Clinical experience with a single-nucleotide polymorphism-based non-invasive prenatal test for five clinically significant microdeletions

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Single-nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT) can currently predict a subset of submicroscopic abnormalities associated with severe clinical manifestations. We retrospectively analyzed the performance of SNP-based NIPT in 80,449 referrals for 22q11.2 deletion syndrome and 42,326 referrals for 1p36, cri-du-chat, Prader-Willi, and Angelman microdeletion syndromes over a 1-year period, and compared the original screening protocol with a revision that reflexively sequenced high-risk calls at a higher depth of read. The prevalence of these microdeletion syndromes was also estimated in the referral population. The positive predictive value of the original test was 15.7% for 22q11.2 deletion syndrome, and 5.2% for the other 4 disorders combined. With the revised protocol, these values increased to 44.2% for 22q11.2 and 31.7% for the others. The 0.33% false-positive rate (FPR) for 22q11.2 deletion syndrome decreased to 0.07% with the revised protocol. Similarly, the FPR for the other 4 disorders combined decreased from 0.56% to 0.07%. Minimal prevalences were estimated to be 1 in 1,255 for 22q11.2 deletion syndrome and 1 in 1,464 for 1p36, cri-du-chat, and Angelman syndromes combined. Our results show that these microdeletions are relatively common in the referral population, and that the performance of SNP-based NIPT is improved with high-depth resequencing.

KEYWORDS
aneuploidy, microdeletion, non-invasive prenatal testing, single-nucleotide polymorphism, submicroscopic chromosome abnormality

1 | INTRODUCTION

Commonly encountered fetal chromosomal abnormalities include both whole-chromosome aneuploidies, such as Down syndrome (trisomy 21), and submicroscopic anomalies. The latter include copy-number variants (CNVs) <10 Mb (microdeletions and microduplications) that may be associated with clinically significant phenotypes. Such CNVs are found in approximately 1% of pregnancies undergoing amniocentesis or chorionic villus sampling (CVS). The most common microdeletion is at 22q11.2, with a population prevalence between 1 in 3,000 and 1 in 6,000; recent reports indicate the rate may exceed 1 in 1,000 in the prenatal population. As microdeletions are not thought to be correlated with maternal age, the most common may have a frequency comparable to that of Down syndrome in the pregnancies of younger women.

Chromosomal microarray analysis (CMA) is the preferred diagnostic tool used to detect submicroscopic CNVs. CMA is recommended for fetuses undergoing invasive prenatal testing after major structural abnormalities are detected by ultrasound, and is an option for any pregnant woman undergoing amniocentesis or CVS. However, because CMA requires invasive procedures that carry a risk
of fetal loss to obtain fetal cells for analysis, or because it may identify CNVs of uncertain clinical significance, some women may decline CMA. Analysis of cell-free DNA in maternal plasma potentially offers an alternative, non-invasive prenatal-testing path toward the identification of targeted microdeletions; only women found to be at high-risk need to be offered invasive testing.

Currently, single-nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT) can screen for 5 clinically significant microdeletions—22q11.2, 1p36, cri-du-chat (5p15.3), Prader-Willi (paternal 15q11-q13), and Angelman (maternal 15q11-q13). Previous analysis of a cohort of nearly 22,000 women referred for screening for the 22q11.2 deletion demonstrated a 0.5% screen-positive rate (SPR) and an 18% positive predictive value (PPV). Here, we report for the first time the screening performance of this SNP-based NIPT for 1p36, cri-du-chat, Prader-Willi, and Angelman microdeletion syndromes. We also update our clinical experience in screening for 22q11.2 deletion syndrome to now include over 80,000 referrals.

Earlier studies indicated that reflex sequencing of high-risk microdeletion test results at a higher depth of read (HDOR) may substantially reduce the false-positive rate (FPR). In this study, we assess the screening performance of this modification, combined with a higher quality-control confidence threshold. We compare the performance of the original and revised protocols, and use these results to estimate the prevalence of the 5 microdeletions in the test population.

2 | MATERIALS AND METHODS

2.1 | Study period and cohorts

A SNP-based NIPT screen for fetal chromosome abnormalities was offered clinically for the 22q11.2 deletion, or for all 5 deletions. A retrospective analysis of test performance was carried out for 80,449 referrals received for microdeletion screening, of which 42,326 were screened for all 5 deletions and the remainder were screened only for the 22q11.2 deletion, between February 19, 2014 and February 18, 2015. The 22q11.2 screening cohort reported in this study included 21,948 cases (received between February 19, 2014, and August 18, 2014) that were reported previously (Table S1, Supporting Information).

For each maternal blood sample, the maternal date of birth, maternal weight, gestational age, reason for testing, and informed consent were collected. Test-interpretation information, guidance on confirmatory testing options, genetic counseling, and disease-specific literature were available to test providers and patients.

2.2 | SNP-based analysis

Samples were analyzed at a Clinical Laboratory Improvement Act-certified, College of American Pathologists-accredited laboratory using previously described methodology. Briefly, samples were screened for chromosomes 13, 18, 21, X, and Y aneuploidy, and samples that received a low- or high-risk call for aneuploidy screening (ie, excluding samples that did not receive a test result) underwent multiplex polymerase chain reaction (PCR) amplification for the selected microdeletions. This amplification covered 672 SNPs within a 2.91 Mb section of the 22q11.2 region that is deleted in approximately 87% of individuals diagnosed with 22q11.2 deletion syndrome, and 1152 SNPs in each of the following: a 10 Mb region deleted in approximately 60% of patients diagnosed with 1p36 deletion syndrome, a 20 Mb region deleted in approximately 65% of patients diagnosed with cri-du-chat syndrome, and a 5.85 Mb region deleted in approximately 28% of patients diagnosed with Prader-Willi/Angelman syndromes. Amplified samples were sequenced to ≥3.2 million reads/sample. Deletions in each region were predicted using an algorithm that calculates and reports the maximum likelihood copy number for the region based on the allele-distribution pattern of the amplified SNPs, along with a confidence value that describes how well the data for the sample fits the expected data profile for the copy-number hypothesis.

2.3 | Definitions of risk

For the 22q11.2 deletion, samples with ≥90% confidence for the deletion at either allele were reported as "high-risk"; those with ≥90% confidence for no 22q11.2 deletion at both alleles were reported as "low-risk"; those with lower confidence values at the maternal allele were analyzed for paternal allele only; and those with lower confidence values at both alleles were reported as "risk unchanged." For the other microdeletion syndromes, samples with ≥80% confidence for a deletion were reported as "high-risk"; those with lower confidence values at the maternal allele were analyzed for paternal allele only; and those with lower confidence values at both alleles were reported as "risk unchanged." The reported risk status for each microdeletion syndrome was accompanied by a numerical estimate of fetal risk based on the population birth prevalence of the syndrome and the SNP analysis, and whether 1 or both alleles were successfully analyzed (Table S2). Cases in which a maternal deletion was suspected were assigned a fetal risk score of 50%. For cases with fetal fractions <6% (22q11.2 deletion) or <7% (other microdeletions) the reported numerical risk reflected analysis of only the paternal allele. Cases with >25% maternal or fetal haploblocks (genomic regions with identical genotypes on homologous chromosomes) on autosomal chromosomes are reported as ‘risk unchanged.

For 22q, any contiguous loss of heterozygosity involving >80% of the region was reported as "high-risk." For other genomic regions, the full region was required to show loss of heterozygosity to be reported as "high-risk."

2.4 | Pregnancy follow-up

Follow-up information, including results of ultrasound examinations performed either prior to, or following NIPT, was requested from providers for all high-risk cases via phone or email. If the initial outreach while the pregnancy was ongoing was uninformative, at least 2 more attempts were made after the estimated delivery date. A de-identified copy of the genetic testing report was requested whenever
diagnostic testing was performed. Providers were also requested to report false-negative results.

Cases identified as high-risk by NIPT were categorized as true positive (TP) or false positive (FP) based on the results of confirmatory diagnostic testing (CMA, fluorescence in situ hybridization, or methylation analysis for the 15q region). Samples without follow-up information, either because the patient did not have any additional testing or because there was no follow-up information available, were classified as "unknown" outcome.

2.5 | Performance metrics

The PPV, SPR, and FPR were calculated for each deletion syndrome and for combinations of syndromes. PPV = number of TPs/number of (TPs + FPs). Upper and lower boundary PPV values were calculated by assuming all unknown cases were either all TP or all FP, respectively. SPR = number of fetal high-risk results/number of cases that received a high-/low-risk result. FPR = unaffected high-risk calls/all unaffected cases. The FPR was estimated by assuming that the ratio of FPs to TPs for cases without diagnostic confirmation was equal to that for cases with diagnostic confirmation. For combinations of microdeletion syndromes, the number of TP, FP and unknown outcome cases were based on a proportionate prorating of the numbers for each individual disorder to allow for the different numbers of test calls. The SPR and FPR for combinations of syndromes were the sums of the individual syndrome rates. For PPV and FPR, the upper and lower limits were calculated for the boundary conditions in which all unknown cases were TP or FP, respectively.

2.6 | Post-hoc analysis

Post-hoc analysis of test performance was performed in an internally blinded manner using a revised protocol with a higher (95%) confidence threshold for reporting a case as high-risk for a microdeletion, and reflex sequencing of high-risk cases at HDOR (≥6 million reads/sample). Because the presence or absence of deletions on the paternally inherited chromosome are readily discerned at normal depth of read, only cases with a suspected deletion on the paternally inherited chromosome were reflexed to HDOR. To allow for cases without post-hoc analysis results (due to samples being unavailable for re-sequencing) in the estimation of PPV, SPR, and FPR for the revised protocol, a proportionate adjustment was made to the total number of cases tested.

2.7 | Estimation of disease prevalence in the study population

The prevalence of each microdeletion syndrome in the population was calculated as: number of affected pregnancies/number of cases with a test result × percentage of syndromic deletions expected to be captured by test), where the numerator is the sum of the number of known TPs, the expected number of affected cases in the group with an unconfirmed outcome, and the number of known false negative cases. Prevalence was calculated using only cases for which both maternally and paternally inherited alleles could be analyzed. For these estimates, it was assumed that the proportion of affected cases among samples with unknown outcome was equal to that of cases with known outcome.

2.8 | Institutional approval

The study was exempted from institutional review board approval (Ethical & Independent Review Services, Corte Madera, CA; Study ID 14064-01).

3 | RESULTS

3.1 | Referrals

A total of 80 449 referrals were received for microdeletion screening during the study period, of which 42 326 were for the full panel of microdeletion syndromes and the remainder were limited to testing for the 22q11.2 deletion (Figure 1 and Table S1). Because microdeletion screening was only performed when aneuploidy screening could be successfully completed, 5511 cases were ineligible for microdeletion screening for reasons including test canceled, draw <9 weeks GA, insufficient blood volume, contamination, multiple gestations, and low fetal fraction (Table S3). Of the 74 938 eligible cases, 39 678 were referred for screening all 5 microdeletions. Characteristics for all cases screened for microdeletions are summarized in Table 1.

3.2 | Test performance

3.2.1 | 22q11.2 deletion

Of the 74 938 eligible referrals, 283 cases (0.38%) received a high-risk result for a fetal deletion, 71 841 cases (95.9%) received a low-risk result, and 2808 cases (3.8%) received a risk-unchanged result (Table 2 and Figure 1). An additional 6 cases received a high-risk result due to a suspected maternal deletion. Of the 283 cases identified as being at high-risk for a fetal microdeletion, follow-up information on copy-number truth was available for 153 (54.1%) cases (via invasive diagnostic testing, n = 117; postnatal diagnostic testing, n = 32; or post-miscarriage products-of-conception testing, n = 4). Twenty-four (8.5%) of the high-risk results were TP (Table S4) and 129 (45.6%) were FP (Table 3).

3.2.2 | 1p36, cri-du-chat, Prader-Willi and Angelman microdeletions

A total of 39 678 samples were screened for all 4 microdeletions, with varying numbers of high-risk, low-risk, and risk-unchanged cases obtained for each microdeletion (Table 2 and Figure 1). Considering the 4 microdeletions together, there were 215 high-risk calls of which 7 were TP, 117 were FP and 91 had unknown outcome (results for each microdeletion and for the combination of deletions are in Table 3). Truth was established on the basis of invasive diagnostic testing (118 cases), postnatal diagnostic testing (5 cases), or post-miscarriage products-of-conception testing (1 case). Additional details of the 7 TPs are in Table S4. One false-negative case was reported (for cri-du-chat syndrome).
3.3 | Maternal deletions

Of the 74,938 total cases screened (whether for 22q11.2 deletion alone or for all 5 microdeletions), a fetal risk score of 50% was assigned in 6 cases due to suspected deletions in the 22q11.2 region in the mother; no case had suspected maternal deletions in any of the other interrogated regions. Follow-up information was available for 3 of these 6 cases. In 2 cases, a maternal deletion of the 22q11.2 region was confirmed, but fetal copy number was not provided. In the third case, a fetal deletion was confirmed, and although the mother’s copy number for the 22q11.2 region was not assessed, she had tetralogy of Fallot and learning disabilities, both of which are associated with 22q11.2 deletion syndrome.

3.4 | Performance based on presence or absence of ultrasound abnormalities

Test performance was compared for high-risk calls with, and without, major fetal structural abnormalities detected by ultrasound prior to NIPT (Table 4). Of the 498 cases determined to be high-risk for a fetal deletion by NIPT, 297 (59.6%) had information available about the presence/absence of ultrasound findings and 201 (40.4%) did not. Fifty-one of these cases had major fetal ultrasound anomalies (Table S5), of which 37 had abnormalities detected prior to NIPT. However, 260 cases had no reported ultrasound abnormalities at the time of NIPT; this includes 14 cases for which ultrasound information became available after NIPT testing was performed. As expected, PPVs were higher in cases with abnormal ultrasound findings identified prior to NIPT (Table 4). Most TP cases (80.6%; 25/31) had major ultrasound anomalies; 71% (22/31) had ultrasound anomalies that were detected prior to NIPT screening (Tables S3 and S4). In contrast, approximately 4.5% of FP cases (11/246) and 5.9% of unknown outcome cases (13/221) were reported to have major ultrasound abnormalities.

3.5 | Pregnancy outcomes for high-risk calls

Information regarding use of invasive testing was available for 84.5% (239/283) cases that were determined to be high-risk for a fetal 22q deletion; 49% (117/239) had invasive testing and 51% (122/239) did not. For the other 4 microdeletions combined, this information was available for 81.9% (176/215) of cases that were determined to be high-risk for a fetal microdeletion; 64.8% (114/176) had invasive testing and 35.2% (62/176) did not.

Post-NIPT pregnancy outcome information was available for 66.5% (331/498) of cases determined to have high-risk for a fetal microdeletion by the assay. Of these, 89% (295/331) of pregnancies were continued, 4% (14/331) miscarried, and 7% (22/331) were terminated. Among confirmed TP cases, 22.6% (7/31) reported terminations. Fifteen other cases reported terminations: 7 were FP, and 8 had unknown microdeletion status. Of the 7 FP, 4 had ultrasound anomalies detected after NIPT screening, 1 had no ultrasound anomalies, and 2 had no information available about presence/absence of ultrasound findings. The latter 3 cases and 1 of the cases with ultrasound anomalies were additionally reported to be at high-risk for trisomy 21 (n = 3) or trisomy 18 (n = 1). Of the 8 cases with unknown microdeletion status, 2 had ultrasound findings prior to NIPT. Pregnancy outcomes for the subset with high-risk results for 22q11.2 deletion
TABLE 1  Pregnancy characteristics for all cases screened for microdeletions

| Screening cohort (n = 74 938) | Maternal agea (year) |
|-------------------------------|----------------------|
| Maternal agea (year)          | Mean ± SD            |
| Mean ± SD                    | 32.0 ± 5.8           |
| Median (range)               | 33.0 (13.0-57.0)     |
| Gestational age (weeks)      | Mean ± SD            |
| Mean ± SD                    | 13.7 ± 4.1           |
| Median (range)               | 12.4 (8.4-41.7)      |
| Maternal weightb (lb)        | Mean ± SD            |
| Mean ± SD                    | 157.3 ± 38.3         |
| Median (range)               | 149.0 (81.0-438.2)   |
| Fetal fraction (%)           | Mean ± SD            |
| Mean ± SD                    | 10.5 ± 4.3           |
| Median (range)               | 9.8 (3.8-50.0)       |

Abbreviation: SD, standard deviation.

a At estimated delivery date.

b Only calculated for US cases. n = 61 536.

( n = 209) were as follows: 188 (90%) were continued, 7 (3%) miscarried, and 14 (7%) were terminated.

3.6 | Prevalence of microdeletions

In this study, the estimated prevalence of 22q11.2 deletion syndrome was 1 in 1255, and those for the 1p36 deletion, cri-du-chat, and Angelman syndromes ranged from 1 in 3624 to 5820 (Figure 2). The prevalence of Prader-Willi syndrome was not estimated due to lack of a TP case. The combined estimated prevalence of 1p36, cri-du-chat, and Angelman microdeletions in this cohort was 1 in 1464, and for all 5 disorders combined it was 1 in 676. Details of the calculations for prevalence are presented in Appendix S1. Because there may have been additional false negative cases that did not come to attention, these prevalence rates are minimal estimates.

3.7 | Post-hoc analysis

3.7.1 | 22q11.2 deletion

The revised protocol was applied to 268 (24 TP, 121 FP, and 123 unknown) of the 283 cases (94.7%) that were determined to have a high-risk call for 22q11.2 deletion using the original protocol (the remaining cases were unavailable for analysis; Figure 1). Of the 121 FP cases, 92 (76.0%) were reclassified as low-risk with the revised protocol. Conversely, for the known TP, 23 of 24 remained high-risk, a drop in detection rate of 4.2%. Of the 123 cases with unknown outcome, 88 (71.5%) were reclassified as low-risk, consistent with the assumption that these cases were a proportionate mixture of TP and FP cases (Appendix S2). Overall, there was a 67.2% (180/268) reduction in high-risk calls. Based on these findings, the SPR for the revised protocol was 0.13% (vs 0.39% with the original protocol), the PPV was 44.2% (vs 15.7% with the original protocol) and the FPR was 0.07% (vs 0.33% with the original protocol; Table 5).

3.7.2 | 1p36, cri-du-chat, Prader-Willi and Angelman microdeletions

Similar improvements were seen for the other microdeletions. Considering the 1p36, cri-du-chat, Prader-Willi and Angelman results together ( n = 215), the revised protocol was applied to 209 (7 TP, 114 FP, and 88 unknown) cases (97.2%) determined to be high-risk.
with the original protocol (the remaining cases were unavailable for analysis; Figure 1). Known FP results were reduced by 86.8% (99/114), all 7 TP remained high-risk calls, and there was a proportionate 83.0% (73/88) reduction in the high-risk calls for cases with unknown outcome. The revised protocol therefore had a SPR of 0.10% (vs 0.59% with the original protocol), a PPV of 31.7% (vs 5.2% with the original protocol) and an FPR of 0.07% (vs 0.56% with the original protocol), a combined minimum estimated prevalence of 1 in 676. The expected frequency is shown above each bar.

Abbreviations: FP, false positive; PPV, positive predictive value; NIPT, non-invasive prenatal testing; TP, true positive.

### TABLE 4
Comparison of positive predictive values for the original and revised screening protocols, stratified by presence or absence of major structural abnormalities detected by ultrasound, prior to non-invasive prenatal testing*

| Deletion syndrome | 22q11.2 | 1p36 | CdC | PWS | AS |
|-------------------|---------|------|-----|-----|----|
|                   | Original | Revised | Original | Revised | Original | Revised | Original | Revised | Original | Revised |
| With abnormal findings, n |         |       |     |     |     |     |     |     |     |     |
| TP, n             | 31      | 24    | 1   | 1   | 4   | 2   | 0   | 0   | 1   | 0   |
| FP, n             | 4       | 0     | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| PPV, %            | 82.6    | 100   | 100 | 100 | 100 | 100 |     |     |     |     |
| Without abnormal findings, or if present, detected after NIPT, n | 124 | 35 | 5 | 2 | 17 | 4 | 4 | 4 | 110 | 17 |
| TP, n             | 5       | 5     | 1   | 1   | 2   | 2   | 0   | 0   | 1   | 1   |
| FP, n             | 73      | 22    | 1   | 1   | 10  | 2   | 1   | 1   | 67  | 9   |
| PPV, %            | 6.4     | 18.5  | 50  | 50  | 16.7| 50  | 0   | 0   | 1.5 | 10  |

*Cases with unknown time of ultrasound relative to NIPT (n = 3) were conservatively counted among cases with anomalies known prior to NIPT.

4 | DISCUSSION

This study extends our initial report of the clinical experience of a SNP-based NIPT for the prediction of 22q11.2 deletions to include additional months of screening for the 22q11.2 deletion, and data for additional microdeletion syndromes: 1p36, cri-du-chat, Angelman, and Prader-Willi. The results show that 22q11.2, 1p36, cri-du-chat, and Angelman microdeletions are relatively common in the referral population, with a combined minimum estimated prevalence of 1 in 676. The performance of the original screening protocol for 22q11.2 deletion syndrome in this cohort is similar to that demonstrated previously. We also show that applying a stricter quality-control confidence threshold and reflexively sequencing high-risk samples to a HDOR with the revised screening protocol reduced FPRs and increased PPVs substantially. As a result of these performance improvements, the revised protocol has been implemented commercially.

Our initial study indicated that screening for 22q11.2 deletion syndrome using the original protocol would be associated with a 0.38% FPR, an 18.0% PPV, and a prevalence of 1 in 962 in the test population. In this study, with the original protocol, the FPR was 0.33%, the PPV was 15.7%, and the estimated prevalence was 1 in 1255. With a revised protocol, performance improved to a projected 0.07% FPR and 44.2% PPV. The revised protocol also exhibited relatively high PPVs for the other 4 microdeletion syndromes, although these had larger confidence intervals (Table 5). Because microdeletions are less prevalent than aneuploidies in NIPT cohorts, the PPVs for screening microdeletions are expected to be lower than those observed for aneuploidy. In fact, observed PPVs for microdeletions using the updated protocol are lower than reported NIPT PPVs for trisomy 18 and trisomy 21, and similar to those for trisomy 13 and monosomy X. Moreover, the PPVs we observed are similar to, or better than, those observed with conventional maternal serum screening for trisomies—a bar that has been considered sufficient to justify routine use.

Prior publications have questioned the use of NIPT as a screening test for microdeletions, citing concerns about high FPRs, low sensitivities, and challenges associated with variants of unknown significance. However, these reports focused on whole-genome sequencing approaches that employ counting-based methodologies. By concentrating on specific genomic regions with clinically significant deletions, the targeted nature of the SNP-based method overcomes many of the limitations discussed in these publications. Furthermore, comparison of detection rates for confirmed microdeletions in clinical cohorts and ratios of maternally inherited vs de novo deletions to published ratios suggests that SNP-based methods have substantially higher sensitivity than counting-based methods.

In this study, the observed FPR for Angelman syndrome (conveyed by the maternally inherited chromosome 15q11-13 region) was substantially higher than that for Prader-Willi syndrome (conveyed by the same
Third, demonstrated no significant bias toward unaffected pregnancies in cases screening. Moreover, comparison of the original and revised protocols trisomy 13 and monosomy X, and greater than that of maternal serum the revised protocol retains PPVs similar to the performance of NIPT for all cases with unknown outcomes are FPs, the SNP-based NIPT with these data indicate that even in the unlikely event values, assuming all cases with unknow no u t nc o m ew e r ee i t e ra lT Po ra l l

This study has a number of limitations. First, this is a clinically derived cohort and the patients who were selected for testing may not reflect a general obstetrical population (ie, the referral population may have been enriched for cases with a high a priori risk for a microdeletion). This was evidenced by the high percentage of TP cases with ultrasound abnormalities identified prior to performing NIPT (Table S5). When we estimated the PPVs for the subset of the cohort without any abnormal ultrasound findings prior to NIPT, we found lower PPVs, as expected. We recalculated the PPVs to adjust for differences in prevalence and found that the test continued to show good performance in low prevalence populations (Appendix S3 and Table S6). Second, we were unable to obtain follow-up data for 44% of cases in this study despite considerable efforts to gather this information. To address this issue, we calculated PPV boundary data for 44% of cases in this study despite considerable efforts to gather and statistical variation.

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This study extends previous work demonstrating that prenatal screening for the 22q11.2 deletion can be effectively performed via a SNP-based NIPT. In this study, the combined estimated prevalence for the set of microdeletion disorders in the screened population (1/676) exceeded that of Down syndrome in younger women, and also that of open neural-tube defects in the US population (1/1886). Additionally, PPVs and FPRs of SNP-based screening appeared to be superior to those of traditional screening methods offered to screen for Down syndrome and open neural-tube defects. The observed prevalences and test performance therefore exceed that of other prenatal screening tests long considered standard of care. Consistent with recent American College of Medical Genetics and Genomics guidelines, our findings support offering microdeletion screening as an adjunct to existing NIPT to refine risks for these 5 clinically significant, well-characterized genetic disorders.

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**TABLE 5 Projected test performance with the revised screening protocol**

| Deletion syndrome | 22q11.2 | 1p36 | CdC | PWS | AS | 1p36, CdC, PWS, AS combined | Rates per 100 000 tests |
|------------------|---------|------|-----|-----|----|-----------------------------|-----------------------|
| Adjusted total calls | 68 307<sup>a</sup> | 37 350<sup>c</sup> | 38 358<sup>d</sup> | 38 673 | 32 095<sup>e</sup> | 100<sup>g</sup> | 228<sup>h</sup> |
| High-risk call, n | 87 | 7 | 7 | 4 | 17 | 0.10<sup>n</sup> | 0.23<sup>h</sup> |
| SPR, % | 0.13 | 0.02 | 0.02 | 0.01 | 0.05 | 19<sup>a</sup> | 53<sup>h</sup> |
| TP, n | 23<sup>f</sup> | 2 | 4 | 0 | 1 | 41<sup>g</sup> | 84<sup>h</sup> |
| FP, n | 29 | 3 | 2 | 1 | 9 | 0.07<sup>n</sup> | 0.14<sup>h</sup> |
| Unk, n | 35 | 3 | 1 | 1 | 7 | 0.07<sup>n</sup> | 0.14<sup>h</sup> |
| PPV (range)<sup>h</sup>, % | 44.2 (26.4-66.7) | 50.0 (28.6-71.4) | 66.7 (57.1-71.4) | 0 (0-75.0) | 10.0 (5.9-47.1) | 31.7 (19.0-59.0) | 38.7 (23.2-63.2) |
| FPR (range)<sup>h</sup>, % | 0.07 (0.04-0.09) | 0.01 (0.005-0.01) | 0.01 (0.005-0.01) | 0.01 (0.003-0.01) | 0.05 (0.03-0.05) | 0.07<sup>n</sup> (0.04-0.08) | 0.14<sup>h</sup> (0.08-0.18) |

Abbreviations: AS, Angelman syndrome; CdC, cri-du-chat syndrome; FP, false positive; FPR, false-positive rate; PPV, positive predictive value PWS, Prader-Willi syndrome; TP, true positive; Unk, cases with unknown outcome.

<sup>a</sup> Adjusted total calls include cases that tested positive with the original screening protocol, but were unavailable for reflex screening or did not have a result on reflex testing.

<sup>b</sup> Fifteen 22q11.2.

<sup>c</sup> One 1p36.

<sup>d</sup> One CdC.

<sup>e</sup> Six AS.

<sup>f</sup> One TP case tested by the original protocol was incorrectly reclassified as low risk by the reflex test.

<sup>g</sup> Sum of the prorated number of cases, allowing for the different number of calls for each microdeletion, calculated for 100 000 total test results.

<sup>h</sup> Upper and lower boundaries assuming all unknown cases were TP or FP, respectively.

<sup>i</sup> Sum of the rates for each microdeletion.
Conflict of interest

K.M., S.J., A.K., C.L., M.S., K.K., H.R., T.T., R.A., A.L.S., and Z.P.D. were employees of Natera, Inc. and hold stock or options to stock in the company. P.B. is a paid consultant for Natera, Inc. and holds options to stock in the company.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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