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Performance of single-gene noninvasive prenatal testing for autosomal recessive conditions in a general population setting

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Abstract

Objective: Carrier screening with reflex to single-gene noninvasive prenatal testing (sgNIPT) is an alternative approach for identifying pregnancies at risk for inherited autosomal recessive conditions without the need for a sample from the reproductive partner. This study is the largest clinical validation of this approach in a general population setting.

Methods: The clinical performance of carrier screening with reflex to sgNIPT for cystic fibrosis, spinal muscular atrophy, alpha thalassemias, and beta hemoglobinopathies was assessed by collecting pregnancy outcome data on patients who underwent this testing and comparing the neonatal outcome to the assay-predicted fetal risk.

Results: Of 42,067 pregnant individuals who underwent screening, 7538 carriers (17.9%) had reflex sgNIPT, and neonatal or fetal outcomes were obtained for 528 cases, including 25 affected pregnancies. Outcomes demonstrated high concordance with sgNIPT, for example, all pregnancies with 9 in 10 personalized fetal risk results were affected (positive predictive value (PPV) of 100% for the sub-group) and the sgNIPT assay showed a sensitivity of 96.0% (95% CI: 79.65%–99.90%), specificity of 95.2% (95% CI: 92.98%–96.92%), average PPV of 50.0% (95% CI: 35.23%–64.77%), and negative predictive value (NPV) of 99.8% (95% CI: 98.84%–99.99%). The end-to-end performance of carrier screening with reflex to sgNIPT was calculated to have a sensitivity of 92.4% and specificity of 99.9%, which are unaffected by partner carrier screening or misattributed paternity unlike a traditional carrier screening workflow, which has a 35% sensitivity and a maximum of 25% PPV (1 in 4) in a real-life setting.

Conclusion: This study builds upon earlier findings to confirm that carrier testing with reflex to sgNIPT is highly accurate for general population screening. Given this high accuracy and an NPV of 99.8%, this workflow should be considered as an option for most of the general pregnant population. When the biological partner sample is unavailable, this workflow should be recommended as the first-line approach.

Key points

What is already known about this topic?

- The utility of single-gene NIPT to assess fetal risk of autosomal recessive (AR) conditions without the need for a partner sample is established.

What does this study add?

- This study replicates and builds on a prior validation study by increasing the sample size four-fold and restricting inclusion to general risk pregnancies—the population for which the test is intended.
- This study demonstrates the clinical validity of a quantitative approach to sgNIPT, demonstrating high sensitivity and specificity to identify affected pregnancies as high risk and unaffected pregnancies as low risk.

1 | INTRODUCTION

Reproductive carrier screening is an important step in determining the risk for a couple to have a pregnancy affected with recessive genetic conditions. The American College of Obstetricians and Gynecologists (ACOG) recommends offering all people considering a pregnancy or who are currently pregnant carrier screening for cystic fibrosis (CF), spinal muscular atrophy (SMA) and beta and alpha hemoglobinopathies (HBB and HBA).[1] Carrier screening maximizes reproductive choices when completed prior to pregnancy, but the vast majority of carrier screening occurs during pregnancy, increasing the urgency of timely screening.[2,3] Carrier screening is further complicated by the need for reproductive partner screening when an individual is identified to be a carrier to provide the complete risk assessment. However, fewer than half of the partners complete the recommended screening due to barriers related to cost, availability, and willingness.[2,4,5] As a result, reproductive management decisions are made with incomplete information. In addition, healthcare inequities may be exacerbated by this traditional approach to carrier screening as many people lack access to preconception healthcare, and underserved populations are those in which reproductive partners may be least likely to undergo carrier screening.[6–11]

The introduction of carrier screening with reflex to single-gene noninvasive prenatal testing (sgNIPT) has the potential to resolve many of the limitations of traditional carrier screening. When a pregnant person is identified to be a carrier of one or more conditions, sgNIPT evaluates fetal cell-free DNA (cfDNA) to determine whether the pregnant person's pathogenic variant was inherited by the fetus and—via next-generation sequencing (NGS) of exons—detects pathogenic variants inherited from the reproductive partner. Single-gene NIPT returns a personalized risk estimate for the fetus to be affected with the condition(s). This testing is typically completed in 9–16 days with no need for a sample from the reproductive partner, compared to a timeline of up to six weeks or more to complete traditional sequential carrier screening.[5,12]

A prior publication provided the initial clinical validation of sgNIPT for AR conditions. It included a clinical sample of over 1600 carriers and demonstrated sgNIPT to have a sensitivity of 93% to detect an affected pregnancy as high risk. Additionally, the personalized risk estimate of sgNIPT was well-correlated with the outcomes.[13] In the current study, the sample size was increased over four-fold, resulting in a more robust and therefore more accurate analysis. More than 75 outcomes were collected for each of the conditions screened, allowing for the investigation of the condition-specific test performance. Most importantly, for the first time, this study validates the performance of this workflow in a general population setting, that is, the intended use population, and the analysis is not affected by the inclusion of cases where both partners were already known to be carriers. The results indicate that carrier screening with reflex to sgNIPT is highly accurate for general population screening and that it should be considered as an approach to first-line screening for AR conditions.

2 | METHODS

2.1 | Sample

The following eligibility criteria were used to identify the study sample: (1) a unique pregnancy in which the pregnant individual was identified to be a carrier of one or more conditions included on the UNITY Carrier Screen, (2) the pregnant person was not part of a high-risk couple (both partners known carriers), (3) the sample was received between July 1, 2021 and December 1, 2022 and the estimated due date was prior to December 1, 2022 for carriers of CF, HBB and/or HBA; (4) the sample was received between February 1, 2022 and January 1, 2023 and the estimated due date was prior to January 1, 2023 for SMA carriers. A different time period was used for SMA carriers to include samples that were analyzed on the current version of the assay. Samples not eligible for sgNIPT were also excluded. These include pregnant individuals with multiple gestations, pregnancies conceived via donor eggs or gestations carried by a surrogate, or pregnancies at less than 10 weeks gestation.

2.2 | Carrier screening with reflex sgNIPT

The UNITY carrier screening with reflex to sgNIPT is a two-step process. First, carrier screening is completed via NGS of all exons, exon-intron junctions, and select intronic regions of the *HBB*, *CFTR*, *HBA1* and *HBA2* genes and copy number analysis of *CFTR*, *SMN1*, *HBA1*, *HBA2*, and *HBB* genes. The alpha-thalassemia carrier screen reports single and double gene deletions, including alpha 3.7, alpha 4.2, SEA, MED-I, SA, 20.5, BRIT, FIL, and THAI. The SMA carrier screen reports *SMN1* silent carrier-linked SNP g.27134 T > G (rs143838139) when two copies of *SMN1* are present.[13]

If the pregnant individual is identified to have one or more pathogenic variants (is a carrier), the second step—sgNIPT—is completed. A sample from the reproductive partner or knowledge of the partner's carrier status is not needed. The methodology for sgNIPT for AR conditions has been described in detail in prior publications.[13,14] Briefly, sgNIPT estimates the likelihood of the fetus having two pathogenic variants and therefore risk to be affected with the AR condition. This likelihood is computed using the following: fetal fraction (FF) of the cfDNA, which is determined through the analysis of multiple polymorphic loci to quantify the paternal allele fraction; molecular counts of the cfDNA from the gene of interest using quantitative counting templates (QCT); identification of non-maternal pathogenic variants through amplicon-based exon sequencing of the *CFTR*, *HBB*, *HBA1*, and *HBA2* genes; and the allele fraction of the maternal variant, which is calculated by dosage analysis of *HBB* exon 1, *CFTR* p.Phe508del (NM_000492.3:c.1521-1523del), and *SMN1* copy number.

The computed likelihood ratio and the *a priori* risk based on the US general population condition-specific prevalences are used to calculate the personalized risk estimate for the fetus (Table S1). When the estimated quantitative risk for the fetus to be affected is greater than the *a priori* risk, a high risk (greater than 1 in 4) or increased risk designation is reported. When the estimated quantitative risk is less than the *a priori* risk, a low risk (<1 in 500) or decreased risk designation is reported. The patient report includes both the quantitative risk and the qualitative risk, and for HBA conditions includes a risk estimate for individuals with and without Asian ancestry. The maximum risk of the sgNIPT screen is 9 in 10, and most low-risk pregnancies have a risk at least 20 times lower than the condition *a priori*, which would correspond to a minimum of 95% sensitivity (Table S2).

2.3 | Outcome collection

The fetal and neonatal outcomes for the pregnancies that had sgNIPT were collected through the Quality Assurance (QA) program and IRB approval was obtained to publish the aggregate results of this program (WCG IRB 13472102). Outcomes were solicited from the ordering provider and/or the patient by the QA team (genetic counselor or nurse) at least a month after the patient's estimated due date. The ordering provider was contacted first for pregnancies that sgNIPT identified as high risk of one or more conditions. The fetal or neonatal outcome was collected when available. If the provider was unaware of the pregnancy outcome for the condition of interest, the study team contacted the patient by phone, email, and/or text. When pregnancies were identified as decreased or low risk of one or more conditions, the patient was contacted by text to complete a survey about the outcome of their pregnancy. Up to four attempts were made to reach a patient to collect the outcome. Patients received a $10–$40 gift card for sharing the outcome of their pregnancy. A small number of outcomes were obtained when the provider and/or patient contacted the laboratory with an unsolicited outcome of the pregnancy.

2.4 | Concordance determination

Fetal or neonatal outcome determinations of affected or unaffected patients were informed by published accepted diagnostic criteria.[15–19] The following factors were considered when determining the outcome: newborn screening (NBS) results, molecular testing (prenatally or postnatally), and diagnostic laboratory testing (Table S3).

In clinical practice, the laboratory assigns a qualitative risk to each quantitative risk, which is specific to the *a priori* risk of the condition. Both the quantitative and qualitative results are included in the test report. However, for the purposes of this analysis and in prior analyses, a single threshold of 1 in 100 was used, which is a conservative approach as it is higher than the condition-specific *a priori* risk of pregnant people identified as carriers for three of the four conditions, but does not falsely inflate specificity.[13] In this analysis, cases with a post-test risk estimate of greater than 1 in 100 were classified as screen positive and those with a risk estimate of less than or equal to 1 in 100 were classified as screen negative. Cases were classified as concordant when the fetus or neonate was affected and the sgNIPT risk was greater than 1 in 100 (screen positive), and when the fetus or neonate was unaffected and the sgNIPT risk was less than or equal to 1 in 100 (screen negative). Cases were classified as discordant when the fetus or neonate was unaffected and the sgNIPT risk was greater than 1 in 100, and when the fetus or neonate was affected and the sgNIPT risk was less than or equal to 1 in 100. When the fetal or neonatal outcome was unknown or there was not sufficient information to make an outcome call, concordance was unknown.

2.5 | Analysis

Cases where a concordance call was made were included in the outcome cohort. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the sgNIPT assay and correlation of PPV with sgNIPT fetal risk were computed for the outcome cohort. The Clopper-Pearson 95% confidence intervals were computed for sensitivity, specificity, PPV, and NPV. The end-to-end clinical analytics of carrier screening with reflex to sgNIPT were estimated for the full eligible cohort according to the methods described in a prior publication and compared to the previous calculation of the sensitivity of traditional carrier screening according to published practices in real-life settings.[13] Finally, the estimated number of

affected fetuses identified as high risk by expanded carrier screening (ECS) was compared to carrier screening with reflex to sgNIPT in a hypothetical sample of 100,000 pregnant individuals. To estimate the affected fetuses identified as high risk by ECS in a real-life scenario, 100,000 was multiplied by the published estimated frequency of HRC for a clinically available panel of 163 AR conditions,[20] and then reduced to account for those cases that would not be identified based on published rates of non-paternity and partners not following up on testing,[2,4,5,21] and divided by 4, as approximately 1 in 4 HRC has an affected pregnancy. To calculate the number of affected fetuses identified in 100,000 pregnant individuals, 100,000 was multiplied by the published estimated frequencies of HRC for SMA, CF, HBB and HBA, divided by 4, and multiplied by the calculated sgNIPT sensitivity.

3 | RESULTS

3.1 | Eligible cohort and outcome cohort characteristics

A total of 42,067 patients underwent UNITY carrier screening during the study period and 7538 (17.9%) were identified as carriers with 8268 sgNIPT eligible cases (there were 713 individuals who were carriers for two or more conditions). The average turnaround time for carrier screening that did not require sgNIPT reflex was 9 and 16 days when sgNIPT was completed. 95% of cases that reflex to sgNIPT are reported within 25 days and outlying turnaround times are edge cases due to delays in receipt of missing clinical information relevant to the testing (e.g. date of draw or gestational age [GA], requests for additional testing after the sample was received, and/or the clinician providing additional clinical information that requires fetal risk re-calculation). All conditions had carrier frequencies greater than those recorded for the US general population, suggesting a clinician bias in submitting cases where the pregnant person was known to be a carrier. However, all known HRC (where both partners were known to be carriers) were removed, therefore, despite the enrichment of carrier individuals, the sample is representative of the general-risk carrier population (Table 1).[22]

A negative carrier result or sgNIPT fetal risk was returned for 98.9% (n = 41,621) of the total samples referred for carrier screening. Of the identified carriers, an informative sgNIPT result was returned to 99.1% (n = 7092/7154) of perfect use cases (those who submitted all requested samples), demonstrating a less than 1% no results rate for sgNIPT. For all carriers, including 384 who did not provide the requested redraw, an informative result was returned for 94.1% (n = 7092/7538).

Outcomes were solicited from 3299 individuals and were obtained from 526 individuals for a total of 528 sgNIPT outcomes from 253 clinical practices across 34 US states and Puerto Rico. The outcomes were successfully obtained for 25 affected neonates of the expected 38 affected fetuses in 42,067 pregnant individuals, a collection rate for affected cases that is greater than other prenatal studies of similar design.[23–25] The average GA at the time the sample was collected was 16.4 weeks (median 13.9 weeks; range 10–37 weeks) and the average FF was 7.8% (median 6.4%, range 1.5%–35%). These characteristics were similar in the full eligibility cohort (average GA: 15.7 weeks, average FF: 7.4%). Self-identified race and ethnicity is not utilized in carrier screening as it is an NGS platform and fetal risk assessment is computed using general population carrier frequencies weighted according to the US reported race and ethnicity frequencies. Outcomes were enriched for high-risk and increased-risk cases (9.7% of the outcomes cohort vs. 2.9% of the eligible cohort) (Table 1). This approach allows for assessment of the sensitivity of an assay when the condition is rare, but artificially inflates the disease prevalence.[26]

3.2 | Clinical analytics of the outcome cohort

SgNIPT correctly identified 24 of the 25 affected pregnancies as having a fetal risk of >1 in 100 and 479 of the 503 unaffected pregnancies as having a fetal risk ≤1 in 100 for a sensitivity of 96.0% (95% CI: 79.65%–99.90%), specificity of 95.2% (95% CI: 92.98%–96.92%), PPV of 50.0% (95% CI: 35.23%–64.77%), and NPV of 99.8% (95% CI: 98.84%–99.99%) (Table 2). The sgNIPT fetal risk estimate was well-correlated with the affected status of the fetus or newborn. All 12 pregnancies assigned 9 in 10 risk were affected (Figure 1). This strong correlation illustrates the validity of the personalized risk assessment. Furthermore, the accuracy of sgNIPT to identify a high-risk fetus was independent of FF (Figure S1).

SgNIPT for CF correctly identified nine pregnancies as high risk. One affected fetus was designated low risk (1 in 2000) for CF by the sgNIPT CF assay and was identified by newborn screening to be homozygous for the p.Arg1066Cys *CFTR* pathogenic variant (NM_000492.4:c.3196 C > T; gnomAD allele frequency: 3.19e-5). The assay is not designed to detect homozygous affected cases for this variant, which is expected to occur in less than 1 in ~10,000,000 pregnancies in the absence of consanguinity. In general, the assay is designed to detect fetuses homozygous for p.Phe508del and compound heterozygote cases involving other *CFTR* pathogenic variants. The approach to CF sgNIPT uses amplicon-based NGS to detect variants different from the known variant in the pregnant individual, as well as dosage analysis for the p.Phe508del variant. Homozygous cases for non-p.Phe508del pathogenic variants constitute less than 1.5%–3.1% of all CF cases, and 1 in 2000 posterior risk reported incorporates this known limitation.[27,28] The nine affected cases identified as high risk included three fetuses predicted to be homozygous for the p.Phe508del variant and six that were compound heterozygous for pathogenic variants. SgNIPT for CF correctly identified 73 cases where the neonate was unaffected as low risk (Figure 2A, Table S4).

The sgNIPT HBB assay correctly identified 11 affected fetuses as high risk (Figure 2B, Table S4). Seven of the identified affected cases were predicted to be homozygous for the p.Glu7Val (NM_000518.5: c.20 A > T) variant implicated in sickle cell disease, and four were predicted to be compound heterozygous for *HBB* pathogenic variants.

TABLE 1 Eligible cohort and outcome cohort and the sgNIPT qualitative results.

	Full sample		Outcomes cohort	
	N	% of total		Response rate
Total eligible/Contacted patients	42,067		3299	
Total unique carriers	7538	17.92%	526	15.94%
Carriers		**Carrier frequency**		**% of carriers**
Total carriers	8268		528	
CF	1620	3.85%	91	17.30%
HBB	1603	3.81%	157	29.85%
HBA	4048	9.62%	205	38.97%
SMA	997 (178)	2.37%	75	14.26%
Reported qualitative risk	***N***	**% of NIPT assays**	***N***	**% of NIPT assays**
High risk	109	1.32%	34	6.44%
Increased risk	111	1.34%	17	3.23%
Decreased risk	169	2.04%	12	2.28%
Low risk	7300	88.29%	465	88.40%

Note: The overall and individual condition specific carrier frequencies for all eligible patients ($n = 42,067$) and the single gene NIPT qualitative results for all identified carriers in the full sample ($n = 7538$). The response rate for the contacted patients ($n = 3299$) and proportion of different carrier types and single gene NIPT qualitative result for the outcomes cohort ($n = 526$). The SMA carriers includes the total number of SMA carriers identified during the study period and (the total number included in the study because they were analyzed on the most recent version of the assay).

TABLE 2 Clinical analytics of the 528 sgNIPT with known neonatal/fetal outcomes.

	Screen positive sgNIPT >1 in 100	Screen negative sgNIPT ≤1 in 100	Total
Affected	24	1	25
Unaffected	24	479	503
Total	48	480	528
			95% CI
Sensitivity	96.00%		79.65%–99.90%
Specificity	95.23%		92.98%–96.92%
PPV	50.00%		35.23%–64.77%
NPV	99.79%		98.84%–99.99%

Note: Clopper-Pearson 95% CI.

There were 139 neonates unaffected with HBB correctly identified by sgNIPT to have a low risk (Figure 2B, Table S4). All sgNIPT HBA fetal risk estimates were <1 in 100 and none of the neonates were affected with alpha-thalassemia disease, consistent with the known prevalence of HBA conditions (Figure 2C, Table S4).[29] Finally, the SMA sgNIPT assay correctly identified four affected neonates as high-risk pregnancies (Figure 2D, Table S4).

3.3 | Estimated end-to-end clinical analytics for carrier screening with reflex to sgNIPT

To understand the overall performance of carrier screening with reflex to sgNIPT the end-to-end sensitivity, specificity, PPV, and NPV were estimated using the condition-specific carrier frequencies in the complete sample and general population, accepted NGS carrier screening sensitivity, and the outcomes cohort sgNIPT sensitivity and PPV.[13] Across the 42,067 pregnant individuals the estimated end-to-end sensitivity was 92.4% and the estimated end-to-end specificity was 99.9% (Table 3).

3.4 | Simulated comparison of expanded carrier screening to carrier screening with reflex to sgNIPT

To estimate the number of affected fetuses identified by ECS versus carrier screening with reflex to sgNIPT, we utilized published estimated frequencies of HRC for 163 AR conditions included on a

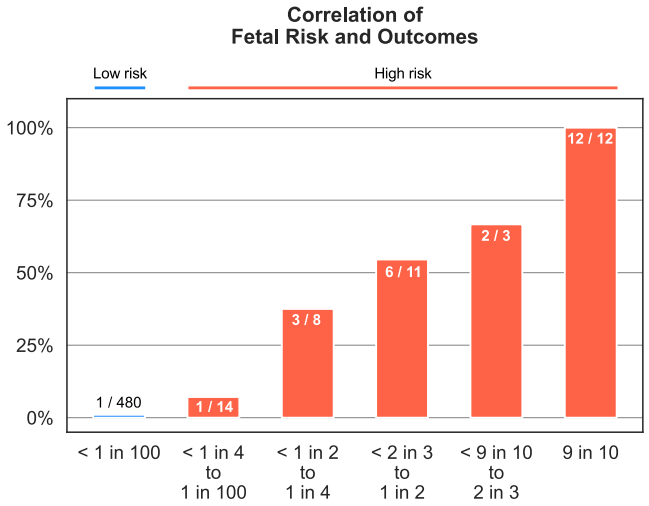

FIGURE 1 SgNIPT fetal risk of ACOG-recommended single-gene recessive disorders. SgNIPT fetal risk was binned into 6 categories (<1 in 100, 1 in 100 to < 1 in 4, 1 in 4 to <1 in 2, 1 in 2 to <2 in 3, 2 in 3 to <9 in 10, and >9 in 10) and correlated with the percentage of affected fetuses in the outcome cohort. Numbers used to calculate the proportion affected (number affected divided by total number in risk category) are indicated in each column. [Colour figure can be viewed at wileyonlinelibrary.com]

clinically available ECS panel and weighted for the race and ethnicity frequencies of the US population, as well as published frequencies of misattributed paternity and partner follow-up for traditional carrier screening. Based on an estimated total frequency of 0.897% HRC for the 163 conditions,[20] 10% misattributed paternity,[21] and 42% partner follow-up,[2,4,5,30] an estimated 85 affected fetuses would be identified as high risk (part of a HRC) per 100,000 pregnant individuals by ECS in a real-life scenario. Comparatively, based on a frequency of 0.402% HRC for CF, SMA, HBB, HBA,[20] carrier screening with reflex to sgNIPT would identify 96 affected fetuses as high risk (and provide an individual fetal risk estimate) per 100,000 pregnant individuals. In contrast, traditional carrier screening for these four conditions would identify 38 affected fetuses as high risk (part of a HRC) per 100,000 pregnant individuals (Figure 3).

4 | DISCUSSION

This study replicates the findings of the prior clinical validation of carrier screening with reflex to sgNIPT in a sample four times the size of the original study.[13,31] These studies demonstrate that sgNIPT has greater sensitivity to identify an affected fetus as high risk than traditional carrier screening for common AR conditions performed in a real-life setting. Together, the findings support the implementation of carrier screening with reflex to sgNIPT for reproductive carrier screening in pregnant individuals and suggest it is preferable to the traditional approach in many circumstances.

For many patients, carrier screening with reflex to sgNIPT provides a more complete reproductive risk assessment than traditional carrier screening. Traditional carrier screening is limited by completion of carrier screening by the biological partner, which is done in less than half of the recommended cases even when the cost of partner carrier screening is waived.[2,4,5] Furthermore, minority individuals—particularly people who identify as Black and/or Hispanic—have higher rates of unplanned and/or unpartnered pregnancies, late presentation to prenatal care, and underinsured or uninsured status, all of which impact the ability to complete traditional carrier screening and may exacerbate healthcare inequities.[7–9,32–34] In this study, a personalized fetal risk estimate was returned to the 99.7% of pregnant individuals who completed the clinical assay, allowing for informed reproductive choices without the barrier of partner carrier screening or the additional delay of sequential testing.

Importantly, even when the traditional carrier screening workflow is executed perfectly with complete partner carrier screening uptake and no misattributed paternity, carrier screening with reflex to sgNIPT has a similar sensitivity and superior PPV and NPV. In the outcomes cohort, sgNIPT had a sensitivity of 96%. For this panel of conditions in the US population, carrier screening has a sensitivity of approximately 96%[13] and therefore a sensitivity of approximately 93% (96%*96%) to identify a high-risk couple. In a real-life setting, the sensitivity decreases to 35% due to incomplete partner uptake and misattributed paternity.[2,4,13,21,35] Comparatively, the carrier screening with reflex to sgNIPT end-to-end workflow has an estimated 92.4% sensitivity to identify an at-risk pregnancy and, unlike traditional carrier screening, it provides a personalized fetal risk assessment of up to 9 in 10, compared to maximum 1 in 4 that traditional carrier screening can achieve. High sensitivity and specificity were achieved despite a conservative approach to the calculation, which included the non-concordant affected case where the implicated variants represented a known limitation of the assay and a risk threshold of 1 in 100. A 1 in 100 risk is reported as *increased risk* in our clinical reports, while a 1 in 4 risk threshold is used for *high risk*. Comparatively, applying a risk threshold of 1 in 4 (equivalent to the risk of HRCs) to this sample would result in significantly improved PPV (79.3%) and specificity (98.9%) and a slightly decreased NPV (99.6%) and sensitivity (92.0%). Importantly, personalized fetal risk scores were accurate and in line with outcome results across all risk ranges, as also previously shown in a prior publication,[13] illustrating the performance of the assay for fetal risk that is relevant to reproductive decision making. Carrier screening with reflex sgNIPT represents a meaningful improvement in the ability to detect pregnancies at high risk while negating the need for a sample from the reproductive partner and facilitates more informed shared decision making, including the decision to pursue diagnostic testing, or enabling rapid postnatal diagnosis and subsequently early treatment initiation. Furthermore, this workflow has 99.8% NPV to return a low-risk result for an unaffected fetus, which can provide a level of reassurance for the patient which is not possible with a traditional approach to carrier screening.

The implementation of carrier screening with reflex to sgNIPT in general obstetrics practices has the potential to streamline care and improve the patient experience. Carrier screening is most often provided by general obstetricians who report a lack of awareness of

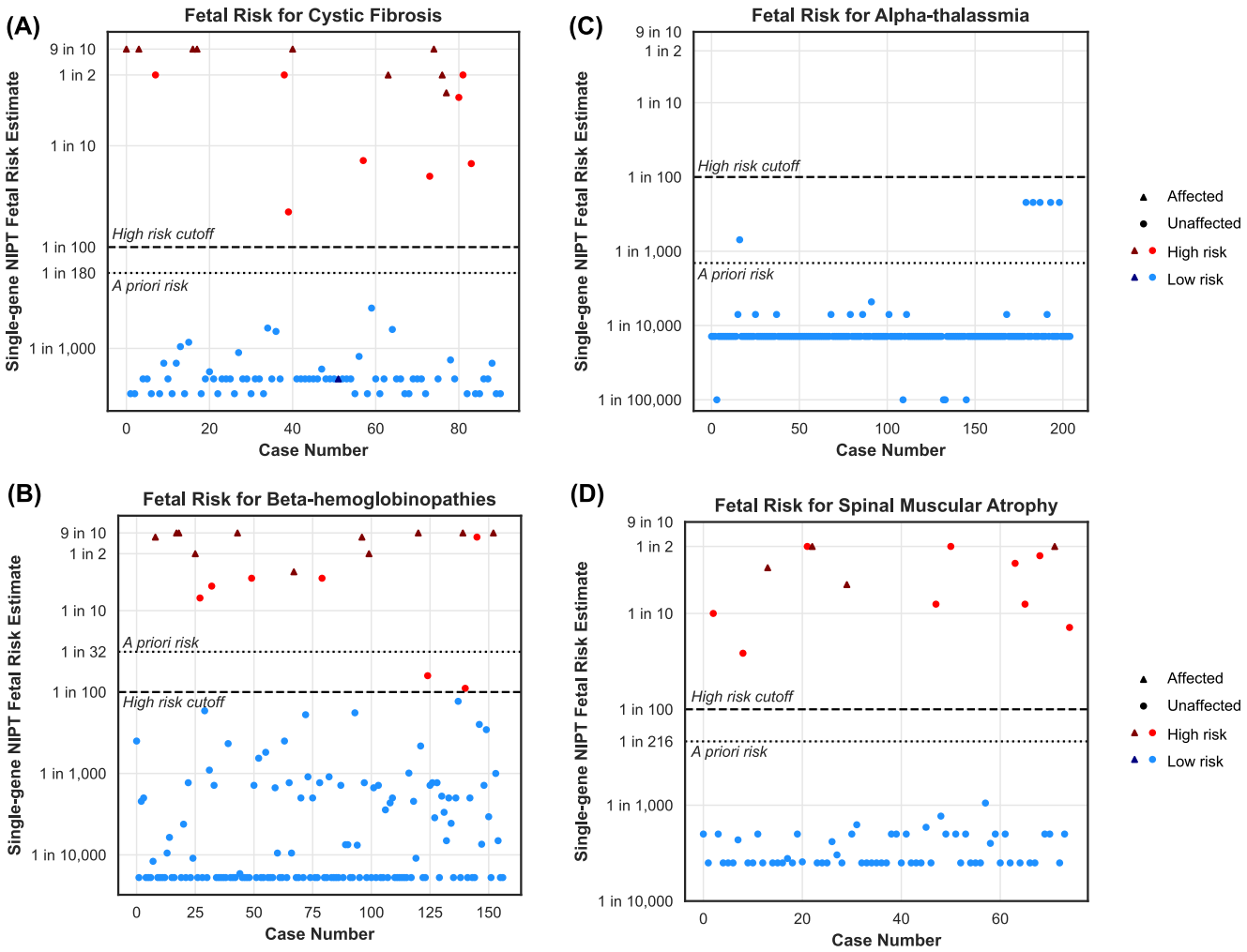

FIGURE 2 Numerical sgNIPT fetal risk and corresponding risk category (high risk, >1 in 100, or low risk, ≤1 in 100) for pregnancies at risk of cystic fibrosis (CF) (A), beta-hemoglobinopathy (HBB) (B), alpha-thalassemia (HBA) (C), and spinal muscular atrophy (SMA) (D). Newborn or fetal disease status (affected or unaffected) was collected from parental or provider reports of diagnostic testing or newborn screening (NBS). For most pregnancies, the *a priori* risk was determined by the general population carrier frequency: 1 in 180 for CF (A); 1 in 32 for HBB (B); and 1 in 216 for SMA (D). The *a priori* for HBA depends on the type of HBA carrier. For three-copy HBA carriers (aa/a-) or two-copy HBA carriers in trans (a-/a-) the *a priori* is 1 in 2280 (1 in 372 for Asian individuals), and for two-copy HBA carriers in cis (aa/--) the *a priori* is 1 in 1140 (1 in 186 for Asian individuals). The higher general population *a priori* is shown on the graph, though most cases are three-copy HBA carriers. For HBB, there were two cases classified as high risk for this study that were clinically classified as decreased risk. For HBA, there were six cases classified as low risk for this study that were clinically classified as increased risk. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Estimated end to end clinical analytics of carrier screening with reflex to single-gene NIPT for the full sample of eligible patients.

	Screen positive sgNIPT >1 in 100	Screen negative sgNIPT ≤1 in 100	Total
Affected	35	3	38
Unaffected	34	41,995	42,029
Total	69	41,998	42,067
End-to-end sensitivity	92.35%		
End-to-end specificity	99.92%		
PPV	50.72%		
NPV	99.99%		

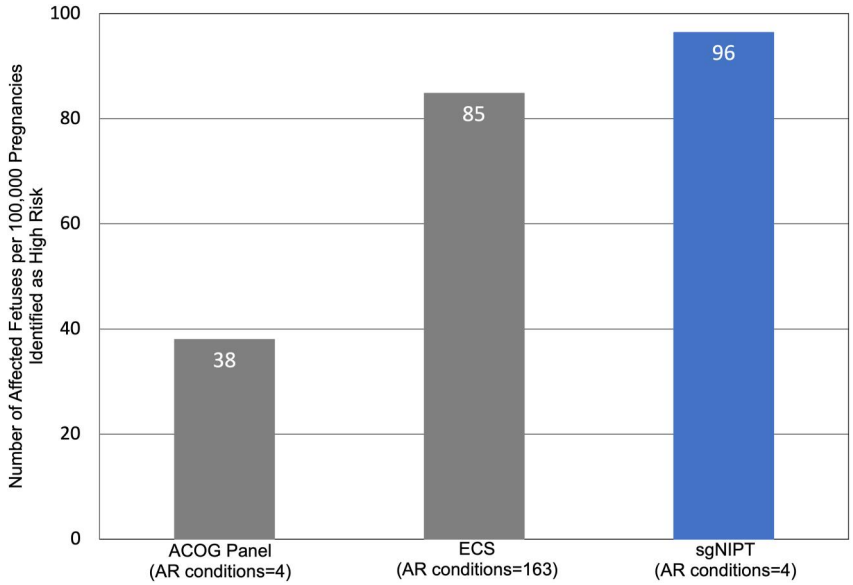

FIGURE 3 Calculated clinical performance of carrier screening per 100,000 pregnancies. The estimated number of affected fetuses identified as high risk by ACOG recommended autosomal recessive (AR) carrier screening (cystic fibrosis (CF), spinal muscular atrophy (SMA), HBB, HBA) in a real-life scenario. The estimated number of affected fetuses identified as high risk by a commercially available expanded carrier screening (ECS) panel for 163 AR genes in a real-life scenario. The estimated number of affected fetuses identified as high risk by carrier screening with reflex to sgNIPT for the ACOG recommended conditions. The real-life scenario includes 10% misattributed paternity and 42% partner carrier screening uptake when recommended based on published frequencies. Carrier screening with reflex to sgNIPT does not require a partner sample, so it is not impacted by these factors. The estimated number of HRC for each condition is based on published estimated frequencies for the US population and the number of affected fetuses was calculated based on a 25% affected fetus rate for these AR conditions in HRCs. [Colour figure can be viewed at wileyonlinelibrary.com]

carrier screening recommendations, lack of confidence in their ability to explain a positive result, and lack of time to provide adequate pre- and post-test counseling.[36–39] Carrier screening with reflex to sgNIPT has the potential to decrease patient counseling and follow-up care needs by proceeding directly to the assessment of fetal risk when an individual is identified as a carrier without the time-intensive logistical complications of screening the reproductive partner. Additionally, the personalized fetal risk assessment enables more individualized, informed post-test counseling. In a general-risk population, more than 98% of pregnancies will have a negative carrier or low-risk sgNIPT result, which reduces unnecessary patient anxiety and allows for triage of the remaining 2% who may need more complex counseling and specialty care.

The use of amplicon-based exon sequencing rather than a genotyping platform to detect variants inherited from the reproductive partner ensures the accuracy of the assay for the diverse US population and paves the way for more equitable access to vital reproductive healthcare. The assay correctly identified low-risk fetuses for a diversity of alpha-thalassemia carriers including 1, 2 and 3 *HBA1/2* gene deletions in both *cis* and *trans* and non-*HBA1/2* gene deletions. All 11 neonates affected with an HBB condition were correctly identified as high risk by sgNIPT. This included detection of pregnancies with the homozygous p.Glu7Val *HBB* pathogenic variant (sickle cell anemia) as well as compound heterozygous cases. The assay also correctly identified fetuses affected with CF as high risk of the homozygous p.Phe508del *CFTR* pathogenic variant as well as a variety of compound heterozygous cases. In this entire cohort of more than 42,067 pregnancies, 7538 carriers, and 25 affected fetal outcomes collected, there was a single affected neonate, detected by NBS, where the pregnancy had been identified as low risk by sgNIPT. Of note, the QCT technology detected 100% of the affected cases that it was designed to detect. The single affected neonate was homozygous for the rare p.Arg1066Cys *CFTR* pathogenic variant. SgNIPT, in the version assessed in this paper, uses exon sequencing to detect compound heterozygote cases and p.Phe508del homozygous cases, but not homozygous cases for non-p.Phe508del variants. The vast majority of CF affected cases (96.9%–98.5%) are homozygous or compound heterozygous for the p.Phe508del variant.[27,28] Therefore, with the detection of all p.Phe508del homozygous cases as well as any compound heterozygote cases, the CF sgNIPT assay, even without the detection of other homozygous affected variants, can still reach a sensitivity of ~97%, higher than most carrier screens, including a panel based on expanded ACMG recommended *CFTR* reportable variants.[40] However, the assay can be further expanded accordingly to detect other homozygous cases in future versions.

4.1 | Study strengths and limitations

The current study examined over 42,000 cases collected from 811 unique practices across 45 US states and Puerto Rico, resulting in a sample representative of the diverse US population. However, the

large study population necessitated an outcome collection procedure reliant on patient and provider reporting. Despite this challenge, over 500 outcomes were collected, with at least 75 outcomes for each condition. Furthermore, outcomes were obtained for 25 of the 38 predicted affected cases, a collection rate that compares favorably with other published studies.[23–25] The prioritization of outcome collection from cases with high-risk NIPT results allowed for estimation of sensitivity for these conditions with US population frequencies of approximately 1 in 2000 for HBB conditions, 1 in 2500 for CF, 1 in 8000 for SMA and less than 1 in 100,000 for HBA conditions; for an overall frequency of approximately 1 in 1000.[41–45] To account for the uneven collection in high-risk and low-risk cases, the end-to-end clinical sensitivity and specificity were modeled and calculated.

Outcomes were obtained for 25 affected cases; however, it was not possible to obtain a high-risk result for alpha-thalassemia, which has a high frequency of silent carriers (aa/a-) but very low disease prevalence in the US (<1 in 100,000).[43] The classification of the outcome for a case was often determined by the reported newborn screening results for the resulting neonate (NBS) and, with the exception of NBS for SMA, first-tier NBS for these conditions are not molecular assays.[46,47] However, NBS is designed with a high sensitivity and therefore remains an excellent comparator for this study.

Carrier screening with reflex to sgNIPT should be considered as a first-line approach in many circumstances, particularly when the patient is already pregnant and early detection of an at-risk fetus will maximize the patient's options as well as opportunities for in-utero treatment where applicable. While ECS is increasingly being incorporated into recommendations for reproductive carrier screening,[48,49] there are known limitations to this approach as have been discussed in this paper and in the literature.[2,4,5,10,30,50,51] Several studies have investigated the improvement of the detection of high-risk couples (and therefore fetuses with a 1 in 4 risk to be affected) by ECS.[52,53] Based on estimated prevalences of HRCs for 163 AR conditions in the US population,[20] misattributed paternity and partner carrier screening uptake rates,[2,4,5,21] the four condition panel carrier screening with reflex to sgNIPT would identify 14% more affected fetuses as high risk than ECS for 163 conditions (including the four conditions on sgNIPT) in a real-life scenario. The higher carrier frequencies of the four conditions result in the detection of more affected pregnancies for a smaller panel composed of just these conditions that is unaffected by partner carrier testing than a more extensive carrier panel of these conditions and other rare conditions that is reliant on partner carrier testing. This comparison illustrates both how these four conditions represent nearly half of the HRC in the US population and the limitations of ECS in real-life settings.[20] Despite the benefit of carrier screening with reflex to sgNIPT for many individuals, there are some circumstances where ECS may be preferable. Per ACOG recommendations, all patients should receive appropriate pre-test counseling about the benefits and limitations of different screening approaches relevant to their particular circumstances, medical, and family history.[1] Carrier screening with sgNIPT has the potential to streamline post-test counseling and care by reducing the number of high-risk pregnancies through direct assessment of fetal risk rather than requiring counseling for all pregnant individuals identified as carriers and by enabling more informed reproductive decisions through personalized fetal risk scores.

5 | CONCLUSION

The findings of this study demonstrate that carrier screening with reflex to sgNIPT performs well in a general population setting. This approach to screening for AR conditions identifies high-risk pregnancies with exceptional sensitivity and specificity; as such, it can increase access to clinically actionable information and significantly improve equity in reproductive healthcare, particularly in the absence of a reproductive partner sample. This workflow should be considered as an option for most of the general pregnant population. When the biological partner sample is unavailable, this workflow should be recommended as the first-line approach.

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CONFLICT OF INTEREST STATEMENT

Julia Wynn and Jennifer Hoskovec are employees of BillionToOne and hold stock or options to hold stock in the company. Meredith J. Ross and Rebecca D. Carter are financially compensated by BillionToOne. Sriram C. Perni is a paid Consulting Prenatal Medical Director at BillionToOne.

DATA AVAILABILITY STATEMENT

All data are available within the manuscript and the supplementary materials.

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