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ORIGINAL ARTICLE

Increased Positive Predictive Value for a Single-Nucleotide Polymorphism-Based Non-invasive Prenatal Test for the 22q11.2 Deletion

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Abstract Non-invasive prenatal testing (NIPT) has expanded its coverage beyond the common aneuploidies to include subchromosomal abnormalities such as the 22q11.2 deletion through the analysis of cell-free DNA. We previously reported a positive predictive value (PPV) of 18% and false-positive rate (FPR) of 0.38% for a single-nucleotide polymorphism (SNP)-based NIPT for fetal 22q11.2 deletion in a clinical cohort. Herein, we assess the performance of the test following methodology changes that include sequencing high-risk calls at high depth of read (HDOR) and raising the confidence threshold of the algorithm from 0.90 to 0.95. At the original confidence cut-off, a PPV of 42.3% upon reflex-sequencing of high-risk samples at a HDOR was reported. Raising the algorithm's confidence threshold further increased the PPV to 52.4% and reduced the FPR to 0.07%, with no loss in test sensitivity. The PPV was 100% for high a priori risk cases, and 20% for low a priori risk cases. The improved assay performance documented with the updated methodology supports the efficacy of the SNP-based NIPT in the detection of fetal 22q11.2 deletion.

Keywords SNP-based NIPT · 22q11.2 deletion syndrome · PPV · Microdeletions · Reflex sequencing

22q11.2 deletion syndrome (22q11.2 DS) is a well-characterized chromosomal abnormality with a high prevalence (1/3000 to 1/6000 live births) and severe phenotype that is independent of maternal age [1]. The syndrome is associated with a spectrum of clinical manifestations including congenital heart defects, hypocalcemia, immune deficiency, palate abnormalities, and intellectual disability [2]. Early detection and prompt intervention can positively impact the clinical outcome of individuals affected with the 22q11.2 deletion. However, detection rates through current screening modalities, including ultrasound examinations, remain low. Thus, alternate methodologies for prenatal screening are necessary to identify pregnancies that are high risk for the 22q11.2 deletion.

Given its superior performance over traditional screening methodologies, non-invasive prenatal testing (NIPT) has altered the landscape of prenatal screening for whole chromosomal aneuploidies [3]. Recently, advances in NIPT technology has allowed for expansion of its coverage of testing to include a range of clinically significant subchromosomal abnormalities such as the 22q11.2 DS [4–7]. A number of validation studies and clinical experience studies indicate the utility of NIPT in the detection of the 22q11.2 deletion [4, 7–10]. Analytical validation of a single-nucleotide polymorphism (SNP)-based NIPT for the microdeletion demonstrated a sensitivity of 97.8% and a false-positive rate of 0.76% [4]. In a clinical experience study (Gross et al. [9]), we report an FPR of 0.38% and a positive predictive value (PPV) of 18.0% of the SNP-based NIPT in a cohort of 20,776 samples. Post-hoc analysis of high-risk samples with a suspected deletion on the maternal homologue at a higher depth of read (HDOR) eliminated two-thirds of the false positive (FP) calls, decreasing the FPR to 0.12% and increasing the PPV to 42.3%; samples with deletions on the paternal homologue are detected at standard depth of read (SDOR).

Here, we extend the findings of Gross et al. by implementing additional methodology changes such as raising the algorithm's positive call confidence threshold from 0.90 to 0.95 in addition to reflex sequencing at a high depth

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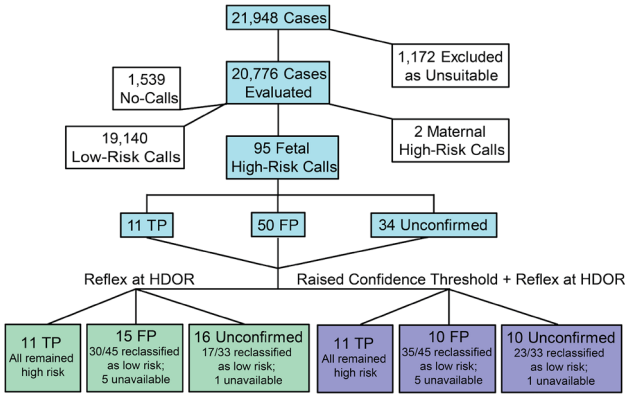

Fig. 1 Study flow chart of samples

of read (HDOR); the impact of the revised protocol on the performance of the SNP-based assay for the detection of the 22q11.2 deletion is assessed.

In the original study of 20,776 reported samples, at the SDOR, the algorithm reported 95 cases as high risk for the fetal 22q11.2 deletion. Follow-up information regarding copy number truth for the 22q11.2 locus was collected for 61 high-risk cases via invasive diagnostic testing (n = 48), postnatal testing (n = 11), or post-miscarriage products-of-conception testing (n = 2). Clinical follow-up revealed 11 true positive (TP) calls and 50 FP calls; 34 calls had no follow-up information available. At HDOR, the algorithm continued to call all 11 TP cases as high risk, while calling only 15 FPs; among cases with no follow-up confirmation, 16 remained high risk. Here we report that raising the confidence cut-off in addition to resequencing high-risk samples at HDOR further decreased the number of FP and unconfirmed calls to 10 each, while preserving all 11 TP calls (Fig. 1). Altogether, these changes eliminated 80% of the FP cases originally reported, yielding an improved PPV of 52.4%. Assuming the cases with unconfirmed copy number status at the 22q11.2 locus were either all FP or all TP resulted in lower and upper PPV boundaries of 35.5–67.8%. Overall, the FPR was reduced to 0.07% and the screen-positive rate to 0.15% (Table 1).

Information on the presence or absence of fetal structural abnormalities detected by ultrasound examinations were collected for 77 (81%) of the 95 high-risk cases, and an a priori risk status was determined. Ultrasound anomalies included tetralogy of Fallot, truncus arteriosus, ventricular septal defect, polyhydramnios, pleural effusion, and oligohydramnios among others, all of which are indicative of the 22q11.2 deletion. Of the original 95 high risk 22q11.2 deletion calls, the updated methodology accurately identified all TP cases for which ultrasound anomalies directly associated with the deletion syndrome had been detected prior to NIPT, as high risk. There were no FPs among this sample subset, yielding a PPV of 100% for cases with high a priori risk. Of note, for cases with low a priori risk (i.e., those for which 22q11.2 DS-associated ultrasound anomalies were not observed prior to NIPT screening), the PPV increased to 20%—a fourfold increase from the PPV originally observed for this group (Table 1). In summary, reflex sequencing at HDOR and raising the algorithm's confidence threshold reduced the FPR and consequently improved the PPV of this SNP-based screening test for the 22q11.2 deletion, in both high-and low-risk populations, without affecting the detection rate.

Advances in technology coupled with continuous improvements in the performance of NIPT have facilitated the expansion of NIPT's coverage to include detection of

Table 1 Improved performance metrics for the 22q11.2 deletion screening NIPT

	Original protocol SDOR	Reflex at HDOR	Raised confidence threshold + reflex at HDOR
High-risk fetal cases			
FPR[a] (boundary FPR)[b] (%)	0.38 (0.24–0.40)	0.12 (0.07–0.15)	0.07 (0.05–0.10)
PPV[c] (boundary PPV)[d] (%)	18 (11.6–47.4)	42.3 (26.2–64.3)	52.4 (35.5–67.8)
SPR[e] (%) (n/N)	0.46 (95/20,776)	0.20 (42/20,776)	0.15 (31/20,776)
High a priori cases			
PPV (%)	81.8	100	100
Low a priori cases			
PPV (%)	5.1	16.7	20.0

FPR false-positive rate, *HDOR* high depth of read, *PPV* positive predictive value, *SDOR* standard depth of read, *SPR* screen-positive rate

[a]FPR was calculated by dividing the sum of known FP cases and projected FP cases [(1 − PPV)*(number of unconfirmed cases)] by 20,776

[b]Lower and upper FPR boundaries were calculated assuming cases without follow-up confirmation were all TP or all FP, respectively

[c]PPV was calculated by dividing the TP cases by the sum of TP and FP cases

[d]Lower and upper PPV boundaries were calculated assuming cases without follow-up confirmation were all FP or all TP, respectively

[e]SPR was calculated by dividing the number of high-risk (n) cases by 20,776 (N)

genetic disorders with high penetrance and severe phenotypes such as the 22q11.2 deletion. Here, we report that the performance of the SNP-based test following improvements in methodology results in substantial reduction in the FPR and improved PPV of the assay, providing additional evidence for the efficacy of the SNP-based NIPT in detecting the 22q11.2 deletion. With its improved assay performance, the SNP-based NIPT offers a valuable addition to the current population-wide prenatal screening approaches for the 22q11.2 deletion.

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Compliance with Ethical Standards

Conflict of interest A.R., S.I., and Z.D., are employees of Natera and hold stock/options to hold stock in the company.

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