardless of counselor comfort with SCA conditions, NIPT with analysis for SCAs has become routine. Based on this survey, 30% of respondents always offered patients NIPT with SCA analysis and 64% indicated they offer NIPT with SCA analysis to patients at least some of the time. Therefore, it is likely that the frequency with which counselors will see patients for this indication will only increase, making increased education about SCAs necessary.

Prenatal Practice

In prenatal practice there was a general consensus that regardless of the indication, it is appropriate to offer diagnostic testing and an anatomy ultrasound to all patients. Offering these options is in line with ACMG and ACOG's suggested testing strategy for NIPT (26, 27). In contrast, there was little consensus about how often or in what situations CMA, single gene testing, maternal testing (karyotype or X,Y FISH), echocardiogram, and postnatal evaluation should be offered. This variability is understandable given the lack of current professional society guidelines for SCA on NIPT, making it harder to determine when to consistently offer these services.

While there was wide variability in frequency, respondents were significantly more likely to offer CMA testing to a patient with ultrasound abnormalities and a normal karyotype ($p<0.01$). A 2012 study by Wapner et al. found that in pregnancies with a normal karyotype result, 1.7% had a relevant deletion or duplication on CMA and 6% had a relevant deletion or duplication on CMA if there was also an ultrasound abnormality (28). Based on this information, offering CMA even when there has been a normal karyotype may be appropriate. Per the written survey responses, some of the nuance in frequency may come from the fact that some respondents only discuss CMA if the patient elects diagnostic testing, "I always discuss CMA if a patient elects a diagnostic test but I do not discuss it if the patient declined diagnostic testing." Respondents may offer either karyotype or CMA but not both, "at our clinic patients typically need to pick a karyotype or CMA."

The offer of maternal testing to uncover an undiagnosed maternal SCA in the prenatal scenarios was also highly variable. For a patient with NIPT positive for 45,X with or without ultrasound abnormalities or a patient with NIPT positive for 47,XXX, maternal

testing was offered significantly more frequently if the fetus had a normal karyotype result ($p<0.03$) or the patient had declined diagnostic testing ($p<0.02$) than at the initial appointment. Some respondents indicated they would offer maternal testing only after a fetus' normal workup, if the mother appeared to have features concerning for Turner Syndrome, or if the testing laboratory indicated that the result might be maternal in origin instead of fetal. Depending on the laboratory's testing methodology, results may be reported as most likely maternal in origin, which is a more recent nuance of NIPT testing. While this may be a helpful way to guide what testing should be offered, this information is not available through every NIPT method. Wang et al. previously reported that of the pregnancies in their sample with NIPT positive for an SCA, 8.6% were due to maternal abnormalities in X chromosome number (14). This number seems sufficiently high to warrant a discussion of routinely offering maternal testing when a patient is seen with NIPT positive for SCA that could be caused by a maternal difference in X chromosome number. Maternal testing is also important because an undiagnosed maternal SCA can have important health implications. Women with 45,X have increased lifelong risks for hearing loss, aortic root dilation, and fractures, and women with 47,XXX may be at risk of primary ovarian insufficiency (20, 21). In addition, women with 45,X have an increased risk for a pregnancy with a SCA (22). Diagnosing these individuals allows for appropriate future screening and management of several serious health problems.

Postnatal evaluation was not offered by prenatal counselors as frequently as had been anticipated given that the PPV for positive NIPT for SCAs generally creates a concern of a true positive of 20-50%. Counselors were most likely to offer this option to patients who declined diagnostic testing during the pregnancy. Even in this situation, not all counselors indicated they would always refer patients for postnatal evaluation. One free

response elucidated a potential reason for this, “we don't have a geneticist within 3 hours of our area. The waiting list for genetics is also 9 months…We encourage patients to share their [NIPT] result with their pediatrician.” Other respondents echoed the ideas that there either was not a geneticist within a reasonable distance of them and/or the waiting list to see medical genetics was anywhere from 6 months to upwards of 12 months. It has been observed that fewer and fewer clinicians are seeking certification in clinical genetics, leading to fewer clinical geneticists today than 30 years ago (29). A 2015 study stated that 50% of available medical genetics residencies remain unfilled (30). Therefore, while ideally all individuals with NIPT positive for SCA should have follow up at birth, referring these patients to a medical geneticist would put increased strain on an already limited resource. While several counselors indicated they encourage their patients to share abnormal NIPT results with their pediatrician following the birth of the baby, there is no guarantee that patients will inform their doctor of their positive NIPT results. Therefore, healthcare teams should consider implementing a system where there is a direct physician to physician hand off of information, such as from obstetrics to pediatrics, to ensure all patients receive appropriate follow up. Pediatricians then need to be involved in ordering a postnatal karyotype and making subsequent referrals to genetics as needed.

Fetal echocardiogram was a common recommendation recorded as an “other” response for scenarios involving NIPT positive for 45,X with or without ultrasound findings (scenario 1 and 2). Specifically, in a scenario where there was NIPT positive for 45,X and no ultrasound findings (scenario 1), several respondents indicated they would also offer an echocardiogram to a patient during the initial appointment or if diagnostic testing was declined. In a scenario where there was NIPT positive for 45,X and a cystic hygroma on ultrasound (scenario 2), greater than 12 respondents indicated they would offer the patient

an echocardiogram regardless of whether it was at the initial appointment, after normal diagnostic studies, or with no diagnostic testing pursued. Since fetal echocardiogram was not included in the original survey, it is not possible to draw conclusions about how frequently it is being offered to patients. Future studies looking to find consensus on SCA practice may wish to include the potential of fetal echocardiogram in the discussion.

Pediatric Practice

In pediatric practice there was a general consensus that a karyotype should be offered to all newborns with a prenatal NIPT positive for an SCA that was unresolved regardless of whether the baby had any abnormal features. This is in line with the ACMG and ACOG statement on NIPT which states postnatal karyotype should be offered to all patients who declined diagnostic testing in the prenatal setting (26, 27). However, there was little consensus about how often or in what situations X,Y FISH, maternal karyotype, CMA, ultrasound (renal/pelvic/abdominal), and echocardiogram should be offered.

Pediatric respondents were more likely to offer a maternal karyotype if the neonate had a normal karyotype result but an unresolved NIPT positive for 45,X with physical findings indicative of 45,X ($p=0.048$) or an unresolved NIPT positive for 47,XXX ($p=0.047$). However, when there was a child with a positive NIPT result for 45,X with no findings and a normal karyotype, respondents were less likely to order a maternal karyotype. These results seem contradictory since the lack of findings for a neonate with NIPT positive for 45,X should increase suspicion for maternal SCA.

Like maternal testing, imaging studies were offered with high variability. Echocardiogram and abdominal ultrasound were offered more frequently at the initial appointment of a newborn with NIPT positive for 45,X and physical findings indicative of

Turner syndrome than following normal karyotype ($p<0.027$), or if there were no physical findings at birth ($p<0.027$). Since approximately 40% of individuals with Turner syndrome have heart defects and 60% have structural renal abnormalities, echocardiogram and imaging of the kidneys/abdomen is a common referral for a baby with potential Turner syndrome (31).

Practice Implications

Overall, this study suggests that many prenatal counselors are following current recommendations by offering diagnostic testing and ultrasound screening to patients with positive SCA results. However, practice differs in offering specialty imaging, maternal karyotyping, and postnatal follow up. Likewise, many pediatric counselors are recommending karyotyping babies with unresolved NIPT positive for SCA. However, there is wide variability in this group regarding subsequent referral for specialty imaging and maternal karyotyping. Therefore, while both prenatal and pediatric counselors self-reported being comfortable with SCA conditions, they are not uniformly recommending appropriate follow up for their patients. Early diagnosis with appropriate follow up and treatment is crucial for mitigating or preventing some of the medical problems associated with SCA conditions. Children with 45,X can have cardiac abnormalities, renal abnormalities, ocular findings, and hearing loss and benefit from being followed by appropriate specialists. Many also benefit from growth hormone treatment which can be started as early as the age of 2. Individuals with 45,X who do not receive growth hormone early do not experience as great a benefit in the social and medical benefits associated with growth hormone treatment (20). Individuals with 47,XXY may need lifelong testosterone supplementation to prevent symptoms and sequelae of androgen deficiency which include osteoporosis, muscle weakness, and increased risk for thromboembolic

events making early diagnosis of 47,XYY key (32). In addition, there is evidence that early intervention improves social and developmental outcomes for children with SCAs making a strong case for the necessity of prompt follow up for all NIPT results positive for a SCA (20, 33, 34).

Both ACMG and ACOG concur that the limitations of NIPT must be discussed with patients, and that it is a reasonable screening option for aneuploidy. While both mention that NIPT may also include analysis for SCA conditions, recommendations about the appropriate testing population and what follow up testing, other than a diagnostic procedure, should be offered following a positive result are not outlined for the SCA conditions (26, 27). NSGC has likewise expressed its support for NIPT screening in the prenatal field but without any information about specific testing or screening that should be offered following a positive result (35).

Because of the variability seen in what is being offered to patients and the benefits of early diagnosis and appropriate follow up for individuals with a SCA, there is a need for professional guidelines to help determine best practice and increase the cohesiveness of care that patients across the country are receiving. While it is unlikely a single policy for follow up testing and screening would be applicable to every situation given the complexities that come with screening for SCA conditions, guidelines could provide a beneficial framework for genetic counseling.

Study Limitations

This study had several limitations. First, there was a relatively poor response rate (8.6%) and thus overall small sample size compared to response rates from other surveys of prenatal and pediatric genetic counselors. However, the study population appears to be representative of the national genetic counselor population based on demographic comparisons to the 2016 NSGC Professional Status Survey. Second, the survey used for this study was not validated and questions could have been misinterpreted by those taking the survey. Finally, the aim of this survey was to describe current counseling practice among prenatal and pediatric counselors for various SCA conditions reported on NIPT. Therefore, conclusions can only be made about current practice and do not reflect clinical practice before or after the survey was administered. However, this study was the first of its kind and gives needed insight into the current practices of a representative sample of prenatal and pediatric genetic counselors who are seeing patients with NIPT results positive for SCAs and is therefore a useful addition to the current literature.

CONCLUSION

Using NIPT to screen for sex chromosome abnormalities is common in prenatal practice. With the increase in screening comes an increase in results positive for SCAs and subsequently more patients being referred for genetic counseling for an unresolved SCA result. While counselors largely report being comfortable counseling patients for this indication and there is general consensus about recommending prenatal or postnatal karyotype, maternal testing, specialty screening, and postnatal evaluation practices are inconsistent. This study demonstrates a need for collaboration among clinicians and governing bodies to create specific practice guidelines from which genetic counselors and other clinicians may base their practice and bring uniformity to the care of patients for this type of indication.

APPENDIX

Survey Questions

Demographic Information

1. Which setting do you currently work in?
    a. University Medical Center
    b. Public Hospital/Medical Facility
    c. Private Hospital/Medical Facility
    d. Diagnostic Laboratory
    e. Physician's Private Practice
    f. Other: (Free response)
2. Do you see prenatal or pediatric patients as part or all of your position?
    a. Yes
    b. No
3. What is your current specialty? (Please select all that apply)
    a. Prenatal
    b. Pediatric
    c. Cancer
    d. General Genetics
    e. Adult
    f. Other: (Free response)
4. How many years have you worked in the prenatal genetic counseling field?
    a. 1 ... 20+
5. How many prenatal patients do you see per week?
    a. 1-10
    b. 11-20
    c. 21-30
    d. 31-40
    e. 41-50
    f. 51+
6. How many years have you worked in the pediatric genetic counseling field?
    a. 1 ... 20+
7. How many pediatric patients do you see per week?
    a. 1-10
    b. 11-20
    c. 21-30
    d. 31-40
    e. 41-50
    f. 51+
8. How many years have you worked in the genetic counseling field overall?
    a. 1 ... 20+

9 What state do you currently work in?
    a. Alabama

b. Alaska
c. Arizona
d. Arkansas
e. California
f. Colorado
g. Connecticut
h. Delaware
i. Florida
j. Georgia
k. Hawaii
l. Idaho
m. Illinois
n. Indiana
o. Iowa
p. Kansas
q. Kentucky
r. Louisiana
s. Maine
t. Maryland
u. Massachusetts
v. Michigan
w. Minnesota
x. Mississippi
y. Missouri
z. Montana
aa. Nebraska
bb. Nevada
cc. New Hampshire
dd. New Jersey
ee. New Mexico
ff. New York
gg. North Carolina
hh. North Dakota
ii. Ohio
jj. Oklahoma
kk. Oregon
ll. Pennsylvania
mm. Rhode Island
nn. South Carolina
oo. South Dakota
pp. Tennessee
qq. Texas
rr. Utah
ss. Vermont
tt. Virginia
uu. Washington
vv. West Virginia
ww. Wisconsin
xx. Wyoming

Pediatric Arm

10. Have you seen at least one patient who was referred postnatally due to a positive/increased risk cell free DNA (cfDNA, NIPT) result for a sex chromosome abnormality during pregnancy?

a. Yes
b. No

11. Please estimate how many patients you have seen in the past year who were referred for a positive cell free DNA result during pregnancy for the below conditions.

a. 45,X _____
b. 47,XXX _____
c. 47,XXY _____
d. 47,XYY _____
e. Other: _____

12. Scenario 1:

You are seeing a newborn with a prenatal cell free DNA result positive for Turner Syndrome who had a normal anatomy ultrasound and no diagnostic testing. At birth, physical findings include a webbed neck and puffy hands.

13. What percentage of the time do you offer or refer the family for the following options at the initial appointment?

a. X,Y FISH
b. Karyotype
c. CMA
d. Pelvic/Renal/Abdominal Ultrasound
e. Maternal Blood Karyotype
f. Echocardiogram
g. Other: (free response)

14. If the family opts for chromosome testing and the results are normal 46, XX, what percentage of the time do you offer or refer for the following options?

a. X,Y FISH
b. CMA
c. Pelvic/Renal/Abdominal Ultrasound
d. Maternal Blood Karyotype
e. Echocardiogram
f. Other

15. If the baby had physical findings including a webbed neck and puffy hands but normal 46, XX diagnostic testing during pregnancy, would your recommendations change?

a. Yes
b. No

16. If yes, what percentage of the time would you offer or refer for the following options?

a. X,Y FISH
b. Karyotype
c. CMA
d. Pelvic/Renal/Abdominal Ultrasound

e. Maternal Blood Karyotype
f. Echocardiogram
g. Other

17. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

18. Scenario 2:

You are seeing a newborn with a prenatal cell free DNA result positive for Turner Syndrome who had a normal female anatomy ultrasound and no diagnostic testing. At birth, no physical signs of Turner Syndrome are appreciated.

19. What percentage of the time would you offer or refer for the following options at the initial appointment?

a. X,Y FISH
b. Karyotype
c. CMA
d. Pelvic/Renal/Abdominal Ultrasound
e. Maternal Blood Karyotype
f. Echocardiogram
g. Other

20. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

21. Scenario 3:

You are seeing a newborn with a prenatal cell free DNA result positive for 47,XXX who had a normal anatomy ultrasound and no diagnostic testing. At birth there are no physical findings.

22. What percentage of the time would you offer the following options at the initial appointment?

a. X,Y FISH
b. Karyotype
c. CMA
d. Maternal Blood Karyotype
e. Other

23. If the family opts for chromosomes and the results are normal 46, XX, what percentage of the time would you offer the following options?

a. X,Y FISH
b. CMA
c. Maternal Blood Karyotype
d. Other

24. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

25. Scenario 4:

You are seeing a newborn with a prenatal cell free DNA result positive for 47,XYY who had a normal anatomy ultrasound and no diagnostic testing. At birth there are no abnormal physical findings.

26. What percentage of the time do you offer the following options to patients at the initial appointment?

a. X,Y FISH
b. Karyotype
c. CMA
d. Maternal Blood Karyotype
e. Other

27. If the family opts for chromosome testing and the results are normal 46, XY, what percentage of the time would you offer the following options?

a. X,Y FISH
b. CMA
c. Maternal Blood Karyotype
d. Other

28. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

29. How comfortable do you feel discussing a positive cell free DNA result for a sex chromosome abnormality with a pediatric patient?

	Extremely Uncomfortable	Somewhat Uncomfortable	Neither Comfortable nor Uncomfortable	Somewhat Comfortable	Extremely Comfortable
45,X					
47,XXX					
47,XXY					
47,XYY					
Other: SCA					

30. Which of the following do you take into consideration when deciding what to discuss and offer when counseling a pediatric patient about a positive cell free DNA result for the below sex chromosome abnormalities? Select all that apply.

	45,X	47,XXX	47,XXY	47,XYY
Lack of Physical Findings				
Risk of Mosaicism				
Potential to Uncover a Maternal SCA				
Potential to Uncover a Paternal SCA				
Other:				

Prenatal Arm

31. Does your office offer cell free DNA testing (cfDNA, NIPT)?

    a. Yes
    b. No

32. When offering cell free DNA testing (cfDNA, NIPT) what percent of the time do you offer each of the following options?

    a. NIPT (18, 21, Y)
    b. NIPT (13, 18, 21, Y)
    c. NIPT (13, 18, 21) with Sex Chromosome Aneuploidy
    d. NIPT with Sex Chromosome Aneuploidy and Microdeletions

33. Does your office offer diagnostic testing to patients?

	No	Yes
CVS		
Amniocentesis		

34. Does your office see patients who are referred in for a positive cell free DNA result for sex chromosome abnormalities?

    a. Yes
    b. No

35. When discussing a positive cell free DNA result for sex chromosome abnormalities how often do you discuss positive predictive value PPV with the patient? (percent of time)

36. If you discuss PPV, how do you determine the PPV used in your counseling? Select all that apply

    a. I calculate my own using the Gil et al, 2015 Meta-Analysis paper (by hand or using an online calculator)
    b. I calculate my own using lab specific sensitivity and specificity
    c. I use the PPV the laboratory reports on the test results
    d. I use PPV published in primary literature
    e. Other: (Free response)

37. Have you seen at least one patient with a cell free DNA result positive for a sex chromosome abnormality?

    a. Yes
    b. No

38. Please list how many patients you have seen in the past year with a positive/increased risk cell free DNA result for the below conditions?

    a. 45,X _____
    b. 47,XXX _____
    c. 47,XXY _____
    d. 47,XYY _____
    e. Other: _____

39. Scenario 1:

You are seeing a 35-year-old woman at 12 weeks who has a cell free DNA result positive for Turner Syndrome and no ultrasound findings.

(For the following questions, consider what you discuss or offer even if it would be performed at a later date)

40. What percentage of the time do you discuss the following options at the initial appointment?

a. CVS
b. Amniocentesis
c. Anatomy Ultrasound
d. CMA
e. Maternal Karyotype
f. Maternal X,Y FISH
g. Postnatal Evaluation by a Geneticist
h. Other

41. If the patient opts for a CVS and the results are normal 46, XX, what percentage of the time would you offer the following for follow-up testing?

a. Amniocentesis
b. Anatomy Ultrasound
c. CMA
d. Maternal Karyotype
e. Maternal X,Y FISH
f. Postnatal Evaluation by a Geneticist
g. Other

42. If the patient declines CVS and opts for amniocentesis at 16 weeks and the results are normal 46, XX, what percentage of the time would you offer the following for follow-up testing?

a. Anatomy Ultrasound
b. CMA
c. Maternal Karyotype
d. Maternal X,Y FISH
e. Postnatal Evaluation by a Geneticist
f. Other

43. If the patient declines diagnostic testing, has a normal 20 week anatomy scan with no ultrasound findings, and is continuing the pregnancy, what percentage of the time would you offer the following for follow-up testing?

a. Maternal Karyotype
b. Maternal X,Y FISH
c. Postnatal Evaluation by a Geneticist
d. Other

44. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

45. Scenario 2:

You are seeing a 35-year-old woman at 12 weeks with a cell free DNA result positive for Turner Syndrome and a cystic hygroma on ultrasound.

(For the following questions, consider what you discuss or offer even if it would be performed at a later date)

46. What percentage of the time do you discuss the following options at the initial appointment?

a. CVS
b. Amniocentesis
c. Anatomy Ultrasound
d. CMA
e. Single Gene Testing Such as Noonan Syndrome
f. Maternal Chromosomes
g. Maternal X,Y FISH
h. Postnatal Evaluation by a Geneticist
i. Other:

47. If the patient opts for a CVS and the results are normal 46, XX, what percentage of the time do you offer the following for follow-up testing?

a. Amniocentesis
b. Anatomy Ultrasound
c. CMA
d. Single Gene Testing Such as Noonan Syndrome
e. Maternal Chromosomes
f. Maternal X,Y FISH
g. Postnatal Evaluation by a Geneticist
h. Other

48. If the patient declines CVS and opts for amniocentesis at 16 weeks and the results are normal 46, XX, what percentage of the time do you offer the following for follow-up testing?

a. Anatomy Ultrasound
b. CMA
c. Single Gene Testing Such as Noonan Syndrome
d. Maternal Chromosomes
e. Maternal X,Y FISH
f. Postnatal Evaluation by a Geneticist
g. Other

49. If the patient declines diagnostic testing and is continuing the pregnancy, what percentage of the time would you offer the following for follow-up testing?

a. Anatomy Ultrasound
b. Maternal Chromosomes
c. Maternal X,Y FISH
d. Postnatal Evaluation by a Geneticist
e. Other

50. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

51. Scenario 3:

You are seeing a 35-year-old woman at 20 weeks with a cell free DNA result positive for 47,XXX and a normal anatomy scan.

(For the following questions, consider what you discuss or offer even if it would be performed at a later date)

52. What percentage of the time do you discuss the following options at the initial appointment?

a. Amniocentesis
b. CMA
c. Maternal Karyotype
d. Maternal X,Y FISH
e. Postnatal Evaluation by a Geneticist
f. Other

53. If the patient opts for amniocentesis and the results are normal 46, XX, what percentage of the time would you offer the following for follow-up testing?

a. CMA
b. Maternal Blood Karyotype
c. Maternal X,Y FISH
d. Postnatal Evaluation by a Geneticist
e. Other

54. If the patient declines diagnostic testing and is continuing the pregnancy, what percentage of the time would you offer the following for follow-up testing?

a. Maternal Karyotype
b. Maternal X,Y FISH
c. Postnatal Evaluation by a Geneticist
d. Other

55. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

56. If the above patient is instead 25 years old would your recommendations change?

a. Yes
b. No

57 If yes, how would they change? (Free response)

58. Scenario 4:

You are seeing a 35-year-old woman at 20 weeks with a cell free DNA result positive for 47,XYY and a normal anatomy scan.

(For the following questions, consider what you discuss or offer even if it would be performed at a later date)

59. What percentage of the time do you discuss the following options at the initial appointment?

a. Amniocentesis
b. CMA
c. Maternal Blood Karyotype
d. Maternal X,Y FISH
e. Postnatal Evaluation by a Geneticist
f. Other

60. If the patient opts for amniocentesis and the results are normal 46 XY, what percentage of the time would you offer the following for follow-up testing?

a. CMA
b. Maternal Blood Karyotype
c. Maternal X,Y FISH
d. Postnatal Evaluation by a Geneticist
e. Other

61. If the patient declines diagnostic testing and is continuing the pregnancy, what percentage of the time would you offer the following for follow-up testing?

a. Maternal Blood Karyotype
b. Maternal X,Y FISH
c. Postnatal Evaluation
d. Other

62. If you would like to provide more information on your counseling/testing strategy for this scenario please do so below. (Free response)

63. How comfortable do you feel discussing a positive cell free DNA result for a sex chromosome abnormality with a prenatal patient?

	Extremely Uncomfortable	Somewhat Uncomfortable	Neither Comfortable nor Uncomfortable	Somewhat Comfortable	Extremely Comfortable
45,X					
47,XXX					
47,XXY					
47,XYY					
Other: SCA					

64. Which of the following do you take into consideration when deciding what to discuss and offer when counseling a prenatal patient about a positive cell free DNA result for the below sex chromosome abnormalities? Select all that apply.

	45,X	47,XXX	47,XXY	47,XYY
Lack of Physical Findings				
Risk of Mosaicism				
Potential to Uncover a Maternal SCA				
Potential to Uncover a Paternal SCA				
Other				
I Offer the Same Options to All My Patients with a cfDNA Result Positive for a SCA				

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VITA

Lauren Elizabeth Fleddermann was born in Albuquerque, New Mexico to Elizabeth and Charles Fleddermann. After completing highschool at Sandia Preparatory School in Albuquerque, New Mexico in 2012, she began her undergraduate education at St. Olaf College in Northfield, Minnesota. She received a Bachelor of Arts degree in biology in June of 2016. In August 2016 she entered The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences Genetic Counseling Program and graduated in May 2018.

Permanent address:

725 Montclaire Dr NE

Albuquerque, NM 87110