VALIDATION OF THE QUALIFI TEST, A PAIRED-END SEQUENCING-BASED GENOME-WIDE NONINVASIVE PRENATAL TEST FOR DETECTION OF FETAL CHROMOSOMAL ABNORMALITIES

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INTRODUCTION

Chromosomal abnormalities in pregnancy can cause miscarriages and congenital birth defects. Some chromosomal abnormalities occur when there is a deletion or duplication of a whole chromosome which is known as a chromosomal aneuploidy [1-3]. Trisomy 21 is probably the most well-known example of a chromosomal aneuploidy and is caused by an extra copy of chromosome 21. This results in a condition called Down syndrome which is the most common genetic disease at birth [4]. The other major chromosomal aneuploidies seen in live birth are Trisomy 18 (Edwards syndrome) and Trisomy 13 (Patau syndrome), as well as conditions caused by missing or extra X and Y chromosomes [5,6]. Therefore, it is important to determine the health of the fetus because knowing this information in advance can help the family make the best health care decisions during and after birth if a baby is affected. Prenatal diagnostic testing involves the testing of amniotic fluid and placental samples and requires an invasive procedure, which carries a small risk of pregnancy loss. Prenatal screening tests are clinically available to all pregnant patients and are simple tests that do not pose a risk to the fetus. In general, screening tests are carried out using ultrasound and a blood test such as maternal serum marker testing or analysis of cell-free DNA (cfDNA) for non-invasive prenatal testing (NIPT). NIPT can determine the presence of fetal aneuploidies from the first trimester onwards based on sequencing of cfDNA originating from the placenta and present in maternal blood [7,8]. In addition, data from a meta-analysis has shown that NIPT performance is superior to conventional prenatal screening methods, with higher detection rates and lower false-positive rates [9]. Consequently, the clinical use of NIPT to screen for fetal aneuploidies is becoming increasingly common. However, there have been differences between studies in estimating the sensitivity, specificity, and false positive/negative rate of NIPT. Thus, the screening performance of NIPT should be validated by each individual laboratory prior to clinical offering. This validation study evaluates the screening performance of the Qualifi test (Next generation genomic, Bangkok, Thailand), which is an internally validated NIPT based on VeriSeq NIPT solution v2, for basic screening (Trisomies 21, 18, and 13 and SCAs), and genome-wide screening (RAAs and partial deletions/duplications ≥ 7 Mb) from maternal frozen plasma samples on Illumina paired-end sequencing platforms.

RESULTS

A total of 284 plasma samples were included in this study, comprising of 255 singleton and 29 twin pregnancy samples. The average maternal age and gestational age for all samples were 35.5 years old and 11.9 weeks, respectively. In this study, most patients (61%) were over 35 years old, and most samples (90%) were collected during the first trimester (Table 1).

Table 1 Demographic characteristics

Characteristic	Total N = 284
Maternal age (years)	
Mean ± SD	35.5 ± 4.1
Median (range)	36 (24-47)
< 35	112 (39%)
≥ 35	172 (61%)
Gestational age (weeks)	
Mean ± SD	11.9 ± 2.1
Median (range)	12 (10-24)
10-14 (1st trimester)	256 (90%)
15-28 (2nd trimester)	28 (10%)

Of the 284 successful NIPT samples, 232 were reported as low risk and 52 were reported as high risk. For high-risk NIPT cases, karyotype results were available for 50 of the 52 samples while the remaining two cases were confirmed by clinical follow-up (Trisomy 22 and 14q deletion). As outlined in the Methods above, low-risk NIPT results were considered to be true negatives unless the physician provided feedback on discordant outcomes. For low-risk NIPT results, confirmation through karyotyping was available for two cases, 156 samples were confirmed by follow-up of clinical outcomes from the health care provider, and there were 74 low-risk NIPT results that had no karyotyping or follow-up confirmation (Figure 1).

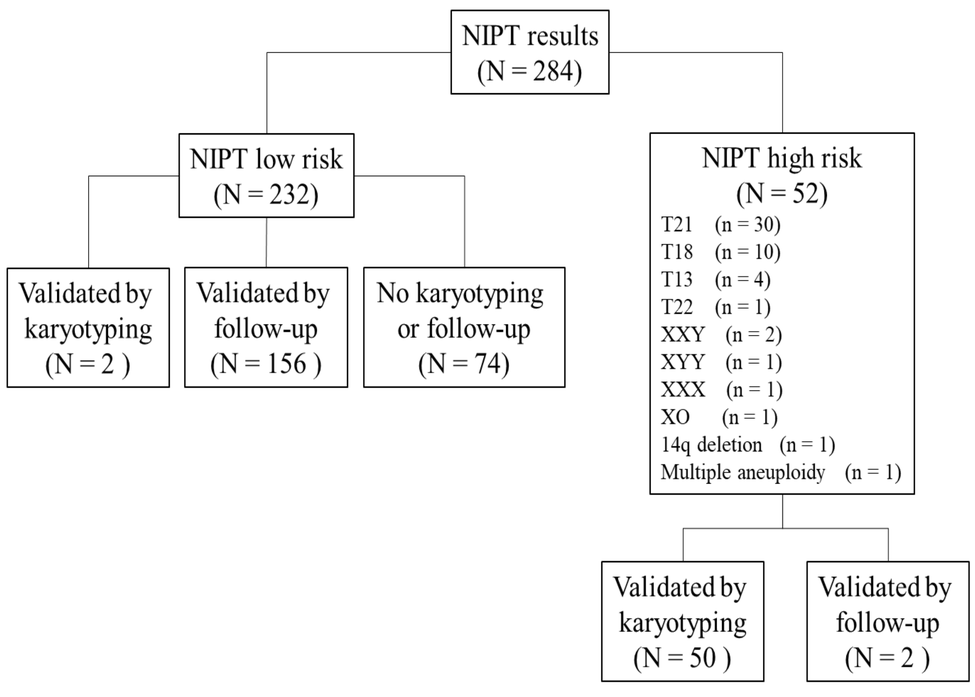

Figure 1. Flowchart of NIPT results of 284 plasma samples from pregnant women with a gestational age ≥ 10 weeks.

RESULTS

The performance of the Qualifi test for basic prenatal screening (Table 2) indicated > 99.9% sensitivity and specificity for Trisomy 21 and Trisomy 18. The estimated sensitivity and specificity for Trisomy 13 were > 99.9% and 99.64%, respectively. There was one false-positive result for Trisomy 13. This case had a NIPT result of multiple chromosome aneuploidies (Trisomy 13, XXY) while the fetal karyotyping after amniocentesis revealed an XXY result (Table 3). The combined sensitivity and specificity for SCAs were 87.5% and > 99.9%, respectively. There was a single monosomy X false-negative, and this case had a mosaic karyotype (mos 45, X [27]/46, XX [73]) but the amniocentesis QF-PCR result revealed a normal chromosomal pattern (Table 3). Mosaicism for monosomy X was confirmed by fetal karyotyping after amniocentesis because of a borderline LLR monosomy value for chromosome X.

Table 2 Performance for basic screening analysis

Condition	Trisomy21	Trisomy18	Trisomy13	SCAs
Sensitivity (n/N; 95% CI)	> 99.9% (30/30; 88.43-100.00%)	> 99.9% (10/10; 69.15-100.00%)	> 99.9% (4/4; 39.76-100.00%)	87.5% (6/7; 47.35-99.68%)
Specificity (n/N; 95% CI)	> 99.9% (254/254; 98.56-100.00%)	> 99.9% (274/274; 98.66-100.00%)	99.64% (279/280; 98.03-99.99%)	> 99.9% (277/277; 98.68-100.00%)

Using genome-wide screening, two samples were reported as high risk (one case of Trisomy 22 and one case of 14q deletion). The trisomy 22 case was recorded as a miscarriage and the 14q deletion case was recorded as inevitable abortion from incompetent cervix at 21 weeks of GA. Fetal karyotyping was not available for these two cases. In addition, one case that was determined to be low-risk by NIPT was confirmed as a false negative for 13q deletion by karyotyping analysis (Table 3); NIPT was performed at 10 weeks' gestation with a fetal fraction of 5% and the sample passed all the QC criteria. A terminal deletion of 13q22 of ~41 Mb in size was detected in fetal blood and placental tissue after termination of the pregnancy because of ultrasound anomalies at routine scanning. In summary, there was one false-positive case (Trisomy 13) in this study, a false-positive rate of 0.35%, and two false-negative cases (mosaic monosomy X and 13q deletion) resulting in a false-negative rate of 0.7% (Table 3). Of the 29 twin samples that were included in this validation study, five were found to be high risk for fetal chromosomal aneuploidies by NIPT. Of these five high-risk twin samples, all were correctly reported as positive for Trisomy 21 (n=4) and Trisomy 18 (n=1). There were no false-positive or false-negative results reported for twin samples.

Table 3 Details of false-positive and false-negative NIPT results

NIPT	Amniotic fluid	Fetal blood	Placental tissue	MA (y)	GA (wk)	%FF	BMI	Types of pregnancy
Low risk	mos 45, X [27]/46, XX [73] (QF-PCR normal)	-	-	34	14	6	25.19	Singleton
Low risk	-	46,XX,del(13)(q22)	46,XX,del(13)(q22)	33	10	5	20.98	Singleton
High risk T13, XXY	47, XXY	-	-	34	12	8	19.83	Singleton

CONCLUSION

This validation study confirms that the Qualifi NIPT is a highly accurate automated method for basic prenatal screening with high sensitivities and specificities reported for trisomies 21, 18, and 13, as well as SCAs. This test can also perform genome-wide screening analysis for rare autosomal aneuploidies and partial deletions/duplications >= 7 Mb.

REFERENCES

1. Polipalli SK, Karra VK, Jindal A, Puppala M, Singh P, Rawat K, and Kapoor S. Cytogenetic Analysis for Suspected Chromosomal Abnormalities; A Five Years Experience. J Clin Diagn Res. 2016; 10(9): GC01–GC05.
2. Zhang R, Chen X, Wang D, Chen X, Wang C, Zhang Y, Xu M, and Yu J. Prevalence of chromosomal abnormalities identified by copy number variation sequencing in high-risk pregnancies, spontaneous abortions, and suspected genetic disorders. J Int Med Res. 2019; 47(3): 1169–1178.
3. Li FX, Xie MJ, Qu SF, He D, Wu L, Liang ZK, Wu YS, Yang F, Yang XX. Detection of chromosomal abnormalities in spontaneous miscarriage by low-coverage next-generation sequencing. Mol Med Rep. 2020 Aug;22(2):1269-1276.
4. Parizot E, Dard R, Janel N, and Vialard F. Down syndrome and infertility: what support should we provide? J Assist Reprod Genet. 2019; 36(6): 1063–1067.
5. Dey M, Sharma S, and Aggarwal S. Prenatal Screening Methods for Aneuploidies. N Am J Med Sci. 2013; 5(3): 182–190.
6. Wagner P, Sonek J, Hoopmann M, Abele H, Kagan KO. First-trimester screening for trisomies 18 and 13, triploidy and Turner syndrome by detailed early anomaly scan. Ultrasound Obstet Gynecol. 2016 Oct;48(4):446-451.
7. Akolekar R, Beta J, Picciarelli G, Ogilvie C, and D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: A systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;45(1):16–26.
8. Carlson LM and Vora NL. Prenatal diagnosis: Screening and diagnostic tools. Obstet Gynecol Clin North Am. 2017;44(2):245–56.
9. Gil MM, Quezada MS, Revello R, Akolekar R, and Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: Updated meta-analysis. Ultrasound Obstet Gynecol. 2015;45(3):249–66.

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