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Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes

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OBJECTIVE: The purpose of this study was to estimate the performance of a single-nucleotide polymorphism (SNP)—based noninvasive prenatal test for 5 microdeletion syndromes.

STUDY DESIGN: Four hundred sixty-nine samples (358 plasma samples from pregnant women, 111 artificial plasma mixtures) were amplified with the use of a massively multiplexed polymerase chain reaction, sequenced, and analyzed with the use of the Next-generation Aneuploidy Test Using SNPs algorithm for the presence or absence of deletions of 22q11.2, 1p36, distal 5p, and the Prader-Willi/Angelman region.

RESULTS: Detection rates were 97.8% for a 22q11.2 deletion (45/46) and 100% for Prader-Willi (15/15), Angelman (21/21), 1p36 deletion (1/1), and cri-du-chat syndromes (24/24). False-positive rates were 0.76% for 22q11.2 deletion syndrome (3/397) and 0.24% for cri-du-chat syndrome (1/419). No false positives occurred for Prader-Willi (0/428), Angelman (0/442), or 1p36 deletion syndromes (0/422).

CONCLUSION: SNP-based noninvasive prenatal microdeletion screening is highly accurate. Because clinically relevant microdeletions and duplications occur in >1% of pregnancies, regardless of maternal age, noninvasive screening for the general pregnant population should be considered.

Key words: microdeletion, noninvasive prenatal testing, single-nucleotide polymorphism

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The discovery in the maternal circulation of cell-free DNA (cfDNA) of fetal/placental origin has led to a revolution in prenatal screening.[1-3] Common whole-chromosome fetal aneuploidies can now be detected with high sensitivity and specificity[4] and have facilitated a significant reduction in the number of invasive diagnostic procedures that have been performed. In the United States, 2 noninvasive prenatal testing (NIPT) approaches have been commercialized: quantitative "counting" that uses massive or targeted parallel sequencing[5-7] and a single-nucleotide polymorphism (SNP)—based approach that relies on the identification of maternal and fetal allele distributions.[8-13] Both methods can detect pregnancies at high risk for trisomy 21 (Down syndrome), trisomy 18, trisomy 13, and sex

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chromosome abnormalities. The SNP-based approach is also able to detect triploidy.[9,11]

Subchromosomal abnormalities (microdeletions and duplications) may result in physical and/or intellectual impairments that can be more severe than whole chromosome abnormalities. Unlike the risks of aneuploidy that is associated with nondisjunction, the incidence of subchromosomal copy number variations (CNVs) is independent of maternal age. Clinically relevant microdeletions and duplications occur in 1-1.7% of all structurally normal pregnancies.[14] In younger women, the risk for a clinically significant deletion exceeds the risk for Down syndrome. Because some infants with subchromosomal abnormalities may benefit from early therapeutic intervention,[15-17] prenatal detection is important for optimal management. In support of this, it is recommended that chromosome microarray analysis be offered to all women who undergo invasive diagnostic testing.[18] However, with the introduction of NIPT for aneuploidy screening, many women who previously would have had invasive testing are choosing to avoid these procedures because of the small risk of pregnancy loss.[3,19]

Submicroscopic genomic alterations are harder to detect noninvasively because of their small size. A small proportion may be identified incidentally through traditional serum and ultrasound screening, but these tests were not designed to screen for these anomalies. The introduction of a highly accurate noninvasive prenatal screening test that would identify women who are at high risk for microdeletions or duplications therefore would be useful. Recently, proof-of-principle studies that used shotgun or whole-genome sequencing reported the detection of subchromosomal microdeletions and microduplications.[20-23] However, these approaches were limited by the requirement for exceptionally high sequence reads, and interpretation was complicated by the identification of variants of unknown clinical significance. Here, we used a targeted SNP-based approach[9-13] to detect the larger deletions that underlie 5 microdeletion syndromes with clinically severe phenotypes.

TABLE 1
Samples used in the main cohort along with the sample deletion sizes

Samples	Sample deletion size	n
Pregnancy samples		
DiGeorge deletion	arr[hg18] 22q11.21(17,010,000-20,130,000)x1	1
DiGeorge deletion	arr[hg18] 22q11.21(17,020,000-20,130,000)x1	1
DiGeorge deletion	46,XX.nuc ish(HIRAx1)	1
Cri-du-chat deletion	46,XX,del(5)(p15.1p15.3)	1
Cri-du-chat deletion	46,XY,del(5)(p14.2)	1
1p36 deletion	46,XY,del(1)(p36.1)	1
46,XX and 46,XY		352
PlasmArt samples: born triads		
DiGeorge deletion	arr[hg18] 22q11.2(17,270,000- 19,810,000)x1	22
DiGeorge deletion	arr[hg18] 22q11.2(16,950,000-20,250,000)x1	22
Cri-du-chat deletion	arr[hg18] 5p15.33p14.1(91,100-29,500,000)x1	22
46,XX and 46,XY		7
PlasmArt samples: cell lines		
Prader-Willi deletion	arr[hg18] 15q11.2q13.1(20,310,000-27,130,000)x1	16
Angelman deletion	arr[hg18] 15q11.2q13.1(20,310,000-27,220,000)x1	22

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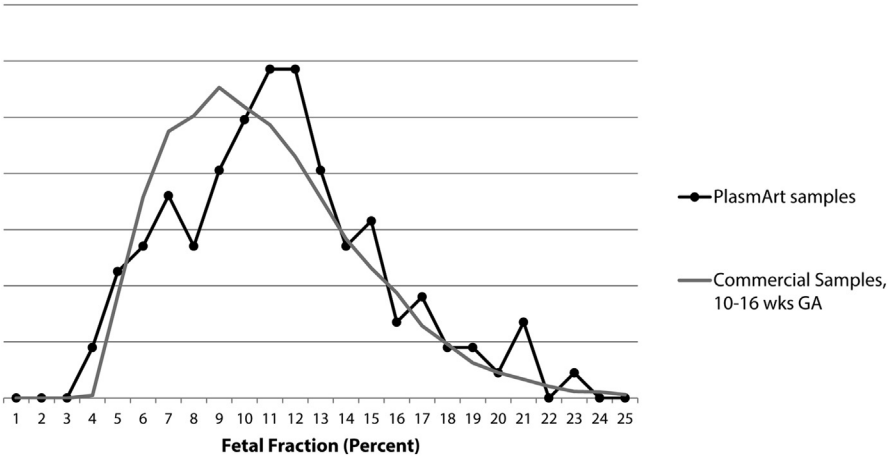
Materials and Methods

Initial validation studies were performed with genomic DNA that had been isolated from 40 characterized cell lines to demonstrate that the SNP-targeted assay

FIGURE 1
Fetal fraction distribution



Distribution of the 111 PlasmArt samples and of 19,910 consecutive commercial samples from 10-16 weeks' gestation.

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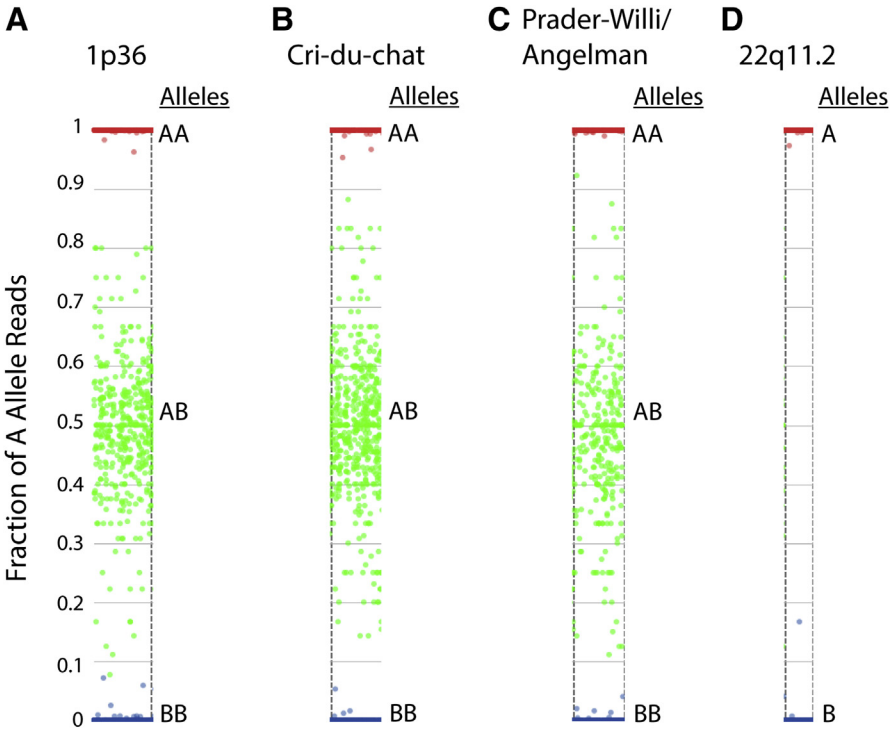
FIGURE 2
Graphic representation of sequencing data



Input: 22q11.2 deletion (Di George) cell line

Data were obtained from analysis of genomic DNA isolated from cells with the 22q11.2 deletion, interrogated for **A,** the 1p36 deletion, **B,** the cri-du-chat deletion, **C,** the Prader-Willi/Angelman deletion, and **D,** the 22q11.2 deletion. Note that this is one way of visualizing the data and is not how the algorithm makes copy number calls. For all plots, single-nucleotide polymorphisms (SNPs) are assumed to be dimorphic and are labeled as A or B. The fraction of A allele reads (*y-axis*) is plotted against the position of each SNP along the chromosome of interest (*x-axis*); each spot corresponds to a single SNP. Spots are colored according to genotype: AA is *red*; AB is *green*, and BB is *blue*. Genotypes are indicated to the right of the plots; **A-C,** SNP plots reveal 2 copies in the 1p36, cri-du-chat, and Prader-Willi/Angelman regions. Homozygous alleles (AA and BB) are associated tightly with the plot's upper and lower limits, respectively. Heterozygous alleles (AB) cluster near the center of the plot, which indicates 2 copies of the chromosome in the interrogated regions. **D,** SNP plots reveal 1 copy in the 22q11.2 region. The lack of heterozygous alleles (AB) identifies 1 copy of the 22q11.2 region; A and B alleles are associated tightly with the plot's upper and lower limits, respectively.

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was capable of detecting the presence or absence of 22q11.2, 1p36, cri-du-chat, Prader-Willi, and Angelman deletions. These cell lines included 7 with 22q11.2 deletions, 19 with 5p deletions (cri-du-chat syndrome), 10 with 15q11-13 deletions (3 with Angelman syndrome and 7 with Prader-Willi syndrome), and 4 with no deletions.

After validation of the SNP-targeted assay, a cohort of 469 test samples was evaluated (Table 1). This included 6 maternal plasma samples from pregnant women in which the fetus had a microdeletion (3 with 22q11.2 deletions, 2 with 5p deletions, and 1 with a 1p36 deletion), 352 unaffected pregnancy plasmas, and 111 artificial DNA mixtures (PlasmArts). Seventy-three of the PlasmArts were generated from DNA derived from 2 individuals with 22q11.2 deletions, 1 with a 5p deletion, and one unaffected child, each of which was diluted into matched maternal DNA. Thirty-eight samples were generated from genomic DNA isolated from two 15q- cell lines (1 Angelman, and 1 Prader-Willi) and the corresponding maternal cell lines. All cell lines were obtained from the Coriell Cell Repository (Camden, NJ). Patients who provided samples were enrolled at prenatal and postnatal care centers under institutional review board–approved protocols (Western Institutional Review Board protocol number: 12-014-NPT), pursuant to local regulations.

Genomic DNA for PlasmArt mixtures was isolated from the buffy coats from mother and child pairs or from paired mother and child cell lines. These DNA preparations were cleaved into internucleosomal fragments of roughly 150 base pairs and multiples thereof with the use of a proprietary reaction that included micrococcal nuclease (New England Biolabs, Ipswich, MA).[24,25] Because fetal cfDNA exists in vivo mainly as mononucleosomal fragments,[26] child DNA of approximately 150 base pairs was isolated using Solid Phase Reversible Immobilization beads (Agencourt Biosciences, Beverly, MA). Maternal genomic DNA was not size purified because maternal cfDNA exists as a nucleosomal ladder.[26] Child DNA was titrated into the corresponding maternal DNA to achieve artificial mixtures with "fetal" fractions that ranged from 3.8-33%, which was a similar distribution to that observed in maternal plasma clinical samples. The "fetal" fraction distribution of these samples is shown in Figure 1; for comparison, the fetal fraction distribution from 19,910 consecutive maternal plasma samples from women at 10-16 weeks of gestation is also shown.

All samples, including maternal and (when available) paternal samples,[9-13] underwent targeted multiplex polymerase chain reaction and were sequenced; the data were analyzed with the Next-Generation Aneuploidy Test Using SNPs (NATUS) algorithm as described previously,[9-13] with the following alterations: a unique set of primers was designed to amplify 4128 SNPs in the regions-of-interest (672 SNPs targeting

TABLE 2
Individual and combined detection rate and false-positive rate for pregnancy plasmas and PlasmArt samples

	Affected (n = 6 plasma; 108 PlasmArt samples)				Unaffected (n = 335 plasma; 108 PlasmArt samples)			
Disorder	Pregnancy plasma, n/N	PlasmArt, n/N	Total, n/N	Analytic detection rate, % (95% CI)	Pregnancy plasma, n/N	PlasmArt, n/N	Total, n/N	False-positive rate, % (95% CI)
22q11.2 del	2/3	43/43	45/46	97.8 (88.5–99.9)	3/332	0/65	3/397	0.76 (0.1–2.2)
Prader-Willi		15/15	15/15	100	0/335	0/93	0/428	0
Angelman		21/21	21/21	100	0/335	0/87	0/422	0
1p36 del	1/1		1/1	100	0/334	0/108	0/442	0
Cri-du-chat	2/2	22/22	24/24	100	1/333	0/86	1/419	0.24
Larger deletions combined	3/3	58/58	61/61	100 (94.1–100)	1/1337	0/374	1/1711	0.06 (0.0–0.3)

Samples for which the algorithm did not produce a result were not included.

CI, confidence interval.

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2.91 Mb in the 22q11.2 region and 1152 SNPs in each of the other regions, targeting 5.85 Mb in the Prader-Willi/Angelman region, 10.0 Mb in the 1p36 region, and 20.0 Mb in the cri-du-chat region). The assay was not validated for the smaller, less-frequent deletions that are associated with these disorders because positive control samples were not available. The estimated relative prevalence of the targeted deletions in the 22q11.2, Prader-Willi/Angelman, 1p36, and cri-du-chat regions were 87%, 28%, 60%, and 65%, respectively. Samples were analyzed with the NATUS algorithm as previously described,[9-13] and all samples that passed quality control (QC) were included in this cohort. The NATUS algorithm was then used to predict fetal copy number (1, 2, or ≥3 copies) for the microdeletion regions-of-interest. The algorithm was blinded to sample status, and all calls were reported as predicted by the algorithm without subjective modification by laboratory personnel.

RESULTS

Algorithm validation using genomic samples

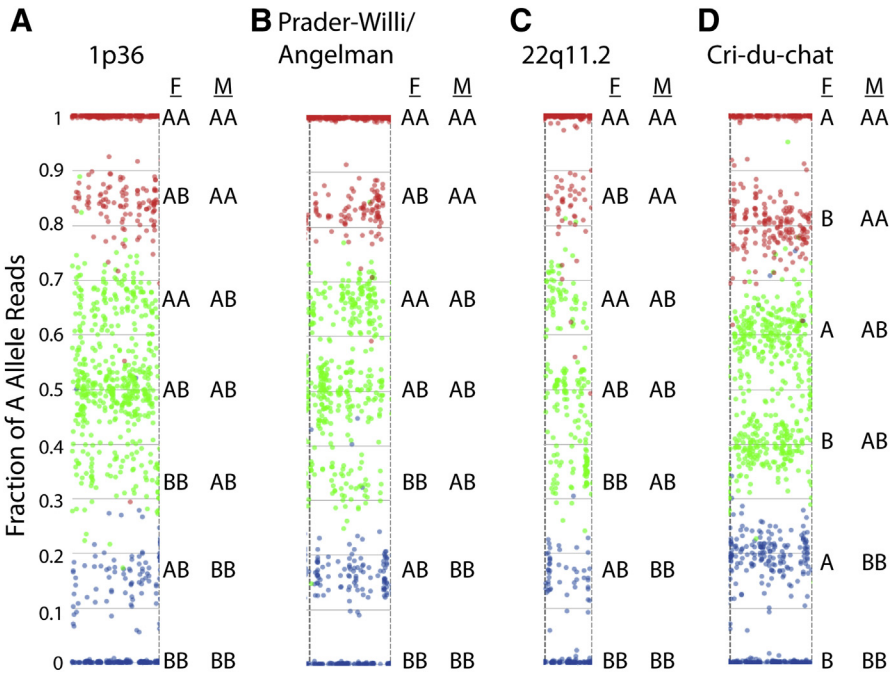
Validation experiments confirmed that the SNP-based technology and the microdeletion-specific primer pools could detect the microdeletions accurately in the 5 syndromes described. Heterozygous SNPs clearly were absent in all affected regions and were present in all unaffected regions; Figure 2 shows the graphic representations of the sequencing data that were obtained from genomic DNA that had been isolated from one cell line with a 22q11.2 deletion. The plots are described in detail in the legend of Figure 2. Briefly, the absence of the central green cluster in the 22q11.2 (DiGeorge) region indicated a lack of heterozygous SNPs, from which it is possible to infer a deletion of one copy of the DNA in this region.

Pregnancy plasma cohort

Of the 358 pregnancy samples, 335 samples passed QC metrics. The algorithm did not return a result for 23 of 358 of the samples (6.4%); all of these

FIGURE 3

Graphic representation of 1 cri-du-chat deletion pregnancy plasma with 33% fetal fraction



Input: Pregnancy plasma with a cri-du-chat deletion on the maternal chromosome

Single-nucleotide polymorphism (SNP) data are represented as described in Figure 2. In this case, spots are colored according to maternal genotype: SNPs for which the mother is homozygous for the A allele (AA) are indicated with *red*; SNPs for which the mother is homozygous for the B allele (BB) are indicated with *blue*, and SNPs for which the mother is heterozygous (AB) are indicated in *green*. Because plasma cell-free DNA is a mixture of fetal and maternal cell-free DNA, the *vertical position* of each spot represents the sum of the contribution of both fetal and maternal allele reads and is a function of the fetal fraction. Because most plasma cell-free DNA is maternal in origin, the spots mainly distribute according to maternal genotype. The contribution of fetal allele reads results in segregation into distinct subclusters. Fetal and maternal genotypes at individual SNPs are indicated with *F* and *M*, respectively, to the right of the plots. **A-C,** SNP plots reveal 2 fetal copies in the 1p36, Prader-Willi/Angelman, and 22q11.2 regions. The presence of 3 green clusters in the center of the plot (centered on 0.335, 0.50, and 0.665), and the presence of 2 red (centered on 1 and 0.835) and 2 blue (centered on 0 and 0.165) clusters, indicate the presence of 2 fetal chromosomes in the interrogated regions. **D,** SNP plots reveal 1 fetal copy of the cri-du-chat region. The center trio of green clusters is replaced with a duo of clusters (centered on 0.4 and 0.6), and the peripheral red and blue clusters have shifted towards the center of the plot (centered on 0.2 and 0.8, respectively). Together, this indicates the presence of a deletion on the maternal chromosome in the cri-du-chat region.

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were unaffected. The detection rates and false-positive rates for those samples that passed QC are listed in Table 2. Of the 6 affected pregnancy plasmas, 1 false negative was reported (22q11.2). Of the 335 unaffected pregnancy plasmas that passed QC, 4 false positives were reported (3 for the 22q11.2 deletion and 1 for the deletion associated with cri-du-chat syndrome). Figure 3 shows a sample with a fetal fraction of 33% having a cri-du-chat deletion on the maternally inherited chromosome 5. In this sample, 2 green clusters in the cri-du-chat region indicate a deletion; 3 green clusters in the 1p36, Prader-Willi/Angelman, and 22q11.2 regions indicate that 2 copies of the fetal chromosomes are present. The patterns are described in detail in the legend of Figure 2.

Artificial mixtures (PlasmArt)

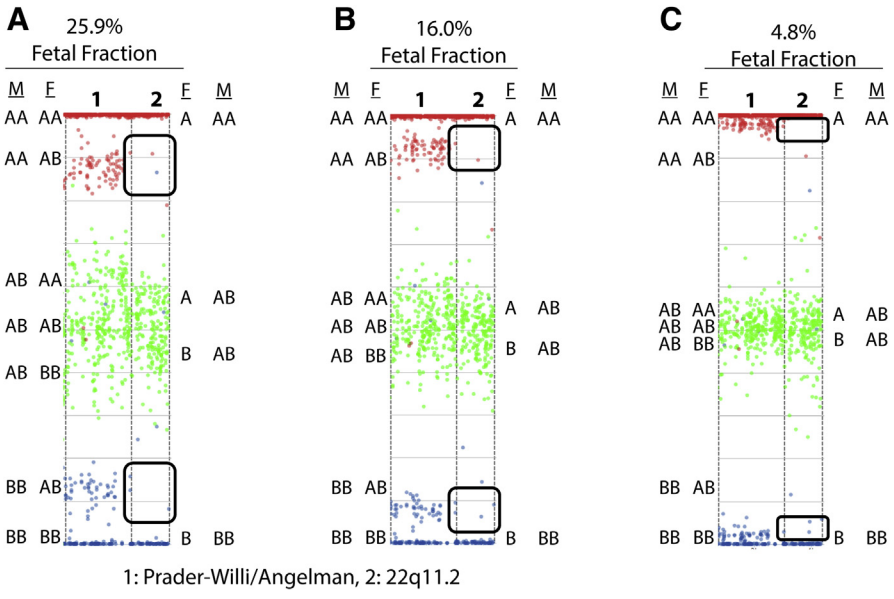
In the cohort of 111 PlasmArt samples, 108 samples passed QC metrics. The 3 samples that did not pass (1 Angelman, 1 22q11.2 deletion, 1 Prader-Willi) were due to low algorithm-generated confidence for the chromosome region of interest (1 Angelman), no-call for the chromosome region of interest (1 22q11.2 deletion), or a fetal fraction below the threshold where the algorithm makes a high-confidence copy number call (1 Prader-Willi). The detection rates and false-positive rates for the samples that passed QC are presented in Table 2.

Figure 4 shows a 22q11.2 deletion on the paternal copy of chromosome 22 that was detected from a set of PlasmArt samples with fetal fractions that ranged from 25.9—4.8%. The absence of the peripheral red and blue clusters where the maternal genotype is homozygous (AA or BB) is the hallmark pattern of a deletion on the paternal copy of the chromosome. The deletion is detectable visually as low as 4.8% fetal fraction (Figure 4).

Comment

We have demonstrated accurate detection of the 22q11.2, 1p36, cri-du-chat, and Prader-Willi/Angelman microdeletions using a SNP-based NIPT approach. Using a large cohort of unaffected samples and artificial PlasmArt mixtures that closely mimic the size profile and fetal fraction distribution of cfDNA that is found in pregnancy plasmas, we were able to estimate sensitivities and specificities for this assay for the microdeletion syndromes that were studied. For evaluations carried out at the 22q11.2 locus, in which the number of SNPs targeted was less than for other locations, we were able to identify the presence of a deletion in 45 of 46 samples with the deletion and absence in 394 of 397 unaffected samples (Table 2). For the other 4 loci, all of

FIGURE 4
Graphic representation of PlasmArt samples



Input: PlasmArt samples with a 22q11.2 (Di George) deletion on the paternal chromosome

Representation with a 22q11.2 deletion on the paternal chromosome at **A,** 25.9% fetal fraction, **B,** 16.0% fetal fraction, and **C,** 4.8% fetal fraction. All PlasmArt samples depicted here were generated from genomic DNA from the same mother-child pair. Three representative plots are depicted to illustrate microdeletion detection across a wide range of fetal fractions. Single-nucleotide polymorphism (SNP) data are represented here as described in Figures 2 and 3. Genomic DNA that represents the fetus and mother are indicated by *F* and *M*, respectively. Plots represent the Prader-Willi/Angelman deletion region (as indicated with 1 above the plots) and the 22q11.2 deletion region (as indicated with 2 above the plots). Genotypes of the Prader-Willi/Angelman region are indicated to the left; genotypes of the 22q11.2 region are indicated to the right. The deletion on the paternal copy of chromosome 22 in the 22q11.2 region is most clearly indicated by the red and blue peripheral subclusters. **A,** At fetal fractions of above approximately 20%, the presence of 3 green subclusters in the center of the plot (centered around 0.63, 0.50, and 0.37) with 2 red (around 1 and 0.87), and 2 blue (around 0 and 0.13) subclusters indicates the presence of 2 fetal chromosomes in the Prader-Willi/Angelman region. By contrast, in the 22q11.2 region, the center trio of green subclusters has condensed into a duo of clusters (centered on 0.57 and 0.43), and the internal peripheral red and blue clusters are absent (as indicated by *black boxes*). Together, this indicates a single fetal chromosome in the 22q11.2 region. **B** and **C,** At fetal fractions of less than approximately 20% in both the Prader-Willi/Angelman and 22q11.2 regions, the center green subclusters condense towards the center of the plot and become difficult to distinguish by eye. In the Prader-Willi/Angelman regions, the internal peripheral red and blue subclusters regress towards the plots' upper and lower limits, respectively. In the 22q11.2 regions, the absence of the internal peripheral red and blue subclusters in the 22q11.2 regions, which indicates a deletion on the paternal chromosome, is still readily apparent (as indicated by *black boxes*), even at fetal fractions as low as 4.8%.

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which targeted the same number of polymorphic loci, deletions were detected in 61 of 61 affected samples and 1 of 1711 unaffected samples.

In clinical practice, samples that do not return a result (no calls) for ≥1 microdeletions are unlikely to be redrawn because of the low previous risk. Under the conservative assumption that such cases would be treated as low risk (negative), the effective detection and false-positive rates for the 22q11.2 deletion would be 45 of 47 and 3 of 422, respectively, and 61 of 63 and 1 of 1813, respectively, for the larger deletions combined (Table 3). Our results suggest that screening for this set of 5 microdeletions could be added to existing NIPT for fetal aneuploidy with a minimal combined incremental false-positive rate of approximately 0.8% (Table 3).

When we combined the data for maternal plasma samples and the PlasmArt samples, the detection rate for 22q11.2 deletions was 45 of 46 (97.8%), and the false-positive rate was 3 of 397 (0.76%; Table 2). Based on a deletion prevalence of 1 of 2000 in the general population, an estimate that the 3-Mb deletion constitutes 87% of all 22q11.2 deletions,[27] and the conservative assumption that this test will not identify any of the other variant deletions, the results would translate into a positive predictive value of approximately 5.3% and a negative predictive value of approximately 99.99% (Table 4).

For the other deletion syndromes (which, in general, constitute larger genomic regions with more SNPs within each region), the combined detection rate was 61 of 61 (100%), and the false-positive rate was 1 of 1711 (0.06%; Table 2). These combined rates were used to estimate positive and negative predictive values for each disorder (Table 4). The deletions included in this study constitute almost 70% of the causal mutations in the 5 syndromes. The positive predictive value conservatively assumed that none of the other variant deletions in patients with these disorders would be identified and also that uniparental disomy would not be recognized. In practice, because some of the other deletions that are seen in these disorders can be large and because uniparental disomy is expected to be recognized, it is likely that >70% of affected pregnancies would be found. Further, it is possible that this assay could detect smaller deletions. Thus, the detection rates described here are considered to be a conservative indication of what could be expected in a clinical setting. To further improve the positive predictive value, reflex testing of samples found to be high risk to higher depth of read currently is being investigated.

TABLE 3
Combined detection rate and false-positive rate for pregnancy plasmas and PlasmArt samples

Variable	Effective detection rate[a] n/N	95% CI	Net detection rate,[b] %	False-positive rate n/N	%	95% CI
22q11.2 del	45/47	95.7: 85.5–99.5	83.3	3/422	0.71	0.1–2.1
Larger deletions combined	61/63	96.8: 89.0–99.6	45.5	1/1813	0.06	0.0–0.3

Samples for which the algorithm did not receive a result are treated conservatively as negatives.

CI, confidence interval.

[a] Detection rates for the specific detected deletions; [b] Net detection rates for each syndrome that take into account the prevalence of each detected deletion.

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Our initial studies have focused on 5 microdeletion syndromes that collectively have a population incidence of approximately 1 in 1000. These disorders are associated with significant morbidity and mortality rates and includes intellectual disability.[14,28-31] Additionally, the SNP method that was used distinguishes between a deletion that has arisen on a paternally vs a maternally inherited chromosome, which will facilitate clinical interpretation when an imprinted gene is involved, such as Prader-Willi or Angelman syndrome, even though patients with these disorders can have identical chromosome 15 deletions.[32] It should be recognized that, for patients who seek comprehensive diagnostic testing, chromosomal microarray analysis is the gold standard. However, for patients who want information about the genetic status of their fetus but who desire to avoid invasive testing, NIPT with broad clinical coverage can be an appropriate first step.

A noninvasive screening test for a defined set of submicroscopic CNVs allows a focus on the common recurrent changes that are known to be associated with well-defined phenotypes. The phenotypes associated with CNVs in other genomic regions are becoming increasingly well-defined, which supports their addition to a screening approach. However, because each CNV will be rare, it is important that the false-positive rate for each is very low. Our study shows that this can be achieved while retaining a high positive predictive value (Table 4).

TABLE 4
Estimated positive predictive value and negative predictive value

Disorder	Incidence (1:n)	Frequency of deletion evaluated	Positive predictive value,[a] %	Negative predictive value,[b] %
22q11.2 del	2000	0.87	5.3	>99.99
Prader-Willi	10,000	0.28	4.6	>99.99
Angelman	12,000	0.28	3.8	>99.99
1p36 del	5000	0.60	17.0	>99.99
Cri-du-chat	20,000	0.65	5.3	>99.99

[a] Calculated by multiplying population incidence, the frequency of the deletion evaluated, and the positive likelihood ratio (detection rate/false-positive rate); [b] Calculated by multiplying population incidence, the frequency of the deletion evaluated, and the negative likelihood ratio ([1-detection rate]/[1-false-positive rate]).

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A significant limitation of this study was the lack of sufficient maternal plasma samples from affected pregnancies at appropriate gestational ages. Because these disorders are relatively rare and because prenatal screening for these disorders is unprecedented, it was not possible to conduct a large-scale study on patient samples. To partially overcome this limitation, we generated artificial pregnancy plasma mixtures, termed *PlasmArts*, over an appropriate range of fetal DNA concentrations. The value of the PlasmArt approach is based on a number of observations. Plasma cfDNA is known to consist of both maternal and fetal fragments. The fetal fragments are mainly mononucleosomal[26,33] that are generated during placental apoptosis[34] and, as such, have specific cleavage sites. Maternal mononucleosomal fragments are approximately 23 nucleotides longer than fetal fragments,[26,33,35,36] and maternal cfDNA is known to consist of a nucleosomal ladder.[26] Additionally, both the fetal and maternal DNA fragments are thought to have associated proteins. Thus, the enzymatic fragmentation and "fetal" DNA size purification used in this study is expected to be superior to the use of sonication,[21] in that it creates fragments similar to those observed in vivo. Additionally, this method will leave proteins intact, in contrast to sonication, which denatures proteins; however, whether this affects cfDNA-based detection of CNVs has not been determined. Research is ongoing to further validate the PlasmArt method as a model for clinical samples.

The exact coordinates and size of some CNVs will differ substantially between individuals. Because of the rarity of positive control samples, this cohort contained a limited number of affected samples, including the use of replicate PlasmArts that were derived from the same mother-child pairs. Further validation of this technology in a larger series is warranted. Performance of this SNP-based method for the detection of well-defined microdeletions is expected to depend primarily on the number of informative SNPs in each region of interest. Although this may limit the detection capabilities for small regions of interest, for larger discrete abnormalities, it should offer a robust and generalizable approach. In other words, because performance is related to the number of informative SNPs in a target region, and not on the identity of the SNPs, validation of rare microdeletions should be possible without the need to collect a large set of positive controls for validation of each microdeletion. Ongoing studies in the clinical population, in which more affected samples are available, will provide insights into the generalizability of performance metrics for different microdeletion syndromes.

Detection of fetal microdeletions has been reported with methods that use shotgun sequencing and counting DNA fragments.[20,21] Because these methods amplify all chromosomes indiscriminately and because these deletion syndromes affect <1% of the genome, large numbers of sequence reads are required for accurate detection of subchromosomal anomalies.[37] In these initial studies that used shotgun sequencing, microdeletions were detected with the use of a depth of sequencing between 2.4×10^8 and 1.3×10^9 sequence reads,[21,38] which is substantially higher than that currently used for aneuploidy detection. The method reported here achieved high sensitivity and specificity with an average of only 8.9×10^6 mapped reads, which demonstrates one of the significant advantages of this targeted sequencing approach. Also, nontargeted sequencing will identify variants of unknown significance,[22,23] which will increase the need for invasive testing and can present counseling dilemmas. Alternatively, the SNP-based method can target specific regions with well-described phenotypes. Although this SNP-based approach offers a number of benefits over counting methods, there are some limitations to this approach. The SNP method requires a longer polymerase chain reaction process, and, as of now, this assay is not appropriate for egg donors.

This report describes the identification of 5 well-defined microdeletion syndromes through noninvasive methods. This next-generation SNP- and NATUS-based NIPT approach routinely identified 22q11.2, 1p36, cri-du-chat, Prader-Willi, and Angelman microdeletions with a low rate of screen positive results. The fact that clinically relevant microdeletions and duplications occur in >1% of pregnancies, regardless of maternal age, challenges the notion of "low-risk pregnancies"[14] and suggests that offering NIPT-based microdeletion screening to the general pregnancy population may be appropriate. Although this report demonstrates the technical ability to identify microdeletions, widespread implementation will require education of care givers and appropriate counseling of patients. Counseling should include the performance and scope of the testing, information about the frequency and phenotype of the disorders, and the fact that testing is voluntary. We recognize that additional validation studies are needed to provide greater confidence in this screening test.

Overall, these genomic alterations occur more frequently than those presently screened for, such as Down syndrome; many microdeletion syndromes, including those in our study, have severe phenotypes. As the technology improves, other microdeletions and duplications should also be identifiable by noninvasive testing. Although some of these may have less severe phenotypes, knowledge of them will allow early interventions, which have been shown to improve greatly a child's development.[15-17] ■

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