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Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome

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KEYWORDS: 22q11.2 deletion syndrome; cardiac defects; microdeletions; NIPT; ultrasound

ABSTRACT

Objectives *To evaluate the performance of a single-nucleotide polymorphism (SNP)-based non-invasive prenatal test (NIPT) for the detection of fetal 22q11.2 deletion syndrome in clinical practice, assess clinical follow-up and review patient choices for women with high-risk results.*

Methods *In this study, 21 948 samples were submitted for screening for 22q11.2 deletion syndrome using a SNP-based NIPT and subsequently evaluated. Follow-up was conducted for all cases with a high-risk result.*

Results *Ninety-five cases were reported as high risk for fetal 22q11.2 deletion. Diagnostic testing results were available for 61 (64.2%) cases, which confirmed 11 (18.0%) true positives and identified 50 (82.0%) false positives, resulting in a positive predictive value (PPV) of 18.0%. Information regarding invasive testing was available for 84 (88.4%) high-risk cases: 57.1% (48/84) had invasive testing and 42.9% (36/84) did not. Ultrasound anomalies were present in 81.8% of true-positive and 18.0% of false-positive cases. Two additional cases were high risk for a maternal 22q11.2 deletion; one was confirmed by diagnostic testing and one had a positive family history. There were three pregnancy terminations related to screening results of 22q11.2 deletion, two of which were confirmed as true positive by invasive testing.*

Conclusions *Clinical experience with this SNP-based non-invasive screening test for 22q11.2 deletion syndrome indicates that these deletions have a frequency of approximately 1 in 1000 in the referral population with most identifiable through this test. Use of this screening method requires the availability of counseling and other management resources for high-risk pregnancies. © 2015 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of the International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

The availability of non-invasive prenatal testing (NIPT) is altering the practice of prenatal genetics and maternal–fetal medicine, resulting in a decline in invasive testing[1]. Similar to conventional screening, NIPT screens for trisomies 18 and 21. NIPT also incorporates screening for trisomy 13 and, in some cases, sex chromosome aneuploidies and triploidy. Multiple studies have demonstrated the improved performance of NIPT compared with conventional screening[2,3]. Recently, a single-nucleotide polymorphism (SNP)-based NIPT was validated for detection of five important, clinically significant microdeletion syndromes: 22q11.2, Prader–Willi, Angelman, cri-du-chat and 1p36 deletion[4]. For all five disorders, the detection rate for the large causal deletions was > 97%, with a specificity of > 99%[4].

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Of the five microdeletion syndromes, 22q11.2 deletion syndrome (i.e. DiGeorge or velocardiofacial syndrome) is the most common, with reported prevalence ranging from 1 in 2000–6000 livebirths[5–7]. The frequency appears to exceed 1 in 1000 in referrals for prenatal diagnosis, even when cases with ultrasound evidence for fetal abnormalities are excluded[8,9].

After Down syndrome, 22q11.2 deletion syndrome is the second most common cause of congenital heart disease and is an important consideration whenever a conotruncal cardiac anomaly is identified, in particular tetralogy of Fallot. Additional developmental disabilities are also often present[10]. Calcium treatment at birth may prevent hypocalcemic seizures, potentially mitigating their negative effects on long-term cognition[11]. Prenatal screening could alert caregivers to an affected neonate, and allow for delivery and treatment at a tertiary-care facility.

Although it is appreciated that prenatal screening for this syndrome could have a positive influence on the lifetime health of the newborn, concerns similar to those expressed for standard NIPT panels have arisen. For example, will women receiving a high-risk result receive adequate counseling and referral to a high-risk center? Will the distinction between screening and diagnosis be explained sufficiently? And will the need for confirmatory testing be fully understood[12]?

Here we report the first 6 months of experience using an SNP-based NIPT to screen for 22q11.2 deletion syndrome. The goals were twofold: to determine the test's performance in clinical practice and to assess follow-up test utilization for women who received a high-risk result.

METHODS

A retrospective analysis was carried out for 21 948 consecutive referrals for fetal aneuploidy and microdeletion screening received over a 6-month period from 19 February to 18 August 2014. This included 10 715 referrals for 22q11.2 microdeletion screening and 11 233 for screening of all five microdeletion syndromes. For each blood sample received, the following patient information was requested: gestational age, maternal date of birth, maternal weight, whether the mother was a known microdeletion carrier, and the reason for testing based on the International Classification of Diseases (ICD-9 codes). Additional detailed information about the reason for referral (beyond that gathered through ICD-9 codes) was not obtained routinely at the time of testing. Some samples were considered outside the specifications for testing for reasons described previously[2]. A paternal buccal swab was requested but not required, and 5912 (26.9%) cases included a paternal sample. The study received a notification of exempt determination from an institutional review board (Ethical and Independent Review Services Assigned Study ID: 14064–01). Prior to testing, women were asked to sign an informed consent that included information about the disorders and the possibility that the test could identify a maternal 22q11.2 deletion.

Samples were sent to our laboratory, which was certified under the Clinical Laboratory Improvement Act and accredited by the College of American Pathologists, in which multiplex polymerase chain reaction (PCR), sequencing and analysis were carried out as described previously[13–16]. Prior to analysis of the 22q11.2 region, the standard panel testing for aneuploidy at chromosomes 13, 18, 21, X and Y was conducted; samples that failed quality control at this step were not evaluated at the 22q11.2 region. For analysis of the 22q11.2 region, samples underwent multiplex PCR with primers designed to amplify 672 SNPs targeting the 2.91-megabase (Mb) portion in the 22q11.2 region associated with the 22q11.2 deletion syndrome[4], located between SNP coordinates 18 835 221 and 21 592 477 (based on human genome build hg19)[17]. A blood redraw was requested when a result was not returned using the standard aneuploidy test panel (except in cases for which there were large regions of loss of heterozygosity or those in which maternal or fetal mosaicism was suspected). For cases that received a result on the standard aneuploidy test panel, but failed quality metrics at the 22q11.2 region, a redraw was not requested but it was analyzed if submitted.

Fetal copy number for the 22q11.2 region was predicted based on the allele distribution pattern for SNPs in the target region[18]. Results for the 22q11.2 region were reported as 'high risk', 'low risk', or 'risk unchanged' with an associated estimate of risk. Numerical risk estimates were based on the population incidence of 22q11.2 deletions, the assumption that the deletion region covered by this assay accounts for 87% of all 22q11.2 microdeletions[19], and the sensitivity and specificity of the assay[4]. The previously described approach to assigning risk was modified to take into account the fact that when the fetal fraction (FF) is $< 6\%$, only the presence or absence of the paternal contribution is reported. A pregnancy considered to be high risk for a *de novo* fetal 22q11.2 microdeletion received a risk of 1/45 if only the paternal contribution could be analyzed or a risk of 1/19 if both the paternal and maternal inherited haplotypes could be evaluated. When a 22q11.2 deletion was suspected in the mother, the fetal status was unclear due to the dominating effect of the maternal deletion, and therefore the fetus was assigned a risk of 1/2. A low-risk result received a risk estimate of 1/13 300 when both parental haplotypes were evaluated and there was no evidence for deletion. When only the paternally inherited haplotype could be evaluated and there was no evidence for deletion, the result was also considered low risk with a score of 1/3031. Samples were sequenced with an average of 8.9 million reads per sample mapped to the genome.

Follow-up information was requested from providers for all high-risk cases; clinical follow-up was completed on 14 April 2015. We also requested details of any abnormal ultrasound findings known to be present. Providers were also encouraged to voluntarily report false-negative results. Based on pre- or postnatal diagnostic test results (chromosomal microarray or fluorescence *in situ* hybridization (FISH) using 22q probes), cases identified

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as high risk were categorized as 'true positive' (TP) or 'false positive' (FP). 'Unknown' samples were those for which confirmatory information was not available.

Sample and patient characteristics were expressed as mean ± SD or median (range), unless otherwise indicated. Positive predictive value (PPV) was calculated for cases in which cytogenetic or molecular cytogenetic confirmation was performed (PPV = TP/(TP + FP)). The PPV was calculated separately for cases with ultrasound anomalies associated directly with the 22q11.2 deletion syndrome identified prior to NIPT, and for cases with no ultrasound abnormalities identified prior to NIPT.

Reports detailing the result obtained, along with the associated risk score following NIPT, were made available to physicians and their patients directly. All high-risk results were communicated directly to the ordering provider by a genetic counselor who discussed the findings and provided contact information for further questions. Providers were also offered supplemental written information designed for patients that described the meaning of a screening test, the result and how to interpret it, core information of the condition, and the options for confirmatory testing. The genetic counseling team was also available to discuss high-risk results with patients, if desired.

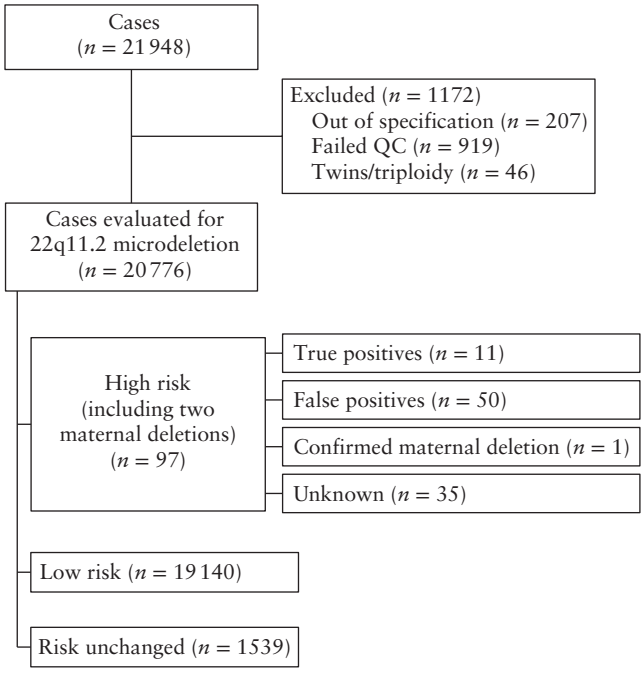

Figure 1 Flow chart of 21 948 samples from pregnant women submitted for screening for 22q11.2 microdeletion using single-nucleotide polymorphism-based non-invasive prenatal testing. True positives, false positives and confirmed maternal deletions were based on diagnostic testing. QC, quality control.

RESULTS

Participants and samples

A flow chart of the 21 948 samples that were submitted for screening for 22q11.2 deletion syndrome is shown in Figure 1. Of the cases submitted, 1172 (5.3%) were excluded after being considered unsuitable; samples were 'out of specification' (Table S1), failed quality metrics (Table S2) or received a twin or triploidy result at NIPT. The remaining 20 776 (94.7%) cases were evaluated for a 22q11.2 microdeletion. Ninety-seven (0.5%) cases were determined to be high risk, 19 140 (92.1%) low risk, and 1539 (7.4%) risk unchanged (i.e. risk was equivalent to population risk). The screen-positive rate was 97/20 776 (0.47%). The average turnaround time for analyses of samples in the study was 7.8 days. Demographics of all patients submitted for screening, those found to be high risk and those that were true positives for 22q11.2 microdeletion are shown in Table 1.

Cases of suspected maternal deletion

Two of the 97 high-risk cases had a suspected deletion in the mother. For one of these cases, diagnostic testing confirmed the presence of the deletion in the mother. Consistent with this finding, the mother had a history of a ventricular septal defect repair, high arched palate, learning disabilities, and dysmorphic craniofacial features. Invasive testing using amniocentesis found that the fetus was negative for the deletion. For the other case of suspected maternal deletion, neither the mother nor the fetus underwent diagnostic testing. However, the mother had previously lost a child affected with the 22q11.2 deletion syndrome.

Follow-up on high-risk cases

Of the 95 cases that received a high-risk result for fetal 22q11.2 microdeletion, information about the provision of invasive testing was available for 84 (88.4%): 57.1% (48/84) had invasive testing and 42.9% (36/84) did not. Clinical outcomes of the 95 high-risk cases are shown in Table 2 and population demographics for the 84 patients with data on invasive testing are shown in Table S3. Of the 36 cases that did not undergo invasive testing, at least 86.1% (31/36) were known to have been offered the option of an invasive test. For the remaining five, it is unknown whether it was offered.

The results for diagnostic testing were available for 61 high-risk cases via invasive testing ($n = 48$), postnatal testing ($n = 11$) or testing products of conception following a miscarriage ($n = 2$). Eleven (18.0%) cases were confirmed to be true positives (Tables 1, 2 and S4) and 50 (82.0%) were considered to be false positives (Table 2). Providers were encouraged to report any false-negative cases, but no such cases were reported.

At the time of writing, pregnancy status was established for 66 (69.5%) cases: 58 had delivered, three experienced a miscarriage, and five chose to terminate the pregnancy (Table 2).

Ultrasound findings in high-risk cases

Ultrasound data were available for 81.1% (77/95) of high-risk cases, with anomalies observed, either before or

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Table 1 Demographic characteristics of total study cohort of 21 948 women undergoing screening for 22q11.2 microdeletion by non-invasive prenatal testing (NIPT), and subgroups of those found to be high risk for the microdeletion following NIPT and those with true-positive result confirmed by diagnostic testing

Characteristic	*Total population* (n = *21 948*)	*High risk* (n = *97*)	*True positive* (n = *11*)
Maternal age (years)*			
Mean ± SD	33.1 ± 5.7	32.8 ± 5.3	31.6 ± 5.7
Median (range)	34.0 (15.0–51.0)	33.0 (19.0–45.0)	34.0 (22.0–39.0)
GA (weeks)			
Mean ± SD	13.9 ± 4.3	15.1 ± 4.9	21.9 ± 6.8
Median (range)	12.4 (5.4–40.0)	13.0 (9.7–31.4)	23.4 (11.9–31.4)
Maternal weight (kg)			
Mean ± SD	72.4 ± 18.5	71.0 ± 16.4	77.8 ± 23.7
Median (range)	68.0 (36.7–200.5)	67.1 (45.4–135.2)	72.6 (56.2–135.2)
Fetal fraction (%)			
Mean ± SD	10.3 ± 4.5	9.4 ± 2.6	11.6 ± 4.3
Median (range)	9.7 (0.8–46.7)	8.9 (5.0–20.9)	10.2 (5.9–20.9)

*At estimated date of delivery. GA, gestational age.

after NIPT screening, in 26/77 (33.8%; Table 3). In all nine true-positive cases with abnormal ultrasound findings observed during their pregnancy, the anomalies were associated directly with the 22q11.2 deletion syndrome (Table S5)[20–22] and, in eight of these cases, the anomalies were observed prior to NIPT screening (Table 4). Of the nine false-positive cases with abnormal ultrasound findings, at least four had findings typically associated with 22q11.2 deletion syndrome. Specific anomalies were unknown for two of the nine false-positive cases.

Positive predictive values

Based on the 61 cases with cytogenetic or molecular confirmatory diagnosis, the PPV was calculated to be 18.0%. Using the assumption that the remaining unconfirmed cases ($n = 34$) were either all false positives or all true positives, a boundary PPV range of 11.6–47.4% was calculated (Table 2). Information about the presence or absence of relevant abnormal ultrasound findings was available for 48 (78.7%) of the 61 cases. When considering only true-positive cases with ultrasound findings associated directly with 22q11.2 deletion syndrome, observed prior to NIPT ('high-risk' referrals), the PPV was 88.9% (8/9). For cases with no ultrasound anomalies identified prior to NIPT ('low-risk' referrals) the PPV was 4.9% (2/41). Only one of the false-positive cases had an ultrasound anomaly associated directly with the 22q11.2 deletion observed prior to NIPT.

Prevalence of 22q11.2 deletion

A minimal estimate of the prevalence of large 22q11.2 deletions in the study population (excluding cases for which only the paternally inherited allele was analyzed) was 10/16 846 = 1/1685. Under the assumption that cases without diagnostic confirmation would show an equal proportion of affected cases to that seen for cases with follow-up, the prevalence of large deletions in this referral population would be at least 15.5/16 846 = 1/1087. Under the further assumption that the assay only found large deletions, which constitute 87% of all deletions present in the 22q11.2 deletion syndrome[19], the prevalence of the syndrome in the study population is estimated to be 1/946.

DISCUSSION

This is the first report on the practical experience of prenatal screening for 22q11.2 microdeletion using the SNP-based NIPT. We found that 22q11.2 deletions are relatively common in the referral population, and that many can be identified using this screening test.

The estimated prevalence of 1/946 reported here is higher than estimates from livebirths[5–7], but is comparable to rates reported in prenatal diagnoses[8,9], which are enriched for affected pregnancies due to the association of the disorder with increased nuchal translucency thickness, cardiac defects and other abnormal ultrasound findings[23,24]. A recent study that evaluated 22q11.2 deletions in an NIPT referral population identified deletions in 1/8770 mothers and 1/8352 fetuses (including cases in which a deletion was also present in the mother)[25]. NIPT referrals are also likely to be enriched for cases with increased nuchal translucency thickness, cardiac defects or other ultrasound findings suggestive of 22q11.2 deletion syndrome. Although current ACOG guidelines recommend offering invasive testing when there is ultrasound evidence of fetal anatomic abnormalities[3], such findings were present in nine of our true-positive cases, indicating that these women chose to use NIPT instead of invasive testing. Some of these cases also had late gestational ages (Table S4), suggesting that physicians may have been offering women with ultrasound anomalies NIPT for the 22q11.2 deletion syndrome screening as an alternative to a definitive invasive test. Our observed high prenatal prevalence compared to livebirth rates might also be partially attributable to reduced viability of severely affected fetuses. It is also possible that prevalence in the general population may have been underestimated[22,26].

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Table 2 Use of invasive prenatal testing, diagnostic testing, pregnancy outcome, and positive predictive values (PPV) for 95 cases determined to be at high risk for 22q11.2 microdeletion based on non-invasive prenatal testing (NIPT) results

Clinical outcome	*Value*
Use of invasive prenatal testing	
Invasive test*	48 (50.5)
No invasive test†	36 (37.9)
Unknown‡	11 (11.6)
Diagnostic test result§	
TP	11 (11.6)
FP¶	50 (52.6)
No follow-up	34 (35.8)
Pregnancy outcome	
Delivered	58 (61.1)
Miscarriage	3 (3.2)
Termination**	5 (5.3)
Unknown/pending	29 (30.5)
PPV	
Cytogenetic/molecular cytogenetic tested cases	18.0 (11/61)
Untested cases, assumed FP (lower bound)	11.6 (11/95)
Untested cases, assumed TP (upper bound)	47.4 (45/95)
High-risk cases††	88.9 (8/9)
Low-risk cases‡‡	5.1 (2/39)
FP rate	
Predicted PPV§§	0.38 (78/20 776)
Lower bound¶¶	0.24 (50/20 776)
Upper bound***	0.40 (84/20 776)

Data are given as *n* (%) or % (*n*/*N*). *Six true positive (TP) and 42 false positive (FP). †Eleven of 36 patients had confirmatory postnatal diagnostic testing. ‡Three of 95 patients experienced miscarriage; two had diagnostic testing on products of conception, both of which were false positive. §Diagnostic testing included invasive ($n = 48$), postnatal ($n = 11$) and testing of products of conception following miscarriage ($n = 2$). ¶Eighteen had follow-up testing based on chromosomal microarray, 25 on FISH, three on chromosomal microarray and FISH, four had unknown molecular cytogenetic test type. **Two TP and two FP (one with NIPT result high risk for trisomy 21 and confirmed mosaic trisomy 21 and one with ultrasound evidence for short long bones suggestive of achondroplasia). One terminated for unstated reasons and did not receive confirmatory testing; however she did receive counseling at the facility in which NIPT was ordered. ††Cases with ultrasound anomalies directly associated with 22q11.2 deletion syndrome observed prior to NIPT screening. ‡‡Cases with no ultrasound anomalies observed prior to NIPT screening. §§Applying PPV of 18.0% to the 34 cases with no diagnostic follow-up results in 28 additional FPs; therefore there were an estimated 78 FPs (50 + 28) out of 20 776 cases evaluated for 22q11.2 deletion. ¶¶Assuming all 34 cases with no diagnostic follow-up were TP, there were 50 FP results out of 20 776 cases evaluated for 22q11.2 deletion. ***Assuming all 34 cases with no diagnostic follow-up were FP, there were 84 FP results out of 20 776 cases evaluated for 22q11.2 deletion.

Our assessments of true- *vs* false-positive results were based on the assumption that follow-up diagnostic tests detected reliably the presence of all clinically significant deletions. However, follow-up testing on at least 25 high-risk cases considered to be false positives, including four with ultrasound anomalies directly associated with this syndrome, relied on standard FISH methodology.

Table 3 Presence of abnormal ultrasound findings, either before or after non-invasive prenatal testing for a 22q11.2 microdeletion, in 95 cases at high risk

Ultrasound anomaly	*True positive*	*False positive*	*Unknown*	*Total*
Yes	9	9	8	26
No	2	33	16	51
Unknown	0	8	10	18

Data are given as *n*.

Table 4 Presence and description of ultrasound findings, either before or after non-invasive prenatal testing (NIPT) for 22q11.2 microdeletion, in 11 true-positive cases confirmed diagnostically

Case	*Ultrasound anomaly*	*Before NIPT*	*After NIPT*
1	Yes	Increased NT	Polyhydramnios
2	Yes	Tetralogy of Fallot	—
3	Yes	Tetralogy of Fallot	—
4	Yes	Tetralogy of Fallot	—
5	Yes	VSD	—
6	Yes	Omphalocele	Tetralogy of Fallot
7	Yes	Tetralogy of Fallot	—
8	Yes	Truncus arteriosus	—
9	No	—	—
10	No	—	—
11	Yes	Cardiac defect*	—

*Coarctation of the aorta or interrupted aortic arch. NT, nuchal translucency; VSD, ventricular septal defect.

Because current FISH probes may not be able to detect all 22q11.2 deletions in the critical region[22,27], it is possible that some cases that appeared to be false positive were in fact true positive.

Another limitation of this study was the lack of follow-up data on both high-risk and low-risk cases. Although attempts were made to follow-up all high-risk cases, confirmatory diagnostic information was unavailable for 36%. This included cases for which patients chose not to have any confirmatory testing or were reluctant to share confirmatory testing results, as well as cases for which the patient was lost to follow-up. Providers were encouraged to report false-negative cases, but no such cases were reported. However, because follow-up on low-risk cases was not carried out, calculation of the negative predictive value was not possible. Based on the estimated prevalence of 1/946, and assuming that our assay was only able to detect cases with the full 2.9-Mb deletion, which accounts for approximately 87% of all 22q11.2 deletion cases[19], we would expect approximately three cases in the low-risk group to have the smaller 22q11.2 deletions. Further prospective studies are required to determine actual prevalence and negative predictive values.

There has been concern that screening for microdeletions could lead to a substantial increase in positive calls[28]. In our study, the observed positive-call rate was less than 0.5% and the PPV was 18.0%. For cases with no abnormal ultrasound findings prior to NIPT,

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the PPV was 4.9%. This estimate is based on a small sample size (2/41) and could be subject to an ascertainment bias because detailed information about ultrasound findings were not comprehensively gathered at the time of testing.

Recently, we reported that resequencing high-risk samples at a higher depth of read (i.e. reflex testing) increased the PPV[29].This analysis was also applied to 89 available high-risk samples identified in this current study (Table S6). Based on these findings, we estimate that a protocol that includes reflex higher depth of sequencing will exhibit the same detection rate and will reduce the false-positive rate to 0.12%, yielding an overall PPV of 42.3%.

Prior to screening for the 22q22.1 microdeletion, pretest patient counseling and consent is necessary. This needs to emphasize the wide phenotypic variability, most frequently including a combination of congenital heart disease, palatal anomalies, immunodeficiency, endocrine abnormalities, gastrointestinal differences, genitourinary problems, developmental delay, cognitive deficits, and psychiatric illness. Also, there is a risk that a 22q11.2 deletion will be identified in a previously undiagnosed parent. Following a high-risk result, women should undergo follow-up confirmatory diagnostic testing, which may result in a need for high-risk pregnancy management and appropriate delivery resources. The potential benefits of screening for the 22q11.2 microdeletion as early as possible include identifying the presence of neonatal hypocalcemia that could go unrecognized, potentially resulting in long-term cognitive deficits[11]; identifying critical cardiac defects, such as ductal-dependent lesions (interrupted aortic arch type B), which could go undetected on prenatal ultrasound and would be missed using postnatal pulse oximetry; and preventing a 'diagnostic odyssey' for parents in search of a unifying explanation for their child's disparate clinical findings.

The decision to add 22q11.2 deletion screening as an adjunct to existing NIPT needs to balance the medical benefits of early diagnosis of 22q11.2 deletions against efficacy of the test, the prevalence in the referral group (which would be expected to be higher when NIPT referrals include patients with positive combined test results and abnormal ultrasound findings), additional clinical service considerations and cost. The data on clinical experience presented in this study may be helpful in this assessment.

DISCLOSURES

S.J.G., M.S., A.N., R.D., K.K., E.K., B.Z., N.W., J.E.B., A.R., K.N.J. and Z.D. are or were employees of Natera and hold stock or have the option to hold stock in the company. D.M.M.M. has presented talks on 22q11.2 deletion syndrome for Natera and P.B. is a paid consultant to Natera. This study was funded by Natera.

REFERENCES

1. Larion S, Warsof SL, Romary L, Mlynarczyk M, Peleg D, Abuhamad AZ. Uptake of noninvasive prenatal testing at a large academic referral center. *Am J Obstet Gynecol* 2014; **211**: 651.e1–651.e7.
2. Dar P, Curnow KJ, Gross SJ, Hall MP, Stosic M, Demko Z, Zimmermann B, Hill M, Sigurjonsson S, Ryan A, Banjevic M, Kolacki PL, Koch SW, Strom CM, Rabinowitz M, Benn P. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. *Am J Obstet Gynecol* 2014; **211**: 527.e1–527.e17.
3. American Congress of Obstetricians and Gynecologists. Committee Opinion No. 545: Noninvasive prenatal testing for fetal aneuploidy. *Obstet Gynecol* 2012; **120**: 1532–1534.
4. Wapner RJ, Babiarz JE, Levy B, Stosic M, Zimmermann B, Sigurjonsson S, Wayham N, Ryan A, Banjevic M, Lacroute P, Hu J, Hall MP, Demko Z, Siddiqui A, Rabinowitz M, Gross SJ, Hill M, Benn P. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *Am J Obstet Gynecol* 2014; **212**: 332.e1–332.e9.
5. Nussbaum RL, McInnes RR, Willard HF, Hamosh A. Thompson & Thompson Genetics in Medicine *(7th edn)*. Saunders Elsevier: Philadelphia, PA, 2007.
6. Sullivan KE. Chromosome 22q11.2 deletion syndrome: DiGeorge syndrome/velocardiofacial Syndrome. *Immunol Allergy Clin North Am* 2008; **28**: 353–366.
7. Botto LD, May K, Fernhoff PM, Correa A, Coleman K, Rasmussen SA, Merritt RK, O'Leary LA, Wong LY, Elixson EM, Mahle WT, Campbell RM. A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics* 2003; **112**: 101–107.
8. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman DA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 2012; **367**: 2175–2184.
9. Grati FR, Molina Gomes D, Ferreira JC, Dupont C, Alesi V, Gouas L, Horelli-Kuitunen N, Choy KW, Martinez-Conejero JA, Gonzales de la Vega A, Piotrowski K, Genesio R, Queipo GF, Malvestiti B, Herve B, Benzacken B, Novelli A, Vago P, Piippo K, Leung TY, Malvestiti F, Quibel T, Tabet AC, Simoni G, Vialard F. Prevalence of recurrent pathogenic microdeletions and microduplications in over 9,500 pregnancies. *Prenat Diagn* 2015; **35**: 801–809.
10. Bassett AS, McDonald-McGinn DM, Devriendt K, Digilio MC, Goldenberg P, Habel A, Marino B, Oskarsdottir S, Philip N, Sullivan K, Swillen A, Vorstman J; International 22q11.2 Deletion Syndrome Consortium. Practical guidelines for managing patients with 22q11.2 deletion syndrome. *J Pediatr* 2011; **159**: 332–339.
11. Cheung EN, George SR, Andrade DM, Chow EW, Silversides CK, Bassett AS. Neonatal hypocalcemia, neonatal seizures, and intellectual disability in 22q11.2 deletion syndrome. *Genet Med* 2014; **16**: 40–44.
12. Vora NL, O'Brien BM. Noninvasive prenatal testing for microdeletion syndromes and expanded trisomies: proceed with caution. *Obstet Gynecol* 2014; **123**: 1097–1099.
13. Samango-Sprouse C, Banjevic M, Ryan A, Sigurjonsson S, Zimmermann B, Hill M, Hall MP, Westemeyer M, Saucier J, Demko A, Rabinowitz M. SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy. *Prenat Diagn* 2013; **33**: 643–649.
14. Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenat Diagn* 2013; **33**: 575–579.
15. Nicolaides KH, Syngelaki A, Gil MD, Quezada MS, Zinevich Y. Prenatal detection of fetal triploidy from cell-free DNA testing in maternal blood. *Fetal Diagn Ther* 2014; **35**: 212–217.
16. Pergament E, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A, Hall MP, Dodd M, Lacroute P, Stosic M, Chopra N, Hunkapiller N, Prosen DE, McAdoo S, Demko D, Siddiqui A, Hill M, Rabinowitz M. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. *Obstet Gynecol* 2014; **124**: 210–218.
17. Human genome build hg19. http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/ [Accessed 9 July 2015].
18. Zimmermann B, Hill M, Gemelos G, Demko Z, M Banjevic, Baner J, Ryan A, Sigurjonsson S, Chopra N, Dodd M, Levy B, Rabinowitz M. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn* 2012; **32**: 1233–1241.
19. Shaikh TH, Kurahashi H, Saitta SC, O'Hare AM, Hu P, Roe BA, Driscoll DA, McDonald-McGinn DM, Zackai EH, Budarf ML, Emanuel BS. Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum Mol Genet* 2000; **9**: 489–501.
20. Noel AC, Pelluard F, Delezoide AL, Devisme L, Loeuillet L, Leroy B, Martin A, Bouvier R, Laquerriere A, Jeanne-Pasquier C, Bessieres-Grattagliano B, Mechler C, Alanio E, Leroy C, Gaillard D. Fetal phenotype associated with the 22q11 deletion. *Am J Med Genet A* 2014; **164A**: 2724–2731.
21. McDonald-McGinn DM, Sullivan KE. Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *Medicine* 2011; **90**: 1–18.
22. Grande M, Jansen FA, Blumenfeld YJ, Fisher A, Odibo AO, Haak MC, Borrell A. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype - a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; **46**: 650–658.
23. Donnelly JC, Platt LD, Rebarber A, Zachary J, Grobman WA, Wapner RJ. Association of copy number variants with specific ultrasonographically detected fetal anomalies. *Obstet Gynecol* 2014; **124**: 83–90.
24. Hume JH, Wardrop J, Boomer T, Almasri E, Paxton WB, Saldivar JS, Dharajiya N, Monroe YJ, Farkas DH, Grosu DS, McCullough RM. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. *Prenat Diagn* 2015; **35**: 999–1004.

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25. Helgeson J, Wardrop J, Boomer T, Almasri E, Paxton WB, Saldivar JS, Dharajiya N, Monroe TJ, Farkas DH, Grosu DS, McCullough RM. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. *Prenat Diagn* 2015; **35**: 1–6.
26. Besseau-Ayasse J, Violle-Poirsier C, Bazin A, Gruchy N, Moncla A, Girard F, Till M, Mugneret F, Coussement A, Pelluard F, Jimenez M, Vago P, Portnoï MF, Dupont C, Beneteau C, Amblard F, Valduga M, Bresson JL, Carré-Pigeon F, Le Meur N, Tapia S, Yardin C, Receveur A, Lespinasse J, Pipiras E, Beaujard MP, Teboul P, Brisset S, Catty M, Nowak E, Douet Guilbert N, Lallaoui H, Bouquillon S, Gatinois V, Joly-Helas G, Prieur F, Cartault F, Martin D, Kleinfinger P, Molina Gomes D, Doco-Fenzy M, Vialard F. A French collaborative survey of 272 fetuses with 22q11.2 deletion: ultrasound findings, fetal autopsies and pregnancy outcomes. *Prenat Diagn* 2014; **34**: 424–430.
27. McDonald-McGinn DM, Emanuel BS, Zackai EH. 22q11.2 Deletion Syndrome. In *GeneReviews*, Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH, Stephens K (eds). University of Washington, Seattle, Seattle, WA, 2013. http://www.ncbi.nlm.nih.gov/books/NBK1523 [Accessed 9 July 2015].
28. Borrell A, Stergiotou I. Cell-free DNA testing: inadequate implementation of an outstanding technique. *Ultrasound Obstet Gynecol* 2015; **45**: 508–511.
29. Gross SJ, Ryan A, Benn P. Noninvasive prenatal testing for 22q11.2 deletion syndrome: deeper sequencing increases the positive predictive value. *Am J Obstet Gynecol* 2015; **213**: 254–245.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Exclusion criteria for out-of-specification samples submitted for screening for 22q11.2 microdeletion by non-invasive prenatal testing, according to whether a redraw was requested

Table S2 Details of samples submitted for screening for 22q11.2 microdeletion by non-invasive prenatal testing with failed quality metrics, according to whether a redraw was requested

Table S3 Demographic characteristics of 84 patients with known decision on invasive testing

Table S4 Details of the 11 cases considered high risk following non-invasive prenatal testing (NIPT) for 22q11.2 microdeletion and confirmed diagnostically as true positive

Table S5 Details of ultrasound findings and their association with 22q11.2 deletion syndrome reported in 95 high-risk cases following non-invasive prenatal diagnosis, according to confirmed diagnosis

Table S6 High depth of read analysis for all available high-risk cases ($n = 89$) for 22q11.2 microdeletion following non-invasive prenatal testing (NIPT) on the basis of a low depth of read analysis

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