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ORIGINAL ARTICLE

Prospective clinical evaluation of Momguard non-invasive prenatal test in 1011 Korean high-risk pregnant women

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ABSTRACT

Clinical performance of the Momguard non-invasive prenatal test (NIPT) was evaluated in a cohort of Korean pregnant women. The foetal trisomies 21, 18 and 13 (T21, T18 and T13) were screened by low-coverage massive parallel sequencing in the maternal blood. Among the 1011 confirmed samples, 32 cases (3.2%) had positive NIPT results. Of these positive cases, 20 cases of T21, all cases of T18 and two cases of T13 had concordant karyotype findings. Only one case out of the remaining 979 negative NIPT samples showed a false negative result. The overall sensitivity and specificity of Momguard to detect the three chromosomal aneuploidies were 96.8% and 99.8%, respectively. Momguard is a clinically useful tool for the detection of T21, T18 and T13 in singleton pregnancy. However, as other NIPT tests, it carries the risk of false positive and false negative results. Hence, the genetic counsellors should provide these limitations to the examinees.

IMPACT STATEMENT

- **What is already known on this subject?** The NIPT approach using massive parallel sequencing (MPS) showed high sensitivity and specificity in various clinical studies. These results are based on analysis systems using their own bioinformatics algorithms.
- **What the results of this study add?** When this NIPT technology was introduced in Korea, the first biological specimens collected in Korea were transported overseas for processing in overseas laboratories and analysed by other country's analysis methods. We needed our own NIPT algorithm and developed Momguard NIPT for the first time in Korea. This study attempted to evaluate this Momguard NIPT protocol prospectively in a large number of samples obtained from three Korean hospitals.
- **What the implications are of these findings for clinical practice and/or further research?** The overall sensitivity and specificity to identify T13, T18 and T21 were 96.8% and 99.8%, respectively. These accuracy values were comparable to that of other studies. From this study, we found that Momguard is a clinically useful tool for the detection of three chromosomal aneuploidies. However, as other NIPT tests, it carries the risk of false positive and false negative results. Hence, the genetic counsellors should provide these limitations to the examinees.

KEYWORDS

Aneuploidy; Down syndrome; Edwards syndrome; non-invasive prenatal testing; Patau syndrome

Introduction

The discovery of circulating foetal cell-free DNA (cfDNA) in maternal blood led to a new era of non-invasive prenatal testing (NIPT). Utilising massive parallel sequencing (MPS) and advanced bioinformatics, NIPT approach has shown promising results with high sensitivity and specificity as validated by multiple clinical studies (Bianchi et al. 2012; Norton et al. 2012; Palomaki et al. 2012; Lau et al. 2014; McCullough et al. 2014; Zhang et al. 2015).

Since NIPT was introduced in Korea by MPS technology, the clinicians showed strong interest and attempted to adopt the technology for diagnosing foetal aneuploidy. Initially, the biological specimens collected in Korea were shipped abroad for processing in the overseas laboratories and were tested following the analytical methods of other countries. Soon, the Korean scientists and bioinformaticians developed their own methods to analyse these samples locally (Jeon et al. 2014; Lee et al. 2015; Kim et al. 2016).

Momguard is the first NIPT protocol developed in Korea. The clinical performance of this test was thoroughly reviewed while collecting cohort samples. The preliminary results have shown that Momguard is highly accurate for the detection of

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trisomy 21, 18 and 13 (Hu et al. 2015; Lee et al. 2015). In this study, we have prospectively evaluated the clinical performance of Momguard in a large number of samples obtained from three Korean hospitals.

Methods

Study design

This was a prospective, large-scale, blinded cohort study conducted in the Asan Medical Centre, Cheil General Hospital and Women's Healthcare Centre and Busan Paik Hospital from August 2014 to March 2016. Approvals were obtained from the institutional review boards of each centre. Only the participants who signed the written informed consent forms were included in this study.

The inclusion criteria were singleton pregnancy in women of >18 years who met at least one of the following additional criteria: (1) advanced maternal age (≥35 years), (2) positive serum biochemical screening test, (3) presence of foetal anomalies as detected by ultrasonography, (4) positive personal/family history of foetal aneuploidy or (5) clinical judgment. The cytogenetic investigations were performed using chorionic villi, amniotic fluid, cord blood or peripheral blood of the neonates. A negative NIPT result was considered as a true negative if the prenatal or postnatal karyotype was normal, or if the neonate was phenotypically normal after birth.

Sample collection

Eight millilitres of maternal blood samples were collected in cfDNA BCT tubes (Streck, Omaha, NE) and shipped to the laboratory within 24 h of collection. Plasma was isolated from the maternal blood using a two-step centrifugation process: centrifugation at 1600 × *g* for 10 min followed by centrifugation at 16,000 × *g* for 10 min at 4 °C. The separated plasma samples were stored in 1 mL aliquots in 1.5 mL tubes having distinct sample codes as labels. The aliquots were frozen at –80 °C until analysis.

cfDNA extraction and sequencing

cfDNA extraction and sequencing were performed in the LabGenomics Clinical Laboratory (Seongnam, Korea) where a multi-platform NGS-based non-invasive test was implemented for foetal aneuploidy. cfDNA was isolated from 2 mL of maternal plasma using a QIAmp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany). The extracted cfDNA was eluted in 40 µL of AVE buffer (Qiagen, Hilden, Germany). According to the Momguard library preparation protocol, the cfDNA library was prepared and evaluated using a PicoGreen assay and 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The resulting libraries were sequenced using a NextSeq 500 instrument (Illumina Inc., San Diego, CA).

Sequencing and data analysis

For each step from sampling to reporting, a quality control system was established. The haemolysed blood samples were excluded from the analysis. The sequencing data were analysed using an in-house bioinformatics pipeline called GeneBro. Briefly, the paired-end sequencing reads were binned according to the index sequence and mapped to the reference human genome sequence (hg19). The Burrows–Wheeler Alignment tool (version 0.6.2) was applied to align the sequence to the human genome. The read counts were corrected for GC-bias and mappability. Using the uniquely aligned reads, the statistical values of euploid pregnancies were calculated. By applying an ensemble algorithm to perform a similarity analysis based on features such as foetal fraction, number of foetuses or guanine-cytosine (GC) content, test samples were evaluated for the risk of foetal aneuploidy.

Foetal cfDNA estimation

For the male foetus, the foetal cfDNA fraction was calculated based on the fractional read counts of Y chromosome referring to previous studies (Fan et al. 2008; Rava et al. 2014). To calculate the foetal cfDNA fraction of female foetus, we first divided each chromosomal region into 50 kbp bins and then we selected the bins based on inter-sample variance and mappability values for further analysis as described in previous studies (Derrien et al. 2012; Jensen et al. 2013). For each bin, the aligned reads were corrected for GC-bias and fractional read counts were calculated. These fractional read counts were applied to establish a regression model to predict the foetal cfDNA fraction. The model coefficients were determined by applying Y chromosome-based foetal fraction of the male samples as a response variable.

Statistical analyses

The continuous variables are expressed as the median with range and the categorical variables are expressed as the frequency and percentage. The sensitivity, specificity, positive predictive value and negative predictive values are shown as the percentage with 95% CI.

Results

Study population

Out of the total 1099 samples, 88 (8.0%) were excluded from the analysis because of test failure, test cancellation and lost to follow-up of patients without confirmation of karyotype or phenotype (Figure 1). The reasons for 10 cases (0.9%) of test failure included low foetal fraction ($n = 6$), a lack of plasma concentration ($n = 3$) and sample haemolysis ($n = 1$).

The demographic characteristics of the remaining 1011 samples are shown in Table 1 and Figure 2. The maximum number of pregnant women who were tested for Momguard were of 35–38 years of age. Most of the women were tested

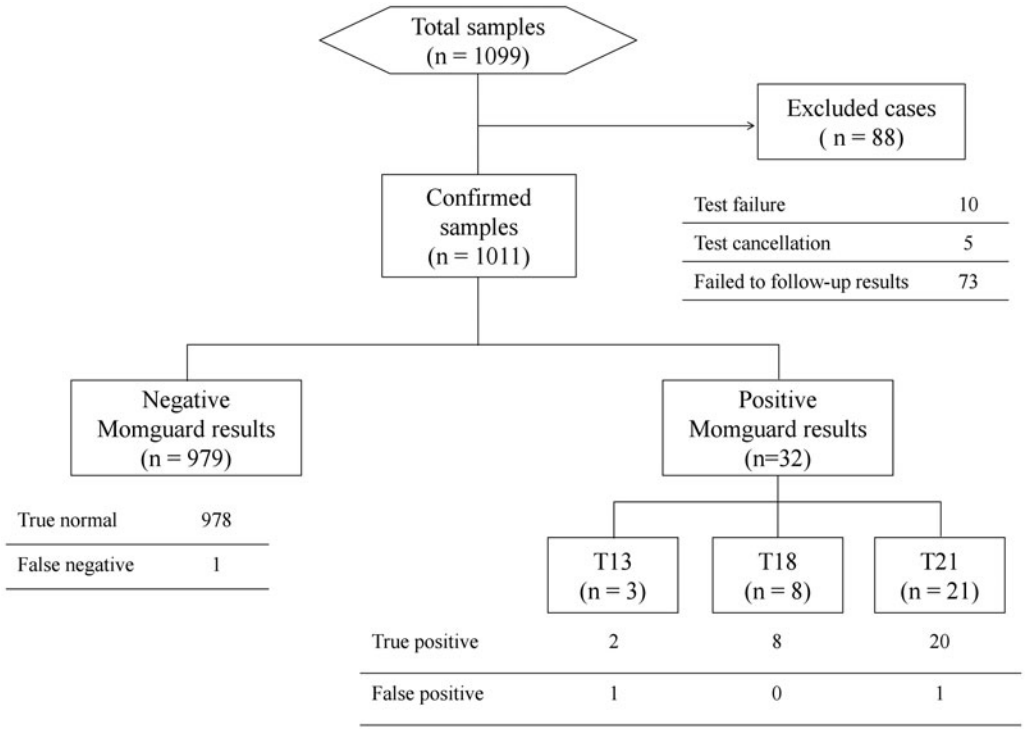

Figure 1. Flow sheet of Momguard non-invasive prenatal test results and clinical outcomes in the Korean women.

Table 1. Demographic characteristics of 1011 confirmed samples.

Variable	Value
Maternal age (years)	35 (20–45)
Gestational age (weeks)	18.0 (7.0–41.0)
First trimester (<14 weeks)	21.2% (214/1011)
Second trimester (14–27 weeks)	72.1% (729/1011)
Third trimester (≥28 week)	6.7% (68/1011)
Indications for non-invasive prenatal test	
Advanced maternal age	67.0% (677/1011)
Positive serum screening test	15.6% (156 /1002)
Presence of ultrasonographic markers	41.5% (419/1009)
Personal/family history of foetal aneuploidy	1.4% (14/1011)
Others	1.3% (13/1011)

Data are represented as the median with range and frequency with percentage.

at the end of the first trimester or at the beginning of the second trimester (Figure 2).

NIPT and karyotyping results

Out of the 1011 cases, 32 (3.2%) showed positive NIPT results, including T21 ($n = 21$), T18 ($n = 8$) and T13 ($n = 3$). Of these positive cases, 20 cases of T21, all cases of T18 and two cases of T13 had concordant karyotyping results (Figure 1 and Table 2). Only one case out of the remaining 979 negative NIPT samples showed a false negative result (Figure 1). The overall sensitivity and specificity to identify three chromosomal aneuploidies were 96.8% (95% CI, 81.5–99.8) and 99.8% (95% CI, 99.2–100), respectively.

Three cases showing discordant results between Momguard and karyotyping, including two false positives and one false negative results, are summarised in Table 3. Case 1 was a 39 years old mother carrying a male baby with a chromosome Y, assumed to have a foetal fraction of 26.3%. The prenatal ultrasonography showed bowel dilatation without any other abnormalities. Although the Momguard result showed a high-risk for T13, the pregnancy outcome was normal. Case 2 was a female baby with a foetal fraction of 10.5% and multiple anomalies were suspected by prenatal ultrasonography. The Momguard result showed an intermediate risk for T21, but both the karyotype and pregnancy outcome were normal. Case 3 was a female baby with a foetal fraction of 8.5%. At 24.4 weeks of gestation, the foetus was suspected to have multiple anomalies. Although the Momguard result showed low risk, the foetus was confirmed as T18 by karyotyping.

Discussion

This prospective study was performed to evaluate the performance of Momguard NIPT in the Korean women. The preliminary results based on a part of this cohort were previously published (Lee et al. 2015). When we started to collect samples in 2014, the concept of NIPT was not familiar to the general public and the utility of next-generation sequencing-based NIPT was questioned in the clinical field. However, as high-quality clinical results based on this new technology have been reported worldwide (McCullough et al. 2014; Willems et al. 2014; Eiben et al. 2015; Lee et al. 2015; Zhang et al. 2015), the awareness and reliability of this test have gradually improved.

The number of cases with positive Momguard NIPT results was 32/1011 (3.2%) which was higher than that reported in other studies, ranging from 1.1 to 2.3% (Lau et al. 2014; McCullough et al. 2014; Willems et al. 2014; Eiben et al. 2015; Zhang et al. 2015). It is due to the fact that our study was initially designed to include mostly the cases of high-risk

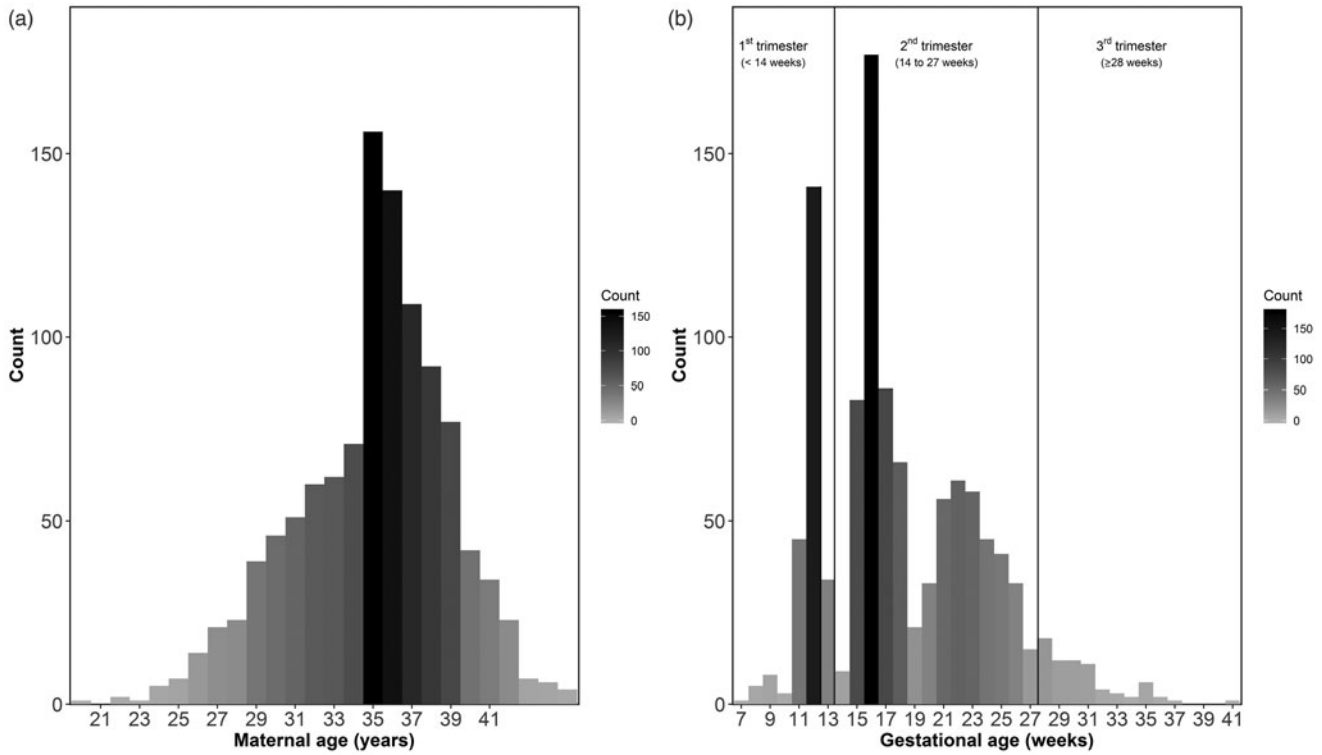

Figure 2. Distribution of maternal age (a) and gestational age (b) in the study cohort.

Table 2. Performance of Momguard non-invasive prenatal test for the detection of trisomy 21, 18 and 13 in 1011 samples.

Trisomy	TP (*n*)	FP (*n*)	TN (*n*)	FN (*n*)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
T21	20	1	990	0	100 (80.0 – 100)	99.9 (99.4 – 100)	95.2 (74.1 – 99.8)	100 (99.5 – 100)
T18	8	0	1002	1	88.9 (50.7 – 99.4)	100 (99.5 – 100)	100 (59.77 – 100)	99.9 (99.4 – 100)
T13	2	1	1008	0	100 (19.8 – 100)	99.9 (99.4 – 100)	66.7 (12.5 – 98.2)	100 (99.5 – 100)
Total	30	2	978	1	96.8 (81.5 – 99.8)	99.8 (99.2 – 100)	93.8 (77.8 – 98.9)	99.9 (99.3 – 100)

TP: true positive; FP: false positive; TN: true negative; FN: false negative; PPV: positive predictive value; NPV: negative predictive value; T21: trisomy 21; T18: trisomy 18; T13: trisomy 13.

Table 3. Summary of the three discordant results between Momguard non-invasive prenatal test and clinical outcomes.

Case	MA (years)	GA at Momguard (weeks)	Foetal fraction (%)	Momguard result	Maternal serum screening	Associated anomalies	Cytogenetic confirmation	Outcome of pregnancy
Case 1	39	18.2	26.3	T13	High-risk for T21	Bowel obstruction	Not tested	LB, normal
Case 2	33	24.2	10.5	T21	Low risk	1. Bilateral microtia 2. Cleft palate 3. Retrognathia 4. VSD with PS 5. Multiple vertebral defects 6. Lipomyelomeningocele	46, XX	LB
Case 3	32	24.4	8.5	Low risk	Low risk	1. Brain maldevelopment 2. Bilateral pulmonary hypoplasia 3. Pulmonary atresia with VSD 4. Right dysplastic kidney 5. Left hydronephrosis 6. Oligohydramnios	47, XX, +18	Neonatal death

MA: maternal age; GA: gestational age; T13: trisomy 13; T21: trisomy 21; LB: live birth; VSD: ventricular septal defect; PS: pulmonary stenosis.

pregnancy for NIPT. Indeed, out of the 1011 cases, 998 (98.7%) were high-risk group fulfilling one of the following criteria; advanced maternal age (>35 years), positive maternal serum screening test, abnormal prenatal ultrasound result, family history of aneuploidy or previous pregnancy with foetal aneuploidy.

Among 1011 women, 645 (64%) underwent the NIPT in less than 20 weeks, and 366 mothers (36%) after 20 weeks. Considering the fact that diagnostic accuracy of NIPT increases with gestational age, our performance would be better if majority of the samples were collected after mid-trimester.

Two false positives and one false negative results were observed in this study. The foetal fraction of these outcomes far exceeded the minimum requirement, the two false positives being >10%, and the one false negative being 8.5%. The same phenomenon was observed in a previous study with more than 140,000 samples where the authors have reported that the mean foetal fraction was 9.74% (range, 3.54–21.94%) in 120 false positive cases and 10.2% (range, 5.18–13.39%) in eight false negative cases (Zhang et al. 2015). Among the several factors known to affect the discordant NIPT results, such as maternal copy number variation (Chudova et al. 2016; Zhou et al. 2017), maternal malignancy (Bianchi et al. 2015), confined placental mosaicism (Mao et al. 2014), foetal mosaicism (Lebo et al. 2015), foetal fraction (Canick et al. 2013) and vanishing twin (Niles et al. 2018), foetal fraction does not seem to be a major factor influencing the false results. This fact is also supported by Hartwig et al. who collected and reviewed 22 studies reporting a total of 206 discordant NIPT results (Hartwig et al. 2017). It was observed that the major factors responsible for false positive results were maternal copy number variation, confined placental mosaicism and maternal malignancy, whereas, the major factor resulting in false negative results was foetal mosaicism.

Out of the two false positive cases, one case showed that the foetal fraction obtained from the overrepresented chromosome 13 was 12%, which was approximately half of the amount of chromosome Y based on the foetal fraction. From this discrepancy, we hypothesised that this strong false positive signal could be explained by foetal or placental mosaicism. Interestingly, Zhang et al. observed similar findings in 12 out of 157 false positive cases (Zhang et al. 2015). They even observed T13 confined placental mosaicism in two out of four placental tissue samples.

Since the foetal fraction was high enough and the statistical values of NIPT showed a stable signal for low risk in case 3, it was unexpected that the karyotyping result was not consistent with Momguard. We tried to re-sample maternal blood to re-perform NIPT, but we were unable to do so because of the mother's refusal. False negative results give us an important message. While NIPT performs well, it should not be considered as a diagnostic or stand-alone test. If there is a strong suspicion of anomalies on prenatal ultrasonography, karyotyping should be recommended even if the NIPT results suggest low-risk.

The innovation of this study is that Momguard NIPT was developed with our own algorithm for the first time in Korea and was prospectively evaluated with over 1000 samples that were difficult to collect in Korea in 2014. The cost of Momguard NIPT service is currently about $300 in Korea.

The limitation of this study is the lack of validation of false positive and false negative results. When we began to perform this prospective study, the concept of NIPT was not familiar to the general public and the usefulness of the NGS-based NIPT was questioned. Unfortunately, when the discordant results came out, we could not get the mothers' consent to confirm the presence of foetal mosaicism or confined placental mosaicism.

In conclusion, this study elucidates the performance of Momguard NIPT in a cohort of Korean women. From our previous and present results, we can infer that although Momguard has high accuracy, it carries the risk of providing false positive and false negative results. Therefore, genetic counsellors should provide sufficient information to the examinees to prevent misinterpretation of these test results.

Disclosure statement

No potential conflict of interest was reported by the authors.

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