PATIENT UPTAKE RATES OF TARGETED VERSUS GENOME-WIDE CELL-FREE DNA SCREENING

By

OLGA LATOSH

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Elena Ashkinadze

And approved by

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ABSTRACT OF THE THESIS

Patient uptake rates of targeted versus genome-wide cell-free DNA screening

By OLGA LATOSH

Thesis Director:

Elena Ashkinadze

Cell-free DNA (cfDNA) screening has been offered to pregnant women for the last decade to screen for aneuploidy. While professional societies have recommended this technology be used to screen for aneuploidies involving chromosomes 21, 18, and 13, and have recommended informing women of its ability to screen for sex chromosome aneuploidies and select copy number variants, there is a genome-wide platform available clinically that is not yet endorsed for routine use by these societies. Our retrospective chart review sought to assess uptake rates of cfDNA screening with a specific focus on patient motivations for selecting targeted versus genome-wide cfDNA screening. In summary, low-risk patients are more likely to either decline cfDNA screening or to opt for the targeted platform, while high-risk patients are more likely to opt for the genome-wide platform. When reviewing data from patients with ultrasound findings, most patients who decline cfDNA screening pursue invasive testing instead. In contrast, patients that opt for cfDNA screening in the presence of ultrasound findings are more likely to choose the genome-wide platform, possibly to get as much information as possible without the risk associated with invasive diagnostic testing. This study also sought to evaluate whether abnormal noninvasive prenatal screening (NIPS) results

affected pregnancy management decisions and had clinically relevant outcomes. While our study echoed a previous study's findings that the genome-wide platform rarely affected pregnancy management, we saw that this screen may provide insight into the underlying cause of an early pregnancy loss which may help grieving patients have closure and reassurance for future pregnancies.

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Table of Contents

Introduction

Several screening and diagnostic measures are available for identifying chromosomal and genetic conditions in pregnancy. Cell-free DNA screening (cfDNA), also referred to as noninvasive prenatal screening (NIPS), is one screening measure that has been offered since 2011, historically for those considered to be high risk.[1, 2] By taking a sample of the patient's blood, laboratories can separate maternal and placental DNA to screen for aneuploidy (autosomal and sex chromosomal). Additionally, screening can include select microdeletions and copy number variants (CNVs), contingent on the platform and technologies utilized. While typically offered in the first trimester after nine to ten weeks of gestation, NIPS can be offered later in pregnancy. Since this is a screening modality with the possibility of false positives, all abnormal results should be confirmed through diagnostic testing such as a chorionic villus sampling (CVS) or amniocentesis before making decisions regarding pregnancy management.[1]

The American College of Medical Genetics (ACMG) recommends informing all pregnant patients that NIPS is the most sensitive screening option for three traditionally screened aneuploidies (chromosomes 21, 18, 13) and informing patients of the availability of the expanded use of NIPS to screen for clinically relevant CNVs, sex chromosome aneuploidies (SCAs), and additional autosomal aneuploidies. However, ACMG explicitly does not recommend NIPS to screen for genome-wide CNVs or additional autosomal aneuploidies.[2] In August 2020, the American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal-Fetal Medicine (SMFM) released a new committee opinion recommending the use of cfDNA for all pregnant women, regardless of maternal age or baseline risk. However, this

recommendation is for the common trisomies and sex chromosomes. ACOG and SMFM also do not recommend routine screening for genome-wide gains or losses with cfDNA or other autosomal aneuploidies.[1] Despite these recommendations, genome-wide NIPS tests are clinically available and accessible to patients, who must decide between the targeted NIPS and the expanded, genome-wide screening.

Several factors impact patient decision-making in regard to genetic testing. Decision-making in a prenatal setting is often influenced by patient perceptions on pregnancy termination options, perceived benefit of ability to prepare for a child with a disability, cost and insurance coverage, clinical utility, value systems, cultural traditions, religious beliefs, ability to accept uncertainty, and desire for information.[2] Numerous studies have looked at factors impacting patient decision-making in regard to pursuing carrier screening, invasive testing, and prenatal genetic testing in general.[3-7] Several studies have also looked at uptake rates of targeted cfDNA screening.[8-10] However, research looking at uptake rates and factors influencing which cfDNA screening platform patients choose is lacking.

One company's genome-wide cfDNA screen has been clinically available since 2015. Internal data from the company shows that throughout the product's first five years on the market, 25% to 30% of positive results are genome-wide only results, including autosomal aneuploidies besides 21, 18, and 13 and subchromosomal CNVs ≥7Mb.[11-13] These results would not have been detected by the company's targeted cfDNA screening platform. Outside of the company's internal research, Porat et al. conducted a retrospective study at Icahn School of Medicine at Mount Sinai in 2018 to analyze outcomes for patients with positive results from that same genome-wide platform

between November 2015 and May 2017. They were interested in better understanding the utility of these results for affecting patient management and found that 0.45% (3/671) of patients had clinically relevant results for antenatal management. Thus, testing through the genome-wide NIPS platform added little to the targeted NIPS platform, could have false-positive results, and rarely affects management.[14] This study also hopes to revisit genome-wide cfDNA screening outcomes with a more recent retrospective analysis with our institution's data.

In this paper, we present data regarding uptake rates of cfDNA screening for targeted and genome-wide platforms. In addition, we will attempt to identify trends and potential barriers for screening uptake rates in a high-volume, diverse patient population Maternal-Fetal Medicine (MFM) practice. We conducted a retrospective chart review of pregnant patients seen in the practice that were offered cfDNA screening and evaluated which screening, if any, they pursued, trends and barriers for NIPS uptake rates by platform, and outcomes of abnormal screening results.

Material and Methods

Subjects

A retrospective chart review was conducted of records from January 1, 2020 to July 1, 2020 of patients seen by MFM genetic counselors at Rutgers, Robert Wood Johnson Medical School in New Brunswick, New Jersey. Women who were seen for a genetic counseling session and offered cfDNA screening were included in this study. Based on this clinic's practice model, all patients were offered both cfDNA platforms, regardless of age or baseline risk. Charts of women who were less than 18 years of age, had multiple gestation pregnancies, or had NIPS ordered elsewhere were excluded. The number of individuals who met eligibility criteria was 624 female patients. All data was de-identified. The Rutgers University New Brunswick Health Sciences Institutional Review Board approved this study.

Data Abstraction

Initially, subjects were identified through the genetic counselors' patient logs, which tabulate patients seen and the indications. Once eligible patients were identified, the consultation summary from their genetic counseling visit(s) was reviewed. For those who met inclusion criteria, the following data was abstracted from the electronic medical record (EMR): genetic counseling provider, referral source, need for translation services, patient and partner's demographics (age, ethnic background, education level), gravida and parity status, mode of conception, insurance (commercial, government program, uninsured), result of other genetic testing (expanded carrier screening (ECS), first-trimester screening (FTS), CVS, and amniocentesis), personal history of mental health conditions, personal and/or family history of a genetic or chromosomal condition,

previous child or pregnancy with a genetic or chromosomal condition, NIPS data (indication, methodology, gestational age at draw, reason for choice, result), if patient opted for diagnostic testing after an abnormal result (if yes, the result of that testing), and outcome of the pregnancy (miscarriage, termination, live birth), if known. Indications for testing were grouped as follows: abnormal serum screen, advanced maternal age (AMA), personal or family history of chromosome abnormality, routine screening, and ultrasound findings. Consult letters and genetic testing results available through the EMR were thoroughly reviewed for the data.

Data Analysis

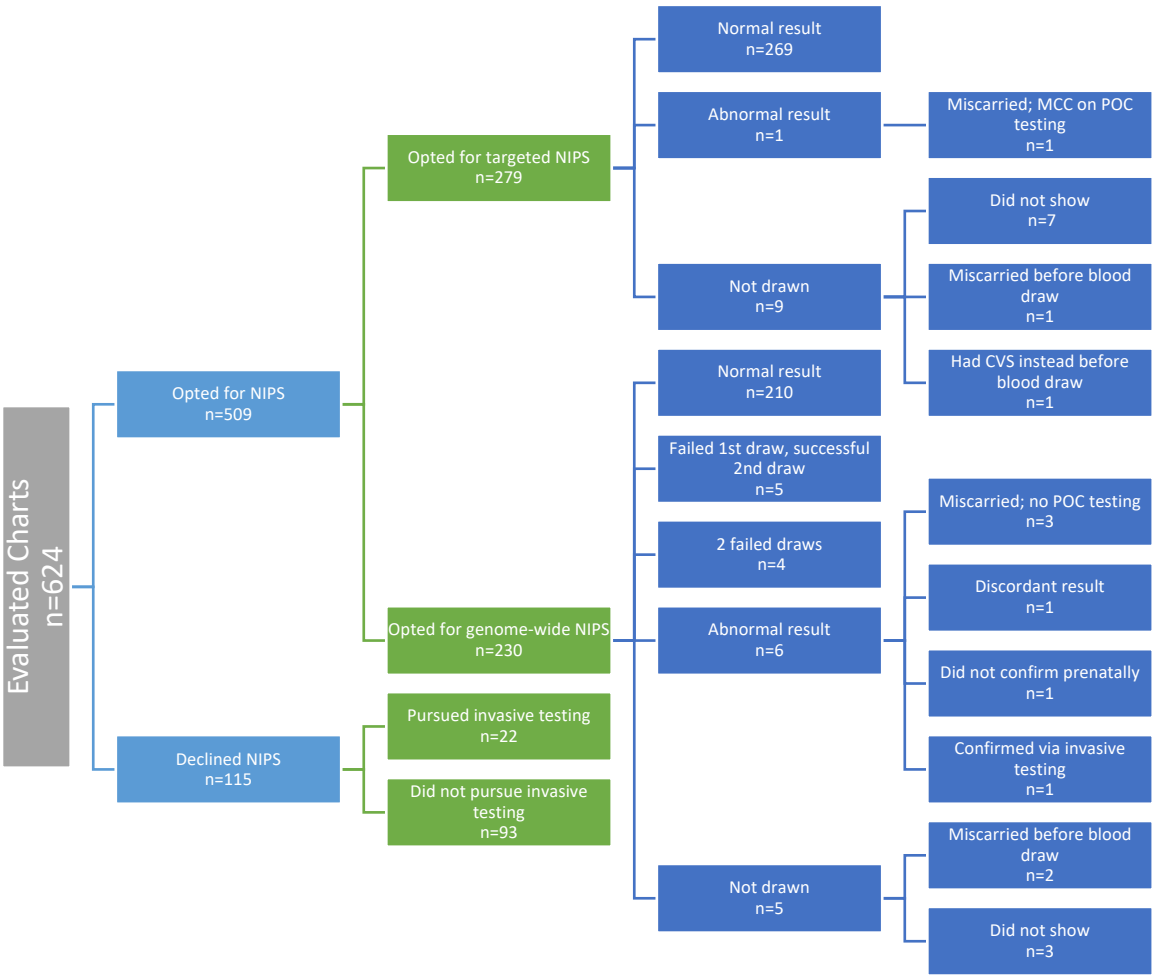
All information was collected and tabulated in Microsoft Excel. The patients were separated into two groups: those that opted for NIPS and those that declined. The group that opted for NIPS was further separated into two groups: those that opted for targeted NIPS and those that opted for genome-wide NIPS. These two groups (opted for NIPS versus declined NIPS and targeted NIPS versus genome-wide NIPS) were separately compared using the chi-square test of independence with a statistical significance set at $p < .05$ to find trends in factors that could influence patient decisions. A chi-square goodness of fit test, with a statistical significance set at $p < 0.05$, was used to see if a statistically significant difference generally existed between which platform patients chose. For each of the two groups, a false discovery rate (FDR) was calculated using p values from the chi-square tests as a multiple testing correction to see which factors were still statistically significant.[15] For patients that had a reported reason for their NIPS decision, common themes were established for each decision to analyze trends. Data

about abnormal results and their outcomes was described, but statistical analyses were not completed for this information.

Results

A total of 624 patients were included in the analysis. Of these 624 patients, 115 (18%) declined NIPS while 509 (82%) opted to pursue NIPS. This difference was found to be significant ($p < .001$), with patients being more likely to opt for NIPS (Table 1). Of those that pursued screening, 279/509 (55%) opted for targeted NIPS and 230/509 (45%) opted for genome-wide NIPS. While this difference was also found to be significant ($p = .03$) on its own, it was not significant by FDR. These choices and subsequent outcomes are summarized in Figure 1.

Figure 1 – Overview of patient decisions and subsequent outcomes



*MCC = maternal cell contamination, POC = products of conception

Table 1 – Patient uptake of NIPS

Uptakes of NIPS	Counts	Percentages
Opted for NIPS	509	82%
Declined NIPS	115	18%
Total	624	100%
χ^2 (1, N = 624) = 248.78, p < .001		

The maternal and paternal demographic information is summarized in Table 2. The majority of patients were between 30 and 34 years of age (228/624 = 37%), Caucasian (237/624 = 38%), and without a history of mental health conditions (507/624 = 81%). Most patients conceived spontaneously (588/624 = 94%) and were multigravidas (375/624 = 60%). Most patients with a reported highest level of education had a post-secondary education (128/221 = 58%). For the partners with reported demographics, the majority were also between 30 and 34 years of age (187/601 = 31%) and Caucasian (247/620 = 40%). Most patients were referred from private practices (475/624 = 76%), did not require translation services (527/624 = 84%), and were commercially insured (418/624 = 67%). For the maternal highest level of education, paternal age, and paternal ethnic background, those unspecified were excluded, so total n values are less than 624. While we hoped to include the paternal highest level of education as a variable, that information was only available for 23 individuals, so we did not include that in our data analysis.

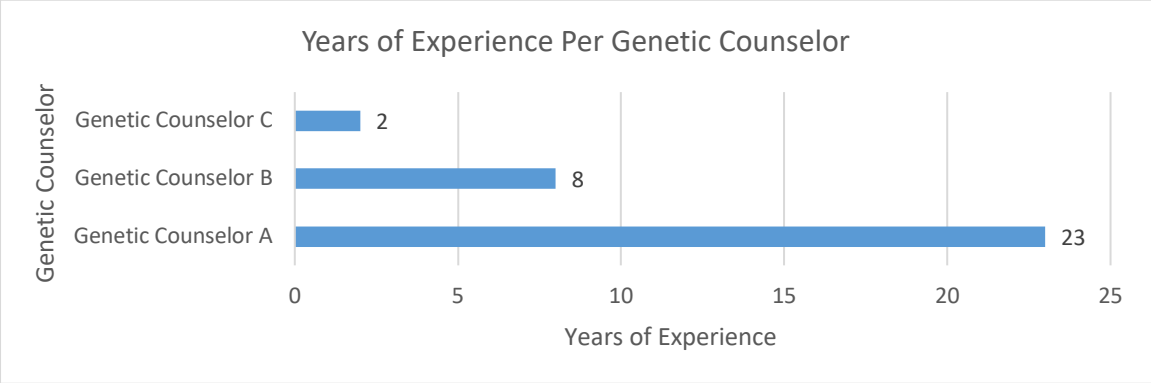
Figure 2 shows the range in years of experience for each of the three genetic counseling providers who saw patients in our time frame. The most experienced provider has been practicing for over two decades, while the other two providers have been practicing for less than one decade each.

Table 2 – Demographic information

Maternal Age	**Reported (n)**	**Percentages**
<30	174	28%
30-34	228	37%
35-39	171	27%
≥40	51	8%
Maternal Ethnic Background	**Reported (n)**	**Percentages**
Asian	93	15%
Black	55	9%
Caucasian	237	38%
Latino	183	29%
Other	56	9%
Maternal Highest Level of Education	**Reported (n)**	**Percentages**
Post-secondary	128	58%
High school	71	32%
Middle school	15	7%
Elementary school	7	3%
Unspecified	403	Not included
Gravida Status	**Reported (n)**	**Percentages**
Primagravida	173	28%
Multigravida	375	60%
Grand multigravida	76	12%
Personal history of mental health condition	**Reported (n)**	**Percentages**
Yes	117	19%
No	507	81%
Mode of Conception	**Reported (n)**	**Percentages**
Artificial reproductive technology (ART)	36	6%
Spontaneous	588	94%
Paternal Age	**Reported (n)**	**Percentages**
<30	122	20%
30-34	187	31%
35-39	172	29%
≥40	120	20%
Unspecified	23	Not included
Paternal Ethnic Background	**Reported (n)**	**Percentages**
Asian	87	14%
Black	65	10%
Caucasian	247	40%
Latino	171	28%
Other	50	8%
Unspecified	4	Not included
Referral Source	**Reported (n)**	**Percentages**
Private	475	76%
Clinic	149	24%

Translation Services Utilized?	Reported (n)	Percentages
Yes	97	16%
No	527	84%
Type of Insurance	**Reported (n)**	**Percentages**
Commercial	418	67%
Government Program	142	23%
Uninsured	64	10%

Figure 2 – Years of experience for each genetic counseling provider



Reasons for patients' NIPS choices were reported in 36% (222/624) of patients' charts. Forty-four percent (51/115) of patients that declined, 30% (84/279) of patients that opted for targeted NIPS, and 38% (87/230) of patients that opted for genome-wide NIPS gave reasons for their choices. Common themes were identified for each group and are reported in Figures 3 – 5. The most common reported reason for declining NIPS was because patients pursued invasive testing instead (21/51 = 41%). The most common reason patients opted for targeted NIPS rather than genome-wide NIPS was related to cost/insurance coverage (52/84 = 62%). For those who opted for the genome-wide platform rather than the targeted platform, being an information seeker or wanting the most amount of information in a noninvasive manner was the most commonly reported reason (70/87 = 80%).

Figure 3 – Reported reasons patients declined NIPS

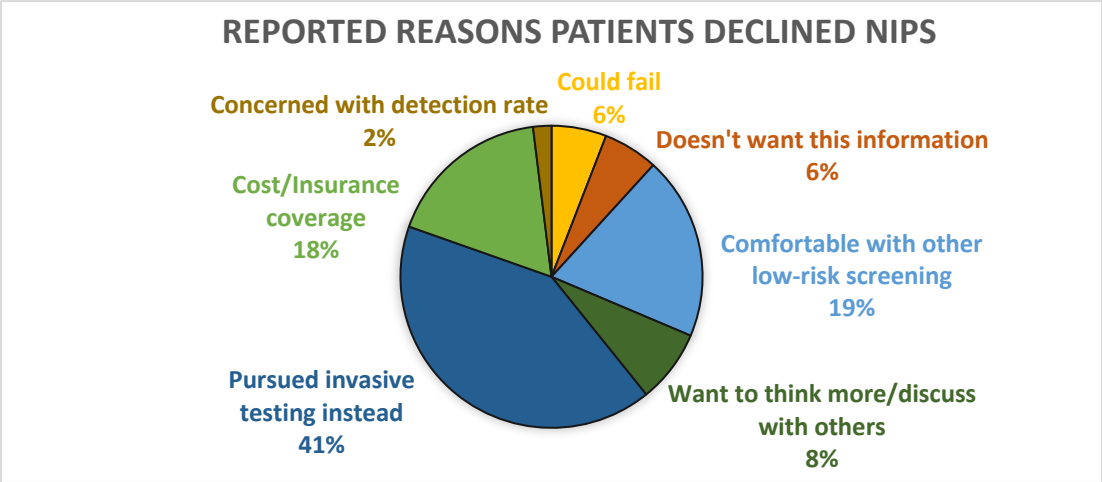


Figure 4 – Reported reasons patients opted for targeted NIPS

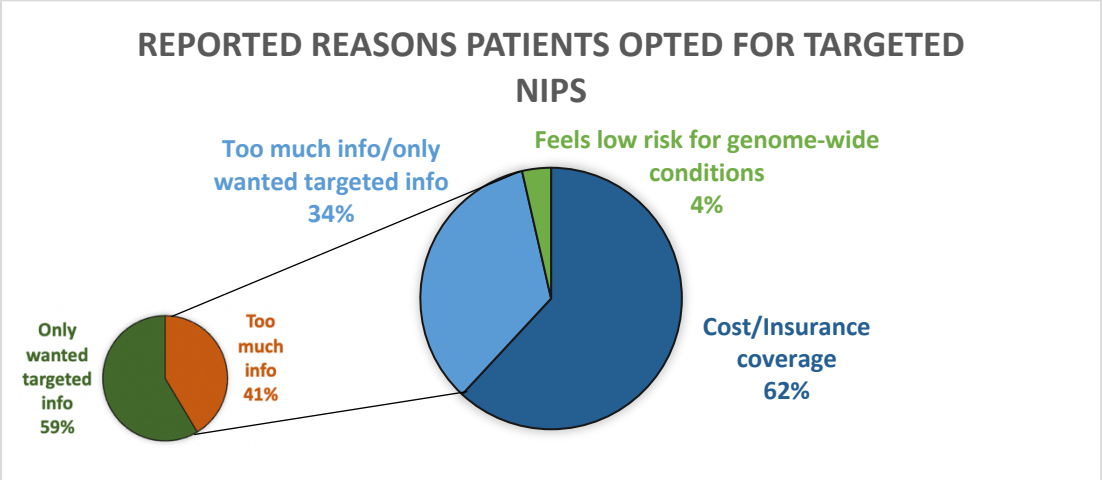
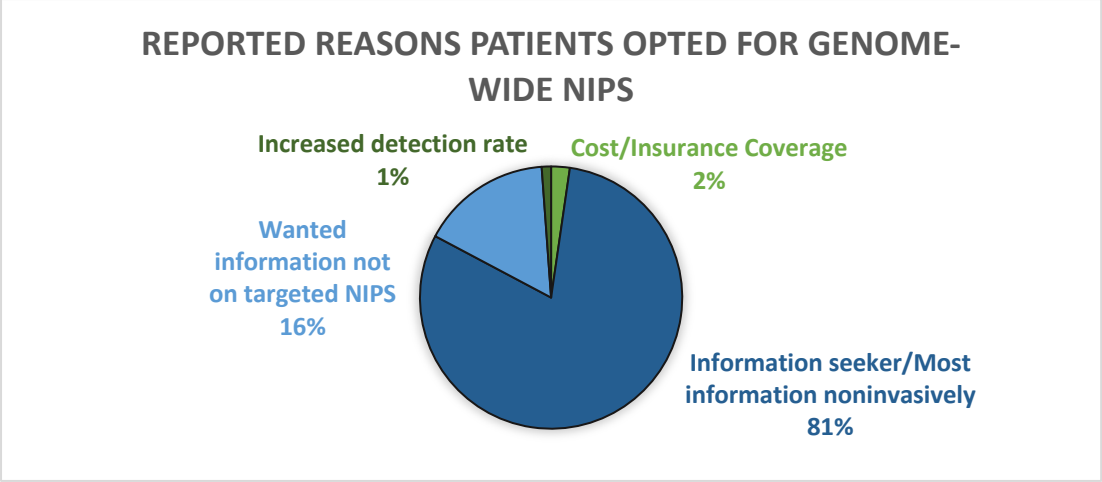


Figure 5 – Reported reasons patients opted for genome-wide NIPS



Patients That Declined NIPS versus Patients That Opted for NIPS

Patients who declined NIPS (n = 115) were compared to patients who opted for NIPS (n = 509) using several variables to assess possible factors influencing patient decision-making. The n values for the maternal highest level of education, paternal age, paternal ethnic background, and ECS uptake were lower due to this information not being available in the chart.

No statistically significant differences were found when comparing patient decisions based on referral source (p = .68), type of insurance (p = .18), maternal ethnic background (p = .21), maternal highest level of education (p = .74), personal history of mental health conditions (p = .35), paternal age (p = .18), mode of conception (p = .58), gravida (p = .77), personal/family history of a genetic condition (p = .57), personal/family history of a chromosomal condition (p = .57), previous child or pregnancy with a genetic or chromosomal condition (p = .15), or whether the patient was alone or accompanied by someone (p = .77). For statistically significant variables, which all remained significant after FDR, the data is summarized in Table 3.

Table 3 – Patient uptake of NIPS compared by specified factors

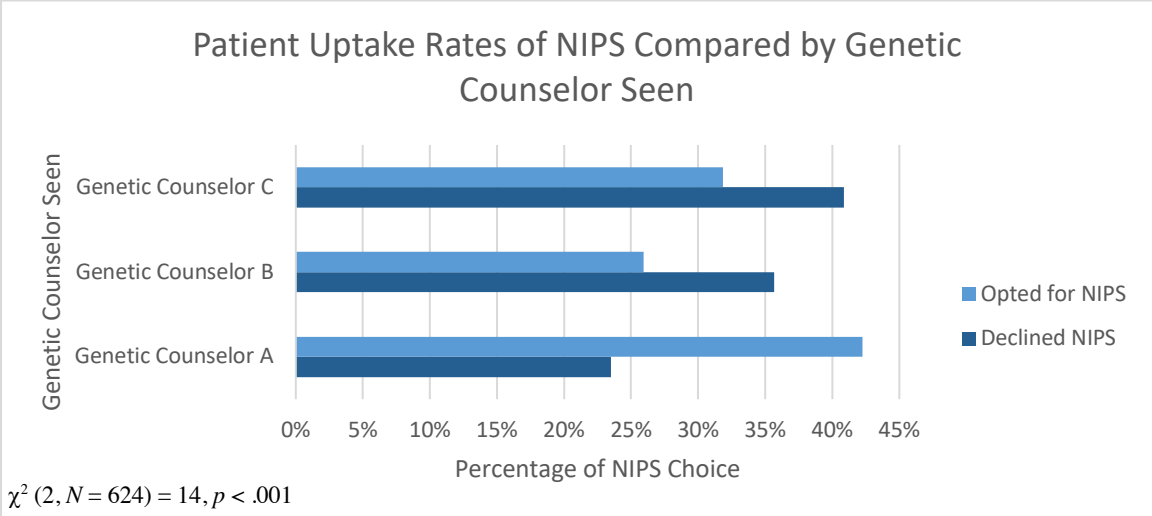
Genetic Counselor Seen	**Declined NIPS**	**Opted for NIPS**
Genetic Counselor A	27/115 (23%)	215/509 (42%)
Genetic Counselor B	41/115 (36%)	132/509 (26%)
Genetic Counselor C	47/115 (41%)	162/509 (32%)
Need for Translation Services	**Declined NIPS**	**Opted for NIPS**
No	87/115 (76%)	440/509 (86%)
Yes	28/115 (24%)	69/509 (14%)
Maternal Age	**Declined NIPS**	**Opted for NIPS**
<30	45/115 (39%)	129/509 (25%)
30-34	40/115 (35%)	188/509 (37%)
35-39	23/115 (20%)	148/509 (29%)
≥40	7/115 (6%)	44/509 (9%)
Paternal Ethnic Background	**Declined NIPS**	**Opted for NIPS**
Asian	19/114 (17%)	68/506 (13%)
Black	9/114 (8%)	56/506 (11%)

Caucasian	34/114 (30%)	213/506 (42%)
Latino	45/114 (39%)	126/506 (25%)
Other	7/114 (6%)	43/506 (8%)
Indication	**Declined NIPS**	**Opted for NIPS**
Abnormal serum screen	8/115 (7%)	37/509 (7%)
AMA	23/115 (20%)	192/509 (38%)
Personal/family history of chromosome abnormality	10/115 (9%)	40/509 (8%)
Routine Screening	64/115 (56%)	229/509 (45%)
Ultrasound findings	10/115 (9%)	11/509 (2%)
Uptake of ECS	**Declined NIPS**	**Opted for NIPS**
Opted for ECS	54/114 (47%)	400/508 (79%)
Declined ECS	60/114 (53%)	108/508 (21%)

A chi-square test of independence was used to see if significant associations existed between uptake of NIPS and the variables specified above. After FDR was applied, it was found that uptake of NIPS differed significantly based on genetic counselor seen ($p < .001$, Figure 6), need for translation services ($p = .0039$, Figure 7), maternal age ($p = .0179$, Figure 8), paternal ethnic background ($p = .0117$, Figure 9), indication for NIPS ($p < .001$, Figure 10), and uptake of ECS ($p < .001$, Figure 11).

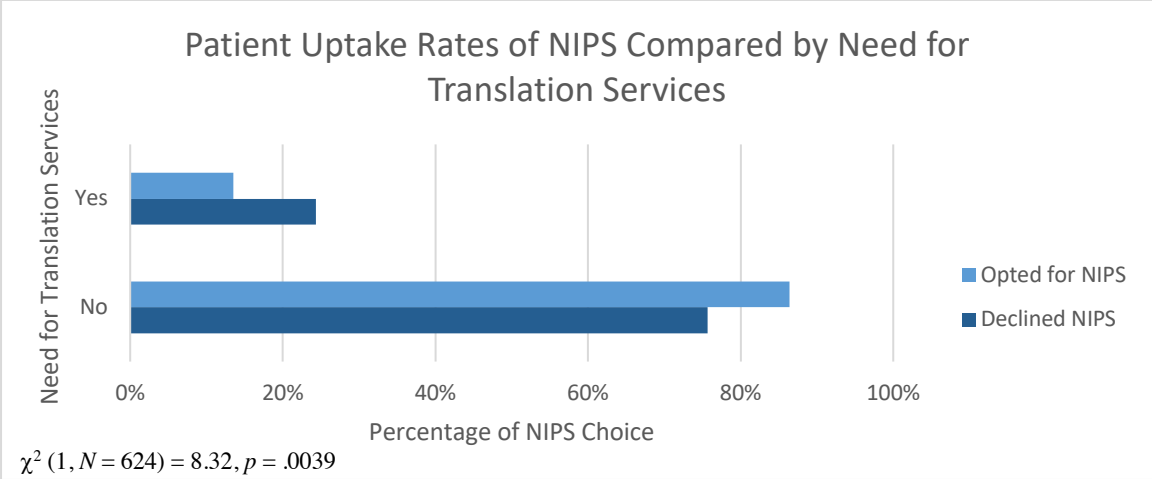
When looking at the most significant differences for uptake rates compared by these factors, it was seen that patients were less likely to decline NIPS if seen by genetic counselor A. Patients were more likely to opt for NIPS if their indication was AMA or if they also opted for ECS. On the contrary, patients were more likely to decline NIPS if they required an interpreter, were under the age of 30, had Latino partners, were offered NIPS due to ultrasound findings, or declined ECS as well. Of the ten patients that declined NIPS and had ultrasound findings, seven pursued invasive diagnostic testing.

Figure 6 – Patient uptake rates of NIPS categorized by genetic counselor seen



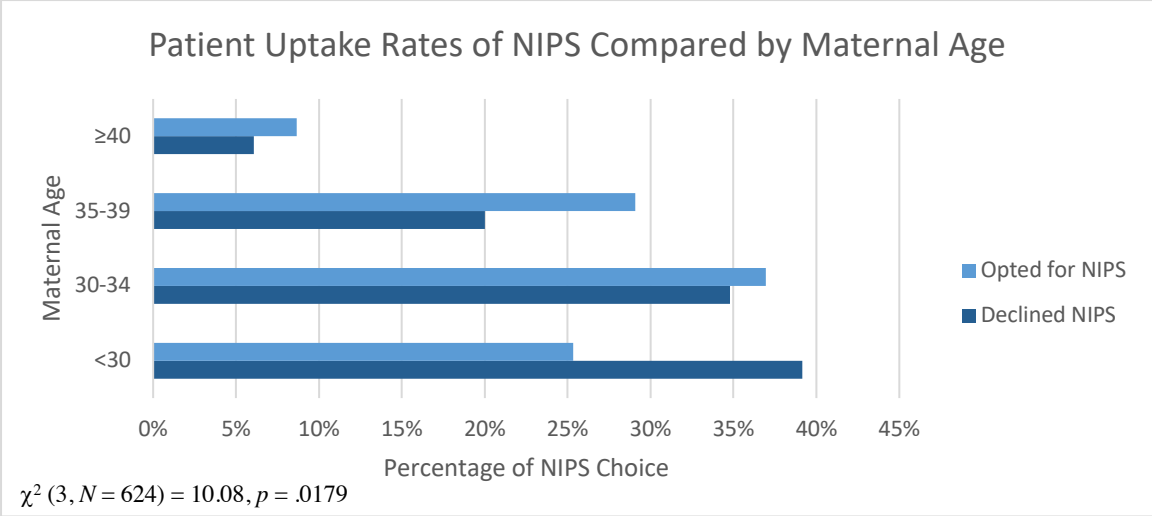
$\chi^2\,(2, N = 624) = 14, p < .001$

Figure 7 – Patient uptake rates of NIPS categorized by need for translation services



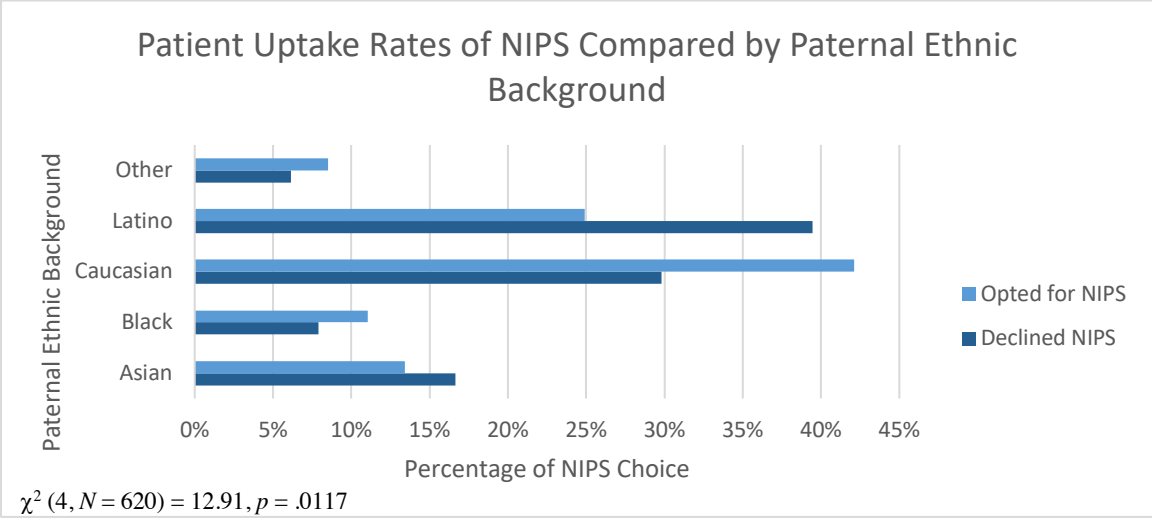
$\chi^2\,(1, N = 624) = 8.32, p = .0039$

Figure 8 – Patient uptake rates of NIPS categorized by maternal age



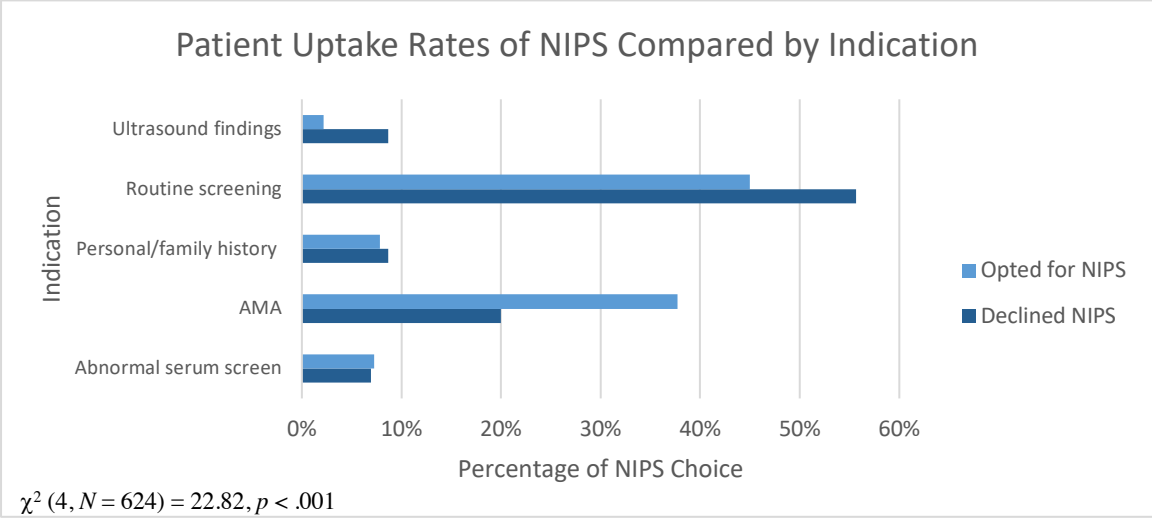
$\chi^2\,(3, N = 624) = 10.08, p = .0179$

Figure 9 – Patient uptake rates of NIPS categorized by paternal ethnic background



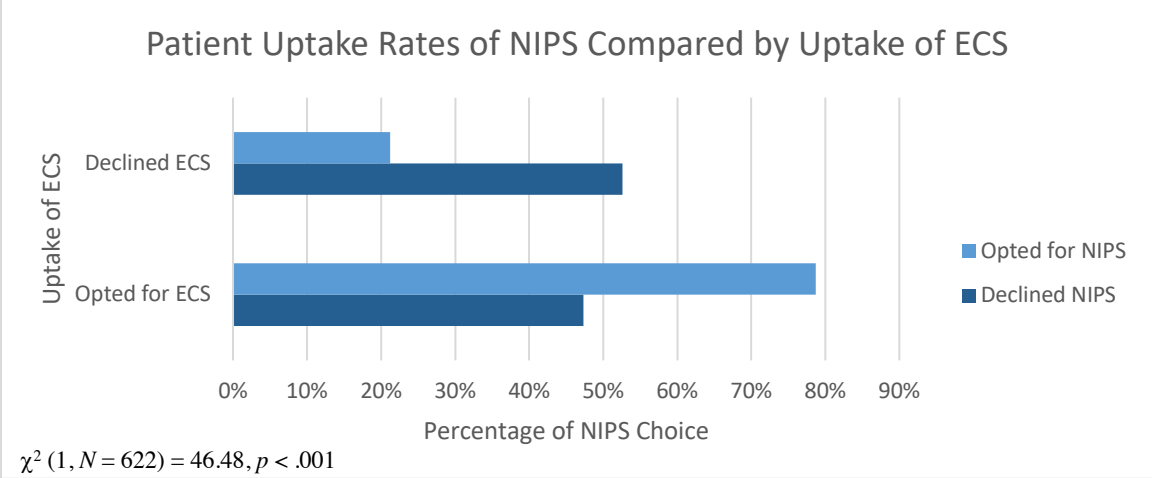
$\chi^2\,(4, N = 620) = 12.91, p = .0117$

Figure 10 – Patient uptake rates of NIPS categorized by indication for NIPS



$\chi^2\,(4, N = 624) = 22.82, p < .001$

Figure 11 – Patient uptake rates of NIPS categorized by uptake of ECS



$\chi^2\,(1, N = 622) = 46.48, p < .001$

Patients That Opted for Targeted NIPS versus Genome-Wide NIPS

Patients who opted for targeted NIPS (n = 279) were compared to patients who opted for genome-wide NIPS (n = 230) using a number of variables to assess possible factors influencing patient decision-making. Again, the n values for the maternal highest level of education, paternal age, paternal ethnic background, and ECS uptake were lower due to this information not being available in the chart. The n values for gestational age at initial blood draw are lower as well since three patients miscarried before getting drawn and eleven patients never had their blood drawn.

No statistically significant differences were found when comparing patient decisions based on referral source ($p = .59$), need for translation services ($p = .18$), type of insurance ($p = .06$), maternal ethnic background ($p = .13$), maternal highest level of education ($p = .41$), personal history of mental health conditions ($p = .70$), gravida ($p = .95$), paternal ethnic background ($p = .18$), personal/family history of a genetic condition ($p = .18$), personal/family history of a chromosomal condition ($p = .22$), previous child or pregnancy with a genetic or chromosomal condition ($p = .13$), whether the patient was alone or accompanied by someone ($p = .73$), or gestational age at initial blood draw ($p = .62$). While a statistically significant difference was found for paternal age ($p = .0207$) by the chi-square analysis, this difference was not significant by FDR. For statistically significant variables after FDR, the data is summarized in Table 4.

Table 4 – Patient Uptake of NIPS by Type Compared by Specified Factors

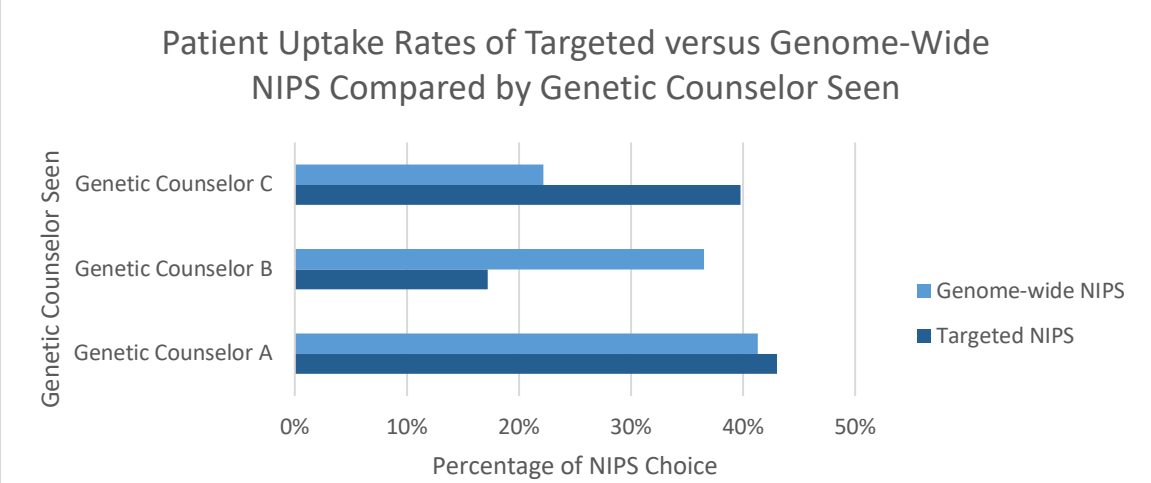
Genetic Counselor Seen	**Targeted NIPS**	**Genome-wide NIPS**
Genetic Counselor A	120/279 (43%)	95/230 (41%)
Genetic Counselor B	48/279 (17%)	84/230 (37%)
Genetic Counselor C	111/279 (40%)	51/230 (22%)
Maternal Age	**Targeted NIPS**	**Genome-wide NIPS**
<30	93/279 (33%)	36/230 (16%)
30-34	103/279 (37%)	85/230 (37%)

35-39	68/279 (24%)	80/230 (35%)
≥40	15/279 (5%)	29/230 (13%)
Mode of Conception	**Targeted NIPS**	**Genome-wide NIPS**
ART	9/279 (3%)	22/230 (10%)
Spontaneous	270/279 (97%)	208/230 (90%)
Indication	**Targeted NIPS**	**Genome-wide NIPS**
Abnormal serum screen	18/279 (6%)	19/230 (8%)
AMA	89/279 (32%)	103/230 (45%)
Personal/family history of chromosome abnormality	17/279 (6%)	23/230 (10%)
Routine Screening	153/279 (55%)	76/230 (33%)
Ultrasound findings	2/279 (1%)	9/230 (4%)
Uptake of ECS	**Targeted NIPS**	**Genome-wide NIPS**
Opted for ECS	208/279 (75%)	192/229 (84%)
Declined ECS	71/279 (25%)	37/229 (16%)

A chi-square test of independence was utilized to see if significant differences existed between uptake of type of NIPS (targeted versus genome-wide) and the variables specified above. After FDR was applied, it was found that uptake of the type of NIPS panel differed significantly based on genetic counselor seen ($p < .001$, Figure 12), maternal age ($p < .001$, Figure 13), mode of conception ($p = .0029$, Figure 14), indication for NIPS ($p < .001$, Figure 15), and uptake of ECS ($p = .0109$, Figure 16).

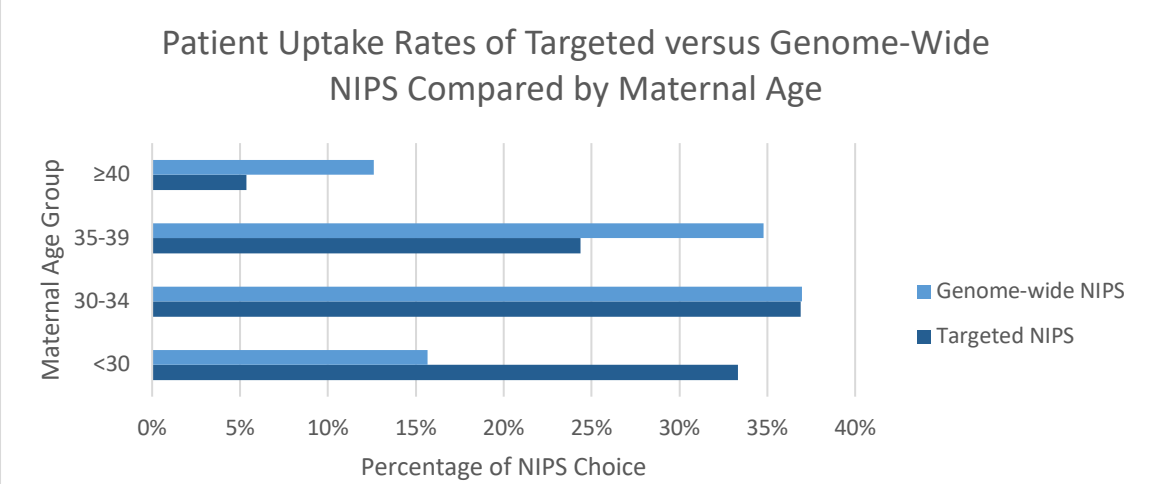
When looking at the greatest differences in NIPS platform uptake rates, patients were more likely to opt for targeted NIPS if they were younger than 30 years of age, were offered NIPS for routine screening, or declined ECS. Patients seen by genetic counselor C were less likely to opt for genome-wide NIPS. In contrast, patients that were more likely to opt for genome-wide NIPS were seen by genetic counselor B, were of advanced maternal age (age 35 and older), conceived via artificial reproductive technology, or were offered NIPS due to advanced maternal age or ultrasound findings.

Figure 12 – Patient uptake rates of type of NIPS categorized by genetic counselor seen



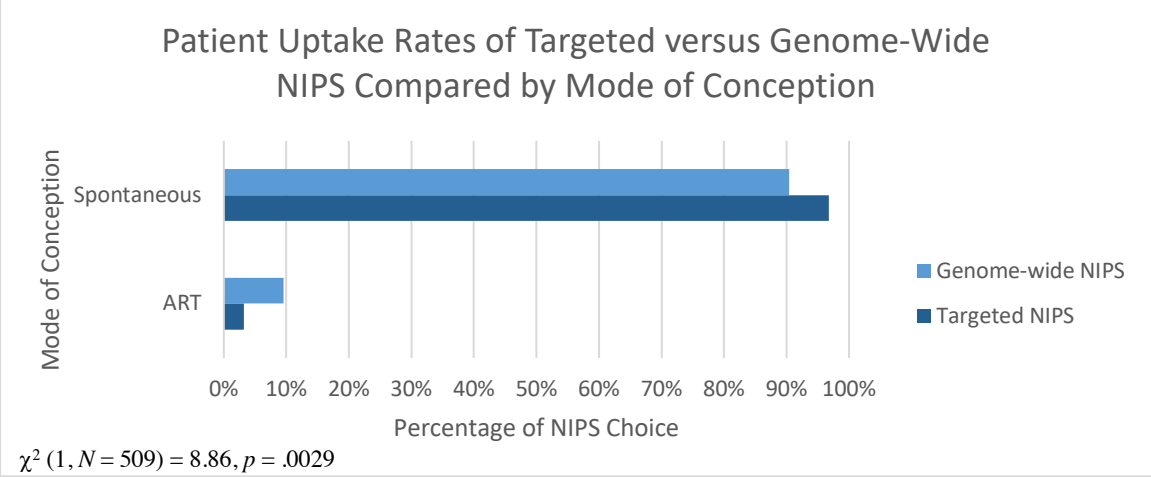
$\chi^2\,(2, N = 509) = 30.51, p < .001$

Figure 13 – Patient uptake rates of type of NIPS categorized by maternal age



$\chi^2\,(3, N = 509) = 27.88, p < .001$

Figure 14 – Patient uptake rates of type of NIPS categorized by mode of conception



$\chi^2\,(1, N = 509) = 8.86, p = .0029$

Figure 15 – Patient uptake rates of type of NIPS categorized by indication for NIPS

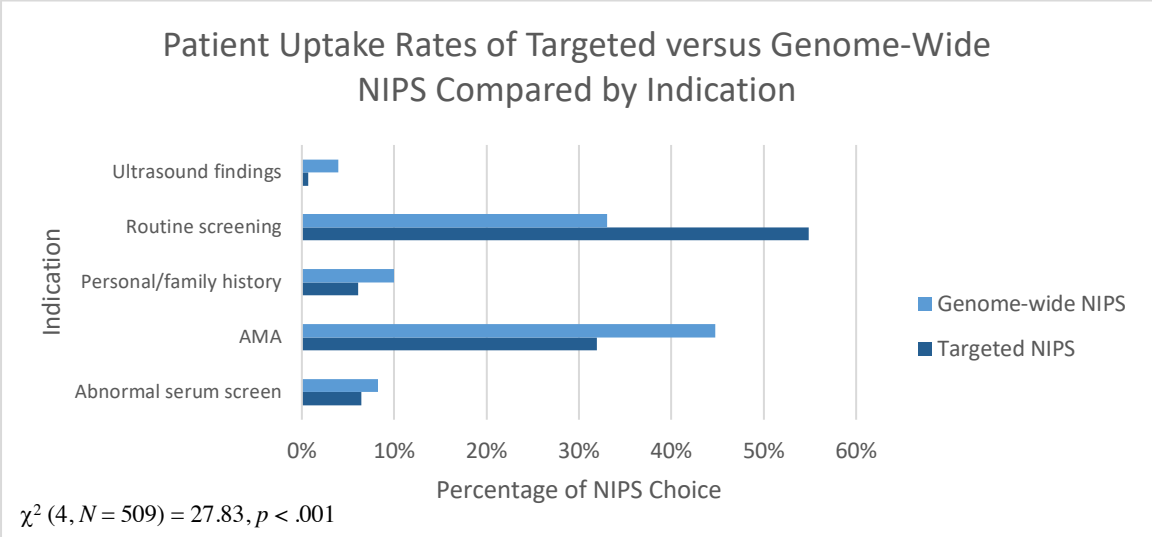
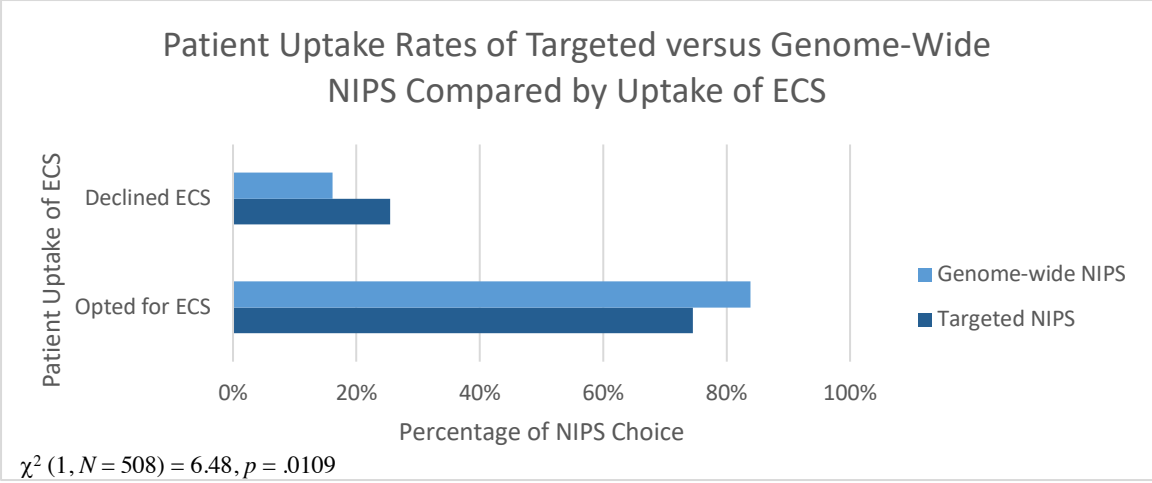


Figure 16 – Patient uptake rates of type of NIPS categorized by uptake of ECS



Abnormal Results

As shown in Figure 1, of the 495 patients that opted for NIPS and had their blood drawn, seven (1.4%) had abnormal results and nine (1.8%) a "no call" result. A no call result occurs when the lab is unable to run the test or the test fails to return a result. This could be due to factors such as a low amount of DNA in the sample. The no call results were all from the genome-wide platform. The outcomes for the screen positive results and the no call results are summarized in Tables 5 and 6 respectively.

Table 5 – Screen positive/uninformative NIPS results and subsequent outcomes

Targeted NIPS Platform			
Age (pt)	**GA at Blood Draw**	**Result**	**Outcome**
21	10 weeks, 5 days	T21, male; suggestive of mosaic T21	Desired CVS to confirm. Fetal demise measuring 8 weeks found during ultrasound. Pursued POC testing but both POC samples had maternal cell contamination.
Genome-Wide NIPS Platform			
Age (pt)	**GA at Blood Draw**	**Result**	**Outcome**
41	11 weeks, 3 days	T15, male	Fetal demise measuring 8 weeks found before results came back. No POC testing pursued.
34	20 weeks, 1 day	T21, male	Opted for NIPS following abnormal quadruple screen (1/9 risk for T21). Thickened nuchal fold present. Did not desire amnio due to risk. Transferred to high-risk clinic; had increased ultrasound surveillance in third trimester. Delivered FT male. Postnatal karyotype: 47, XY, +21.
40	11 weeks, 4 days	T18, male	Opted for NIPS due to AMA. Before results came back, FTS returned screen positive for Trisomy 18 (1/<5). Opted for CVS; multiple anomalies seen at that time. Karyotype: 47, XY, +18. Terminated pregnancy at 13 weeks.
40	10 weeks, 2 days	T15, female	Miscarried two days after blood draw. No POC testing pursued. Pursued chromosome analysis for herself and her partner, which returned 46, XX and 46, XY.
40	11 weeks, 2 days	Monosomy X, female	Miscarried two days after blood draw. No POC testing pursued.
27	12 weeks, 1 day	Negative, male; uninformative SCA due to low levels of mosaic maternal monosomy X	Opted for NIPS following absent nasal bone during first trimester ultrasound. Opted for microarray for herself following result which returned normal female dosage, no mosaicism detected. Delivered FT male. We do not know if postnatal confirmatory testing was pursued.

*pt=patient, GA=gestational age, T=trisomy, POC=products of conception, SCA=sex chromosome aneuploidy, FT=full-term, FISH=fluorescence in situ hybridization

For the seven screen positive/uninformative results, one result was from the targeted platform, comprising 0.37% (1/270) of returned targeted NIPS results. Six of the results were from the genome-wide platform, which results in a 2.8% (6/225) overall rate of abnormal genome-wide results. Two of the seven patients (29%) chose to undergo

confirmatory testing in the prenatal setting. One result was confirmed, while one was discordant. The patient whose male fetus screened positive for T21 pursued diagnostic testing after delivery, which confirmed the result as well. Four (57%) of the patients with abnormal results miscarried, one (14%) patient terminated her pregnancy after diagnostic confirmation, and two (29%) delivered full-term infants. One of the patients that delivered a full-term infant was transferred to the high-risk clinic and had additional ultrasound surveillance to monitor for fetal distress and reduce the risk of stillbirth. One of the patients that miscarried and had a fetus screen positive for Trisomy 15 pursued a chromosome analysis for herself and her husband to assess for a Robertsonian translocation, which returned normal. Overall, three results were relevant to antenatal management, representing 1.3% (3/225) of total genome-wide results and 0.60% (3/495) of all NIPS results.

Two of the six (33%) screen positive/uninformative genome-wide results, both Trisomy 15, were specific to the genome-wide platform and would not be detected by the targeted platform. This equates to 0.89% (2/225) of the total genome-wide NIPS results. Both patients miscarried before the NIPS results returned and were counseled that the NIPS-detected aneuploidy is the most likely explanation for their losses.

The nine no call results consisted of four results that had no calls for both blood draws and five results that returned low-risk results after a second blood draw. This equates to a 4% (9/225) no call rate on the first draw for genome-wide NIPS. For patients that had two draws, there was a 56% success rate. Three of the nine (33%) patients who had a no call result pursued diagnostic testing prenatally. It is not known if the six other patients pursued testing postnatally.

Table 6 – No call genome-wide NIPS results and subsequent outcomes

Maternal Age	GA at 1st blood draw	Result of 1st draw	GA at 2nd blood draw	Result of 2nd draw	Other outcome
38	9 weeks, 2 days	QNS	12 weeks, 2 days	Negative, female	No diagnostic testing pursued. Delivered pre-term female at 36w5d.
38	10 weeks, 4 days	QNS	12 weeks, 3 days	Negative, male	Pursued CVS following abnormal FTS (1/44 T21 risk, 1/36 T18 risk): normal FISH, karyotype, and microarray (46, XY). Delivered FT male.
43	12 weeks, 1 day	QNS	14 weeks, 6 days	QNS	Pursued amnio: normal FISH, karyotype, microarray (46, XX). Delivered FT female.
36	10 weeks, 5 days	QNS	12 weeks, 4 days	Negative, male	No diagnostic testing pursued. Delivered FT male.
18	16 weeks, 4 days	QNS	18 weeks, 5 days	QNS	No diagnostic testing pursued. Delivered PT female at 30 w3d due to PPROM.
35	13 weeks, 0 days	QNS	14 weeks, 6 days	Negative, female	No diagnostic testing pursued. Delivered FT female.
29	10 weeks, 6 days	QNS	12 weeks, 6 days	QNS	Has high BMI. Delivered stillborn female at 41 weeks, 2 days. Microarray on POC returned as normal female dosage, with negative maternal cell contamination.
31	17 weeks, 4 days	QNS	19 weeks, 4 days	QNS	Has high BMI. No diagnostic testing pursued. Delivered FT male.
32	13 weeks, 4 days	Not reportable due to technical or sample error.	16 weeks, 1 day	Negative, female	No diagnostic testing pursued. Delivered FT female.

*QNS=quantity not sufficient, PT=pre-term, PPROM=preterm premature rupture of the membranes, BMI=body mass index

Discussion

Our study sought to assess uptake rates of cfDNA screening with a specific focus on patient motivations for selecting targeted versus genome-wide cfDNA screening. Overall, our study found that patients were more likely to opt for cfDNA screening than to decline it. For those that did opt for screening, no overall statistically significant difference was found in terms of which platform was chosen after FDR. Factors that may predict declining cfDNA screening include younger age and comfort with other low-risk screening results, need for translation services, declining other comprehensive and optional screening, and choosing to pursue invasive testing instead due to ultrasound findings. For patients that did opt for NIPS, the targeted platform was more likely to be chosen by those under 30 being offered this for routine screening, with financial concerns or wanting targeted information (Down syndrome risk or fetal sex) as commonly reported reasons. Factors that may predict opting for the genome-wide platform rather than the targeted platform include a patient being of advanced maternal age, conceiving through artificial reproductive technologies, or having ultrasound findings but not desiring invasive testing.

Based on our sample, cost/insurance coverage was a common reason cited for declining cfDNA or choosing the targeted platform over the genome-wide platform. While it would be expected for this to be an important factor and a barrier for uptake based on previous studies,[16, 17] it was not found to be a statistically significant variable in either of our uptake rate groups. This finding is likely due to our uninsured patients and our patients insured through government programs not having a significant difference in

out-of-pocket costs between the two types of NIPS panels. However, this may differ at other institutions.

When looking at patients that conceived via artificial reproductive technology, our data showed that these patients were more likely to opt for the genome-wide screen over the targeted screen than expected. Of the thirty-one patients that opted for NIPS and conceived via ART, twelve (39%) had preimplantation genetic testing (PGT), and all twelve opted for the genome-wide platform. These twelve patients account for 55% (12/22) of patients that opted for genome-wide screening and conceived via ART. Patients that pursued PGT were counseled that aneuploidy was screened for during that process, so the targeted platform adds little to testing that was already completed. The genome-wide platform, however, can screen for some CNVs and microdeletions, which would be additional information. Given this patient population had trisomy screening on the embryos and their a priori risk reduced, they may have wanted to use cfDNA screening as a tool to assess for sporadic CNVs that could contribute to an abnormal phenotype.

Our study also found differences in patient decision-making based on individual genetic counselor providers that differed in years of experience, and whether translation services were necessary. Patient decisions may be influenced by the styles, affects, and own opinions of those providing patients with information, which can contribute to these findings.[18-20] Each genetic counselor also typically saw different patient populations that varied based on factors that were studied, which may be confounding variables that further contribute to these findings. Despite the overall significant differences in this and

cited studies, it is important not to generalize these findings or stereotype since individuals differ in their values, needs, and motivations when it comes to genetic testing.

All patients in this cohort received genetic counseling prior to cfDNA selection. Patients were counseled regarding age related aneuploidy risk and the 1.6% rate of clinically significant copy number variants, regardless of maternal age.[21] Diagnostic testing was explained in terms of benefits, risks and limitations. CfDNA screening via targeted versus genome-wide platforms was compared in terms of test performance characteristics and limitations of known positive and negative predictive values for genome-wide cfDNA screening. Thus, the strength of this study was that patients were fully informed prior to making their decision about cfDNA and all patients, regardless of risk, were offered the same screening options.

This study also sought to evaluate whether abnormal NIPS results affect pregnancy management decisions and have clinically relevant outcomes. In our study, 4% of first-pass samples were no calls, which is higher than the 2.9% rate from a 2020 study by Kleinfinger et al., but lower than the 4.35% rate from a 2021 study by Soster et al.[22, 23] Our study had a 56% success rate on redrawn specimen, which is lower than the 70.5% success rate reported by Soster et al.[23] We found that 33% of our screen-positive/uninformative results were unique to the genome-wide platform, which is comparable to the 25% to 30% reported by one laboratory and mirrors past findings that the majority of genome-wide positive results are common trisomies or sex chromosome aneuploidies.[11, 12, 23] In our study, for the patients that opted for the genome-wide platform, the three most common indications were as followed: 45% AMA, 33% routine screening, 10% personal/family history. This contrasts Soster et al.'s findings which saw that the

most common indications were: 52.9% AMA, 16.7% ultrasound findings, 9.0% routine screening.[23]

Our findings slightly contrast those of Porat et al., with our study having about half the rate (0.89% versus 1.6%) of genome-wide specific results, a smaller percentage choosing the genome-wide screen over the targeted screen (45% versus 58%), a lower rate of discordant results (50% versus 83%), and about triple the percentage of results relevant to antenatal management (1.3% versus 0.45%).[14] The lower percentage of patients opting for the genome-wide screen may be because all patients were offered that screen in our study, while only high-risk patients were offered the genome-wide screen in theirs. Based on this data alone, our study echoes Porat et al.'s conclusion that genome-wide results rarely affect pregnancy management.

Despite echoing Porat et al.'s findings, our study indicates that genome-wide cfDNA screening may have personal utility for patients experiencing first-trimester pregnancy losses. Three of our patients that pursued genome-wide cfDNA miscarried between the time of their blood draw and their screen positive results. These results, a Monosomy X and two Trisomy 15s, likely explain their losses, with two of the three results being specific to the genome-wide platform. Nikčević and Nicolaides found that having information related to the cause of a pregnancy loss helps women find meaning and adjust psychologically.[24] Since about 50% of early pregnancy losses are due to chromosomal abnormalities, it would be expected that genome-wide NIPS can provide insight into the cause of the miscarriage which help the grieving patient have closure and reassurance for future pregnancies as miscarriages due to sporadic aneuploidy pose the lower risk for recurrence.[25, 26]

Study Limitations and Research Recommendations

Since this was a retrospective chart review, our data relies on reported information from patient charts in EMR for women seen for genetic counseling at a single institution. This sample was identified based on specific criteria, which is not representative of the general population of pregnant patients. Our small sample size had few abnormal results, making generalizations for genome-wide results and comparisons to Porat et al.'s study limited. While this institution offers targeted and genome-wide cfDNA screening for all patients, those deemed to be high-risk based on age, ultrasound findings, or family history may be more likely to be referred to genetic counseling, which may skew our results. Each variable was also treated independently, but it is possible that some studied variables could be confounding variables for other studied variables.

A large, prospective multi-center study over a longer time period that offers all pregnant patients the option of targeted or genome-wide cfDNA would be of interest. Such a study would allow us to track uptake rates over time, which may change as ACOG recommendations are modified in the future. To elucidate further information regarding motivations for their decisions, it may be helpful to offer a survey or to interview patients following their genetic counseling appointments. We hope that future studies analyzing factors influencing uptake rates of genome-wide cfDNA screening can help providers facilitate patient decisions that best align with patients' needs and motivations, as well as identify and address potential barriers for the uptake of cfDNA screening.

Future studies with genome-wide platforms should offer screening as early as possible, around nine or ten weeks of gestation, to see if other likely causes for first

trimester miscarriages could be discovered. For these women, a separate study with a survey or interview six to twelve months after the loss would help evaluate the personal utility of the genome-wide screen and assess whether this information helped women adjust and cope with the loss.

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