Sensitivity and specificity of MGC-NIPS for trisomy 13, trisomy 18, trisomy 21, and sex chromosome aneuploidy screening in 219 Thai pregnant women

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ABSTRACT

Non-invasive prenatal screening (NIPS) has rapidly gained its acceptance as another prenatal screening method in Thailand. It offers higher sensitivity and specificity comparing to the commonly used biochemical tests. We have developed a pipeline called "MGC-NIPS", based-on chromosome read ratio algorithm, to detect all autosomes and sex chromosome aneuploidy (SCA). Validation on 219 archival data, sequenced by Ion Proton platform, was performed. Over 99% of sensitivity and specificity were achieved for all common autosome and sex chromosome aneuploidy. Two false positive SCA and one false negative Trisomy 18 had occurred. We concluded that MGC-NIPS, using simple chromosome read ratio algorithm on semiconductor sequencer data, is highly accurate. Further prospective study is warranted to gain more confirmative evidence before offering it as an alternative pipeline for NIPS service in Thailand.

Keywords: prenatal screening; non-invasive prenatal screening; sensitivity; specificity; semiconductor sequencer

INTRODUCTION

Chromosome aneuploidies, particularly trisomy 13, trisomy 18 and trisomy 21, are commonly occurred in elderly pregnancy. Invasive procedures, e.g. chromosome study from amniotic fluid or chorionic villus sampling, are gold standard diagnosis for these conditions. The invasive procedures, however, are associated with adverse risk either to the mother or to the fetus. Therefore, several screening methods, including maternal blood test and ultrasonogram, have been offered to reduce unnecessary invasive procedure. However, the performance of the existing screening tests is still associated with high false positive rate and relatively low detection rate (Alldred *et al*., 2017). Even combined first and second trimester serum screening test, including ultrasonogram, that can improve Down syndrome detection rate up to 95%, has only 4% positive predictive value (Gray *et al*., 2018). Thus, a large number of screened positive cases will undergo invasive diagnosis procedure with normal results.

The discovery of the fetal DNA in maternal blood has revealed a new avenue for non-invasive prenatal screening (NIPS) and diagnosis (Lo *et al*., 1997). Fetal cell-free DNA are originated from cyto- and syncytio-trophoblastic cells and can be detected in maternal blood as early as 10 week pregnancy (Grati *et al*., 2014; Srebniak *et al*., 2014). Using massively parallel sequencing technology (MPS), fetal cell-free DNA can be analyzed and fetal genome status can be reliably evaluated with high accuracy (Fan *et al*., 2008; Chiu *et al*., 2008). Down syndrome detection rate is more than 99% while positive predictive value reaches 80-99% (Gray *et al*., 2018). Several large scale, multi-site, cohort studies have supported the usefulness of this method as a prenatal screening test (Norton *et al*., 2012; Allyse *et al*., 2015; Palomaki *et al*., 2012; Gregg *et al*., 2016). MPS-based fetal DNA testing has been recognized as an alternative prenatal screening method by several professional societies (Gregg *et al*., 2016).

One of the challenges in analyzing the MPS data from cell-free DNA is the mixing nature of the fetal and the maternal cell-free DNA. The fetal cell-free DNA represents around 5-10% of total cell-free DNA in maternal blood. Therefore, a comprehensive bioinformatics pipeline is crucial to produce a highly accurate fetal genome interpretation out of the maternal DNA background.

Typical NIPS analysis pipelines will include human reference genome alignment, followed by quality control and data correction (mostly GC-Loess correction), fetal DNA estimation and aneuploidy detection (Jiang *et al*., 2012). Aneuploidy detection algorithm is one of the key steps for a highly accurate test result.

Several aneuploidy detection methods have been proposed. Some are publicly published while

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others are proprietary. However, most commonly used method is the Z-score based analysis with some modifications in certain steps, for example, changing data correction method (Chen *et al*., 2011), selection of internal reference chromosome (Lau *et al*., 2012), or even using certain regions on selected chromosomes within the same case as references (Straver *et al*., 2014). Collectively, all methods can achieve very high sensitivity and specificity while they have their own drawback or limitation. Some methods required a large number of normal controls to establish a reference, some methods were not robust across different sequencing platforms, some methods required a large number of sequencing reads; hence, the cost was increased.

During the past few years, NIPS has been increasingly adopted for prenatal screening in pregnant women in Thailand. It offered much higher sensitivity and specificity and broader detection of chromosome abnormalities than regular biochemical tests. Most NIPS services in Thailand were technically transferred from companies abroad, either the whole process or at least the analysis software (Chang *et al*., 2016). We have developed our own NIPS pipeline called "MGC-NIPS" based on Z-score analysis of chromosome read ratio. Here, we report the validated results on the Ion Proton sequencer data. The performances on trisomy 13, trisomy 18, trisomy 21, and sex chromosome aneuploidy (SCA) are presented.

MATERIALS AND METHODS

Study Design

This was a retrospective study aiming to evaluate the performance of MGC-NIPS, an in-house software developed at the Medical Genetics Center, Thailand (MGC). The study used the data generated from routine NIPS service provided by the MGC between 2016 to 2018. During which time, the sequencing was performed by MGC on the Ion Torrent platform while the analysis was conducted by a third party software. The sensitivities and specificities of the software are over 99.9% for trisomies 13, 18, 21, and chromosome X aneuploidies. The positive predictive value (PPV) are 83.3%, 91.4%, 95.1%, and 74.2%, respectively) (16). Indications for the test were mainly high risk pregnancy. All borderline or high risk NIPS results were recommended to be confirmed by karyotyping and/or chromosome microarray from amniotic fluid or fetal cord blood. The low risk NIPS results were assumed as true negative unless there was a report of fetal or neonatal abnormalities back from the health care provider or the patient. Chromosome study and/or chromosome microarray from placenta and the maternal blood were analyzed in the false positive and false negative cases. There were two false negatives reported during the period. Extensive investigation for the cause was able to be performed in one case and the data was incorporated into this study.

Samples from the archival data, only those with confirmed NIPS results or known pregnancy outcomes, were selected by an internal third party. Three hundred samples with normal pregnancy outcome, with equal sex distribution, were used to construct a base line reference for the MGC-NIPS. Two hundred and nineteen samples were further selected as a test group, including two to eleven samples of each common chromosome aneuploidy (trisomies 13, 18, 21, X, XXX). The data for the test group was then anonymized and blinded before sending to the bioinformatic team to re-analyze using the MGC-NIPS software.

Data Analysis by MGC-NIPS

MGC-NIPS is an in-house NIPS analysis pipeline using combination of tools and algorithms publicly available through reviewed literatures. Briefly, the Fasq data was aligned by Burrows-Wheeler Alignment Tool (BWA) (Li *et al*., 2009) against reference human genome. The uniquely aligned DNA fragments were normalized and GC corrected using QDNAseq (Scheinin *et al*., 2014). Chromosome read count ratio, with some minor modifications, was used to detect autosome aneuploidy and gender classification (Chiu *et al*., 2008; 2011). For all autosomes, Z-score above/below +/- 3SD, comparing to the baseline of each chromosome, was considered abnormal and called as a "high risk" result. For sex chromosomes, Z-score for 2X chromosomes were +/- 3SD of the baseline X chromosome, while Z-score for one Y chromosome was below -2.6 SD of the baseline Y chromosome. Fetal fraction was calculated by SeqFF (Kim *et al*., 2015) with combined WRSC and Enet algorithms.

Sensitivity and Specificity Calculation

The previous NIPS results, karyotype or microarray results, and the pregnancy outcome were combined and used to establish gold standard results. MGC-NIPS ability to detect true positive, true negative, false positive, and false negative were tabulated. The sensitivity and specificity of the MGC-NIPS for chromosomes 13, 18, 21, X, and Y were then determined.

RESULTS

Characteristics of the samples and the data in the test group are summarized in table 1. Common autosome aneuploidy samples were included relatively to their prevalence. All available archival samples of sex chromosome aneuploidy were added into this group. There were only 45,X and 47,XXX in our archive. Mapped median read counts were around 5.9 million reads per sample, ranging from 3,332,178 to 8,542,444 reads. Median fetal fraction was 12%, the lowest value was 3.81 %, and the highest value was 26.29%.

MGC-NIPS was able to pick up all true positive samples except for one trisomy 18 (Table 2). Two false positive for sex chromosome abnormalities [SCA] were observed. One was called 45,X and the other was called 47,XXY. There was not any "no call" incidence from MGC-NIPS analysis.

Sensitivity and specificity for trisomies 13, 18, 21 and SCA were calculated (Table 3). Except for trisomy 18, all common autosomes and SCA aneuploidy were above 99%.

Table 1 Characteristics of the samples and the data in the test group.

Sample	**Number**	
Male	126	
Female	93	
Trisomy 13	2	
Trisomy 18	5	
Trisomy 21	11	
X	3	
XXX	3	
Data	**Median**	**Min-Max**
Raw read count	6,413,839	3,621,933-9,285,266
Mapped read count	5,900,731	3,332,178-8,542,444
Fetal fraction	12%	3.81%-26.29%

Table 2 Performance of MGC-NIPS in detecting chromosome aneuploidy.

	True Positive	**False Positive**	**True Negative**	**False Negative**	**Total**
Trisomy 13	2	0	217	0	219
Trisomy 18	4	0	214	1	219
Trisomy 21	11	0	208	0	219
SCA	6	2	211	0	219

Table 3 Sensitivity and specificity of MGC-NIPS.

Chromosome	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value (%)**	**Accuracy (%)**
13	100	100	100	100
18	80	99.53	80	99.09
21	100	100	100	100
SCA	100	99.09	75	99.09

DISCUSSION AND CONCLUSION

MGC-NIPS demonstrated high sensitivity and specificity for detection of trisomies 13, 18, 21, and sex chromosome aneuploidy. Simple chromosome read count ratio algorithm could classify the abnormal cases accurately. Example of the positive trisomy 21 was shown in Figure 1. The Z-score value (+8.95) was much higher than the +3SD cut off value, making unambiguous interpretation of the result.

Sensitivity, specificity and accuracy of MGC-NIPS were above 99% for chromosomes 13, 18, 21 and sex chromosomes [Table 3], except for trisomy 18 (details discussed below). The results were comparable to those published and reviewed literature. Pooled sensitivity from the meta-analysis was 99.3% for trisomy 21, 97.4% for trisomy 18 and 97.4% for trisomy 13, with pooled specificity 99.9% (99.9% to 100%) for all three trisomies (Taylor-Phillips *et al*., 2016). In addition, positive predictive value was varied according to the prevalence of the disease. In the general obstetric population, the PPV for Trisomy 21 was 82% and increasing to 92% in high-risk population. The number of which was also comparable to our results.

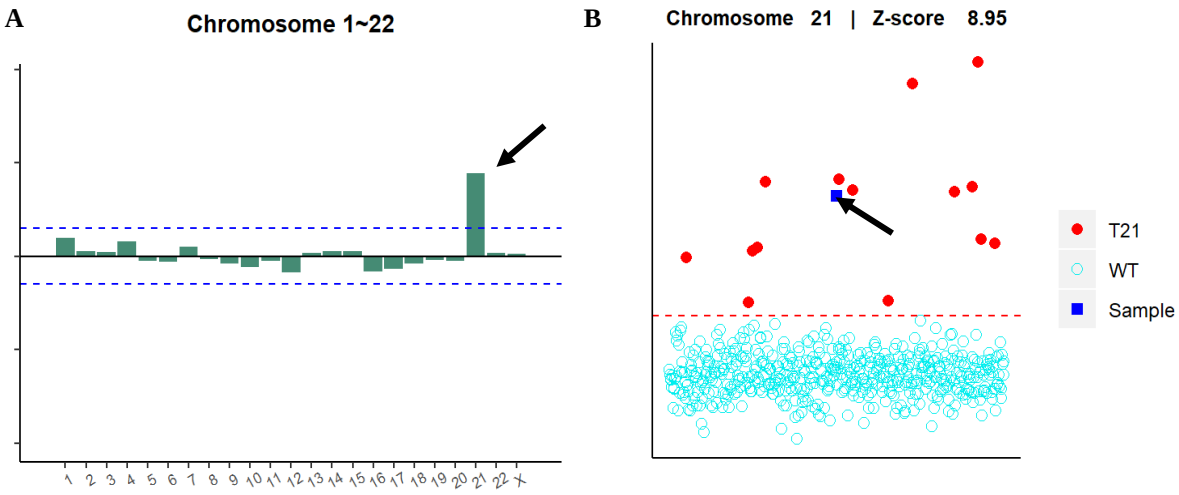

Figure 1 (A) All chromosome view and (B) chromosome 21 view demonstrated a high Z-score (arrow head) over the cut-off value.

In the two SCA false positive cases, the algorithm assigned both cases into the responsible class. However, they located rather close to the normal XX and XY class (Figure 2). Several factors may contribute to the borderline or misclassify result including maternal or fetal mosaicism for SCA, maternal or fetal CNV, vanishing twin, internal variation of the laboratory process, or even cross sample contamination (Bianchi *et al.*, 2018; Wilkins-Haug *et al.*, 2018; Hartwig *et al.*, 2017). Further study into these factors along with revision of the cut-off value may help to reduce the false positive result for the SCA.

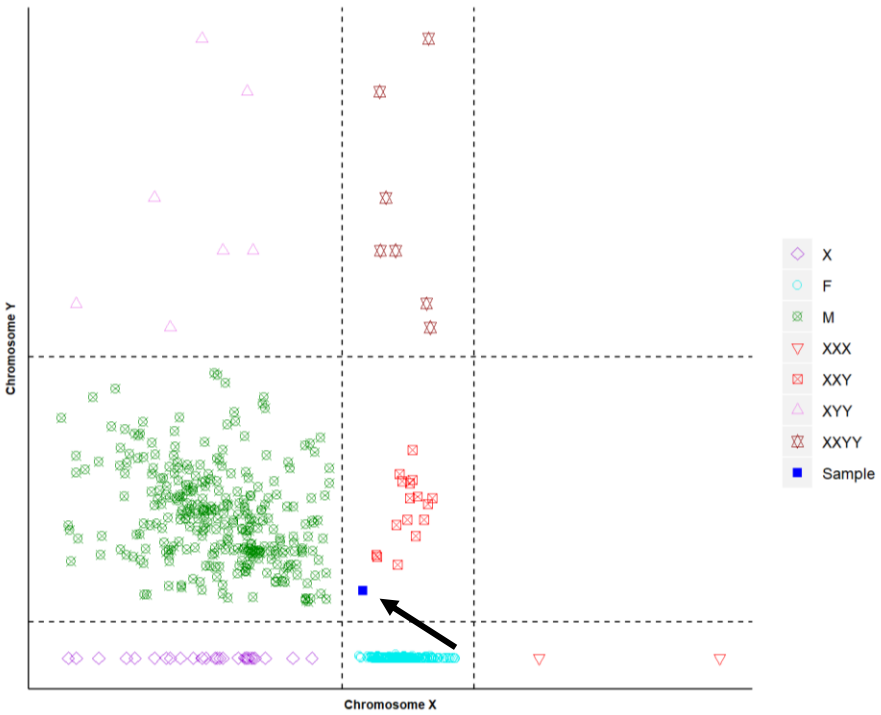

Figure 2 Location of the false positive XXY case (arrow head).

The case of false negative trisomy 18 was also re-analyzed by WISECONDORE (15), which yielded similar false negative result. This case was further investigated extensively. Chromosome study from the infant cord blood confirmed trisomy 18. However, chromosome study from placenta tissue showed a normal karyotype. Further investigation, using Affymetrix CytoScan Optima array, revealed 10% mosaic trisomy 18 on the placenta tissue (Figure 3). These findings support that a low-level mosaicism event had occurred in the placenta. Since the embryonic trophoblastic cells are the main source of the fetal cf-DNA, low-level of chromosome abnormality in these cells may escape the lower detection limit of MGC-NIPS (Wang *et al*., 2014). When removing such case from the study, the sensitivity and specificity of MGC-NIPS for chromosome 18 would approach 100%.

The limitation of this study is a relatively small number of sample size and small number of positive common autosome trisomy, e.g. trisomies 13, 18, 21. This may affect the sensitivity of the test. However, the encouraging result would support its further validation in a prospective study. The result from which will provide more relevant data to support its use in clinical applications.

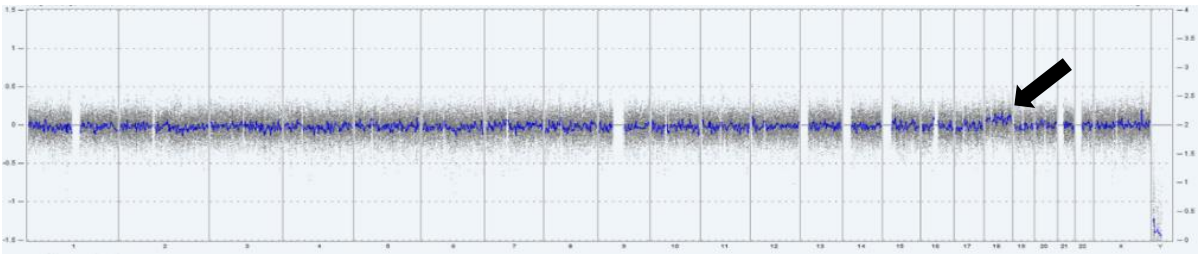

Figure 3 10% mosaic trisomy 18 (arrow head) from placenta tissue detected by chromosome microarray.

In conclusion, MGC-NIPS, using simple chromosome read count ratio algorithm on low-pass whole genome sequencing data generated by a semiconductor sequencer, was very robust and accurate. Over 99% of sensitivity and specificity on major autosomes and sex chromosomes were achieved. The required read count around 6 million reads make it possible to pool 10-12 samples onto one P1 chip of the Ion Proton sequencer (Tian *et al*., 2018). This is considerably economical and convenient for a laboratory that process a small number of samples to issue the result within a short turn-around time. Further prospective clinical study will be helpful to support the value and strength of the MGC-NIPS pipeline and provide more insightful data for a better application in real world clinical service.

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