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Simultaneous detection of fetal aneuploidy, *de novo FGFR3* mutations and paternally derived β-thalassemia by a novel method of noninvasive prenatal testing

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Abstract

Objective: The aim is to develop a novel noninvasive prenatal testing (NIPT) method that simultaneously performs fetal aneuploidy screening and the detection of *de novo* and paternally derived mutations.

Methods: A total of 68 pregnancies, including 26 normal pregnancies, 7 cases with fetal aneuploidies, 7 cases with fetal achondroplasia or thanatophoric dysplasia, 18 cases with fetal skeletal abnormalities, and 10 cases with β-thalassemia high risk were recruited. Plasma cell-free DNA was amplified by Targeted And Genome-wide simultaneous sequencing (TAGs-seq) to generate around 99% of total reads covering the whole-genome region and around 1% covering the target genes. The reads on the whole-genome region were analyzed for fetal aneuploidy using a binary hypothesis T-score and the reads on target genes were analyzed for point mutations by calculating the minor allelic frequency of loci on *FGFR3* and *HBB*. TAGs-seq results were compared with conventional NIPT and diagnostic results.

Results: In each sample, TAGs-seq generated 44.7–54 million sequencing reads covering the whole-genome region of 0.1–3× and the target genes of >1000×depth. All cases of fetal aneuploidy and *de novo* mutations of achondroplasia/thanatophoric dysplasia were identified with high sensitivities and specificities except for one false-negative paternal mutation of β-thalassemia.

Conclusions: TAGs-seq is a novel NIPT method that combines the fetal aneuploidy screening and the detection of *de novo FGFR3* mutations and paternal *HBB* mutations.

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Key Points

What's already known about this topic?

- Recently, the improvement of noninvasive prenatal testing (NIPT) allowed the expanded detection of chromosomal copy number variants (CNVs) and monogenic diseases in fetus. However, the expanded detection of CNVs and monogenic disease usually requires separate experiment procedures such as quantitative polymerase chain reaction (qPCR), digital PCR or haplotype-assisted approaches, which could not be integrated with the screening of chromosome aneuploidy and lack of laboratory efficiency

What does this study add?

- We developed a novel NIPT method that could simultaneously detect fetal aneuploidies, autosomal dominant diseases, and the paternally inherited mutations of autosomal recessive diseases without additional experiments. We validated this method using the plasma samples of pregnant women carrying fetal achondroplasia, thanatophoric dysplasia, and β-thalassemia, which showed promising results

1 | INTRODUCTION

Since its discovery in maternal blood, cell-free DNA (cfDNA) originated from placenta trophoblasts was soon used for fetal disease testing, and rapidly adopted in clinical practice known as the noninvasive prenatal testing (NIPT).[1] With higher sensitivity and specificity than conventional biochemical approaches, NIPT was initially used to screen for fetal trisomy 21 (T21), trisomy 18 (T18), and trisomy 13 (T13).[2,3] Not long after that, cfDNA was used to screen for sex chromosome aneuploidy and chromosome copy number variants (CNVs).[4–6] Recently, noninvasive detection of the *de novo* mutations causing certain autosomal dominant disease has been determined to have clinical significance.[7] Meanwhile, several studies have demonstrated the detection of fetal monogenic diseases by analyzing cfDNA using quantitative polymerase chain reaction (qPCR), digital PCR, or haplotype-assisted approaches.[8–13] The detection of monogenic diseases with such methods provides the full genotype assessment of fetus, and certain method such as the haplotype-based approaches can even interpret the inheritance of the maternal allele. However, detecting monogenic disease usually requires separate experiment procedures that are different from screening for chromosome aneuploidy. Thus, it is not easy to perform chromosome screening and monogenic disease detection simultaneously.

We intended to develop a novel NIPT method with improved library construction and sequencing strategy, so that fetal chromosome aneuploidy, *de novo* mutations and paternally inherited mutations can be detected simultaneously. Herein this study, we reported the proof-of-concept validation of simultaneous detection of fetal T21, T18, T13, achondroplasia (ACH), thanatophoric dysplasia (TD), and the paternal mutations of β-thalassemia by using a Targeted And Genome-wide simultaneous sequencing (TAGs-seq) method.

2 | METHODS

2.1 | Patients

Pregnant women participating in this study were recruited through collaborative hospitals. Each hospital was involved in patient recruitment, routine prenatal healthcare, prenatal diagnosis of disease, and follow-up of outcomes. Institutional review board approval was obtained from each hospital, and an written informed consent was obtained from each participant before collecting samples. To be included in the study, pregnant women had to be singleton pregnancy with gestational age of 12 weeks or larger and at least 18 years old at the time of sample collection. Participants were recruited if they have pregnancy with confirmed fetal T21, T18, T13, ACH, or TD. As an internal control, pregnant women who had no *FGFR3* mutations in fetus, yet with abnormal ultrasound suspicious to ACH/TD, or previous history of an ACH/TD pregnancy were also recruited. Pregnant women and spouses who were carriers of β-thalassemia were also recruited to evaluate the fetal risk of inheriting paternal mutations. Women with normal singleton pregnancy were recruited as healthy controls.

2.2 | Sample collection and processing

Five milliliter of blood sample was taken from each pregnant woman using an ethylene diamine tetra-acetic acid containing tube. Plasma was extracted from blood samples by a two-step centrifugation procedure at 4°C within 8 h after blood collection.[14] Plasma and buffy coat of each sample were then separately barcoded, and delivered with dry ice to BGI-Shenzhen for sequencing and bioinformatics analysis. CfDNA was extracted from maternal plasma using an QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany) following the manufacturer's instructions. As a quality control, only cfDNA with peak size

of around 167 base pairs and yield of more than 10 ng was used for further experiments.

2.3 | Sequencing for conventional NIPT

MGIEasy cfDNA Library Prep Set (MGI, China) was used to prepare cfDNA library for conventional NIPT following the manufacturer's instructions. Briefly, cfDNA was end-repaired and ligated with adapters containing 10 nt barcode sequences (Table S1) for multiplex sequencing. The ligated products were subjected to 12 cycles of PCR using the Kapa HIFI hotstart ready master mix (Kapa Biosystems, South Africa). PCR products were then normalized and processed for circularization[15] (Figure S1). Briefly, PCR products were heat-denatured at 95°C for 3 minutes to make a single strand DNA circle (ssDNA circle), which were then mixed reagents of MGIEasyTM DNA Library Prep Kit (MGI, China) and incubated at 37°C for 30 minutes to complete the circularization. The resulting ssDNA circle was then used to generate DNA nanoballs (DNBs) by rolling circle amplification (RCA).[16] After RCA and the formation of DNBs, the final product was measured by Qubit using the ssDNA HS Assay kit (Invitrogen, US) and loaded on a DNBSEQ-500 platform (MGI, China) for sequencing[17] following the manufacturer's instructions.

2.4 | Targeted And Genome-wide simultaneous sequencing

TAGs-seq used a proprietary library construction and sequencing strategy to simultaneously amplify the whole-genome region of 0.1–3× depth and target genes of >1000× depth. CfDNA was firstly processed with end-repair and ligated with DNBSEQ-500 adapters (Table S1). The ligated products were subjected to two rounds of nested multiplex PCR, each round of 10–20 cycles, using a Kapa HIFI hotstart ready master mix kit (Kapa Biosystems, South Africa) for the amplification of whole-genome and gene-specific regions (Figure 1). At the first round of multiplex PCR (PCR1), 20 μl of ligased products were mixed with 25 μl of 2× Kapa HIFI hotstart ready master mix, 2.5 μl of 10 nmol/L universal primer 1 (UP1, Table S1), and 2.5 μl of 0.5 nmol/L gene-specific primer 1 (GSP1, Tables S2 and S3) for 10 cycles of PCR. This allowed the linear amplification of genome-wide regions and exponential amplification of the target regions. At the second round of multiplex PCR (PCR2), 2.5 μl of 10 nmol/L UP1 (Table S1), 2.5 μL of 10 nmol/L UP2 (Table S1), and 2.5 μl of 0.5 nmol/L GSP2 (Tables S2 and S3) were added to the amplification product of PCR1 for another 20 cycles of PCR. This allowed the exponential amplification of both the genome-wide regions and target regions. After beads clean up, the products were quantified using the Qubit dsDNA High Sensitivity assay on Qubit 3.0 (Invitrogen, US). The amplification libraries were then normalized and processed for circularization and sequencing on a DNBSEQ-500 platform, following the same procedures as described above. After sequencing, the whole-genome region with low sequencing depth was used to analyze chromosome aneuploidy, while the selective regions with an ultra-high sequencing depth were used to analyze *de novo* or paternal mutations. All TAGs-seq sequencing data were uploaded to China National Gene Bank Nucleotide Sequence Archive upon the request of accession code.

2.5 | Primer design

Nested gene-specific primers used in TAGs-seq were designed to amplify 17 reported mutations on *FGFR3* responsible for ACH and TD,[7] and common mutations on the coding and flank region of *HBB* responsible for β-thalassemia in Chinese population,[18] respectively (Tables S2 and S3). Primer3 (v. 0.4.0) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to design the primers.

2.6 | Data analysis

Data analysis was conducted in blind of the diagnostic results. Paired-end (PE) reads were generated with a read length of 50 bp or 100 bp using DNBSEQ-500. The clean reads were aligned to the human hg19 reference genome using the BWA tool (version 0.7.15). The aligned reads were then divided into two parts: the reads mapping to the target genes for genotyping analysis and the reads of whole-genome region for chromosome analysis. For the reads mapping to the target genes, the minor allelic frequency (MAF) of the loci known to cause ACH, TD, and β-thalassemia was calculated. To avoid the influence of sequencing errors, the MAF of above 2% was used to determine the presence of a *de novo* or paternal mutation. In the other hand, if the MAF was above 20%, the mutation was determined as a maternal mutation, because the allelic frequency of paternal alleles is half of the fetal fraction according to the equation $ff = \frac{2dA}{dA+dB}$, where ff is the fetal fraction and d is the depth of the paternal original allele A or maternal original B. Our previous study in over 23,000 pregnancies has showed that only 0.9% of samples have a fetal fraction higher than 25% and extremely rare samples have fetal fractions higher than 40%.[19] Thus, the MAF of a *de novo* or paternal mutation ought to be less than 20%.

Chromosome analysis using the genome-wide reads followed the method described previously.[20] Briefly, the GC bias introduced by the sequencing process was corrected, and the *k*-mer (*k* refers to the length of the sequencing reads) coverage was calculated with the corrected GC content for each chromosome. A binary hypothesis *t*-test and logarithmic likelihood ratio *L*-score between the two *t*-tests were used to determine the risk of chromosome aneuploidy.

Fetal fraction was calculated in male pregnancies using method described before.[20] Briefly, formula Fetal fraction $= 2 \times \frac{\overline{UR_Y}}{\overline{UR}}$ was used to calculate the fetal fraction estimate by chromosome Y, where $\overline{UR_Y}$ represents the unique reads on chromosome Y, and $\overline{UR}$ represents the unique reads on autosome chromosomes.

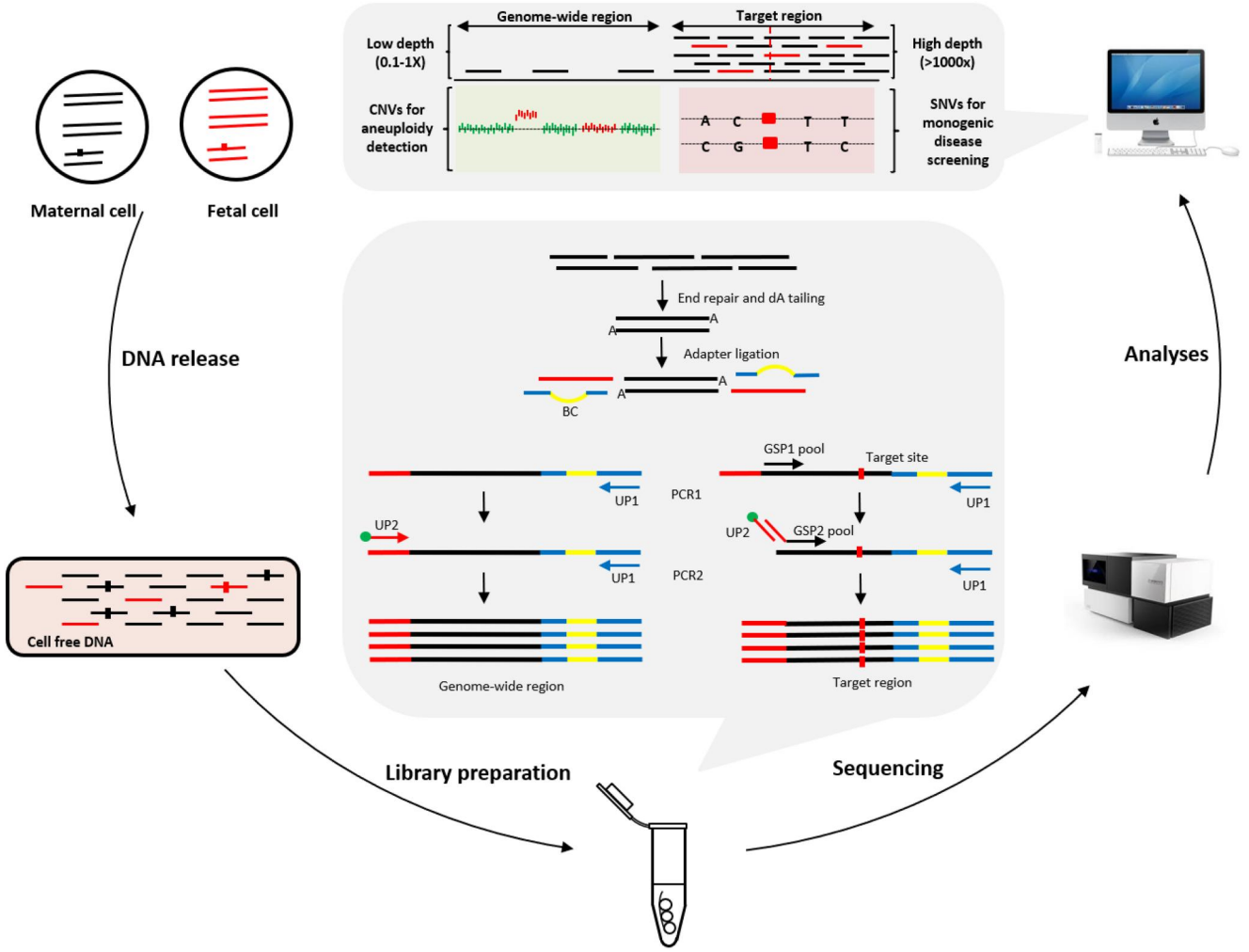

FIGURE 1 A schematic illustration of simultaneous amplification of whole-genome and target regions of cfDNAby TAGs-seq. BC, barcode; GSP1, gene-specific primer 1; GSP2, gene-specific primer 2; TAGs-seq, Targeted And Genome-wide simultaneous sequencing; UP1, universal primer 1; UP2, universal primer 2 [Colour figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

A total of 68 pregnant women were recruited (Figure S2), including 26 normal pregnant women without known high-risk of fetal aneuploidy or ultrasound abnormalities, 7 pregnant women with fetal trisomy, 7 pregnant women with fetal ACH or TD, 18 pregnant women with ACH/TD-like ultrasound findings, and 10 pregnant women with fetal risk of β-thalassemia. The mean maternal age was 31.1 years old, and the mean gestational week was 24.6 weeks (Table S4).

3.1 | TAGs-seq analysis in pregnancies with fetal trisomy

Plasma cfDNA of 26 normal pregnancies and 7 pregnancies of fetal trisomy were tested by TAGs-seq and conventional NIPT in parallel, generating about 54 million and 40 million of mean clean reads per sample, respectively (Table S5). In each sample, about 98.9% of the TAGs-seq reads was distributed on 23 pairs of chromosomes as well as mitochondrial genome, with a mean sequencing depth of 1.7 × ± 0.2 (95% CI). The coefficient variance (CV) and chromosome distribution percentage between TAGs-seq and conventional NIPT showed no statistical difference (Figure 2). Using the genome-wide reads of TAGs-seq, we calculated the fetal fraction of each sample, which increased by 20% comparing with the fetal fraction of conventional NIPT (Figure S3). Consistently, reads generated by TAGs-seq contained higher percentage of short cfDNA fragments than conventional NIPT (Figure S3).

Among 33 plasma samples, three cases of T21, two cases of T18, and two cases of T13 were correctly identified by TAGs-seq using the T-score of 3 as the cut-off value. This was consistent to the results of conventional NIPT (Figure 3A, B) and diagnostic results by amniocentesis and G-banding karyotypes (Table S5). Meanwhile, fetal genders deduced using TAGs-seq data were also consistent to that of conventional NIPT (Table S5).

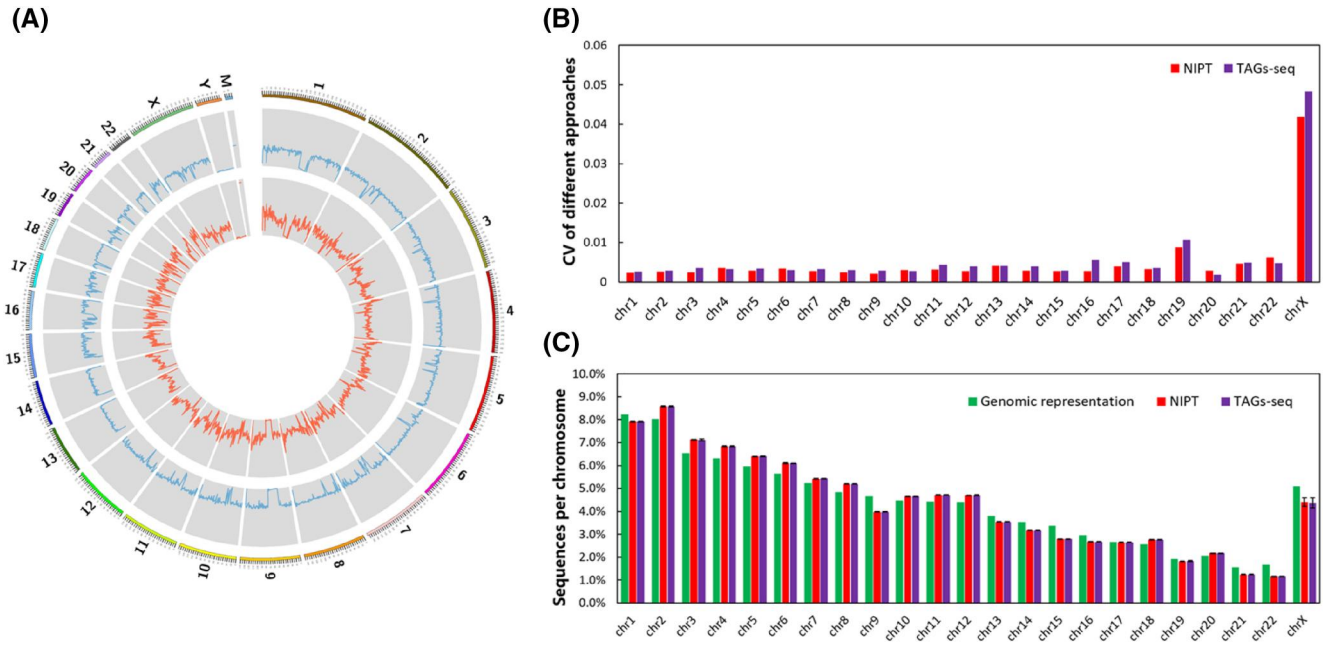

FIGURE 2 Comparison of the whole-genome reads obtained by Targeted And Genome-wide simultaneous sequencing (TAGs-seq) to conventional noninvasive prenatal testing (NIPT). (A) Comparison of genome distribution on each chromosome (1–22, X, Y) and mitochondria genome (M) between TAGs-seq (red) and conventional NIPT (blue); (B) coefficient of variation (CV) of sequencing reads on different chromosomes between TAGs-seq (purple bars) to conventional NIPT (red bars). (C) The percentage of sequencing reads on each chromosome between TAGs-seq (purple bars) to conventional NIPT (red bars) [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | TAGs-seq analysis in pregnancies with fetal ACH and TD

Plasma cfDNA of 7 pregnant women with fetal ACH or TD, and 18 pregnant women with ACH/TD-like ultrasound findings or pregnant history were analyzed for *FGFR3* mutations. On average, TAGs-seq generated 44.7 million clean reads per sample, and the mean mapping rate on human genome was 93.0% (Table S6). In each sample, around 98.8%–99.3% of the TAGs-seq reads were distributed on the whole-genome region with mean sequencing depth of 1.4 × ± 0.2 (95% CI). Around 0.5%–2.6% of reads were aligned to *FGFR3* with 100% of coverage and mean sequencing depth of 55,625.3 × ± 8717.1 (95% CI; Figure S4A, B). Chromosome analysis using the reads on the whole-genome region showed low-risk of fetal trisomy (Figure 3C). Thirteen samples were determined as male pregnancy, and the fetal fraction calculated with TAGs-seq data ranged from 14.3% to 35.9% (Table S6).

Using the high-depth reads on *FGFR3*, seven samples with allelic frequency higher than 2% were identified, including five samples of ACH of *FGFR3*: c.1138G > A, one sample of TD of *FGFR3*: c.1118A > G, and one sample of TD of *FGFR3* c.742C > T (Figure 4A). No remaining samples were found to contain *FGFR3* mutations with the allelic frequency higher than 2%. All TAGs-seq results were consistent with Sanger results using amniocentesis samples. In 26 normal pregnancies as normal controls, 0.4%–1.9% of the TAGs-seq reads were mapped to the *FGFR3* gene, and no allelic frequency higher than 2% was detected (Table S5 and Figure 4A).

3.3 | Analysis of β-thalassemia using TAGs-seq

Ten pregnancies with high risk of fetal β-thalassemia were tested by TAGs-seq, each generating 53.9 million of mean clean reads (Table S7). Around 98.2% of the TAGs-seq reads in each sample were distributed on the whole-genome region with mean sequencing depth of 2.0 ± 0.7× (95% CI). Chromosome analysis showed no samples with T-score of 3 or above, and thus no fetal aneuploidy of T21, T18, and T13 was detected (Figure 3D). In each case, about 1.1%–2.5% (mean = 1.8%) of clean reads were mapped to the coding and flank region of *HBB* with mean sequencing depth of 42,603.9× ± 18,901.8 (95% CI), and were used to calculate the MAF of mutations (Table S7).

In two samples (H170 and X391), paternal mutations were identified by TAGs-seq in plasma cfDNA, showing 9.81% of c.52A > T in H170 and 2.96% of c.-78A > G, respectively (Figure 4B and Table S7). Both H170 and X391 also contained high MAF of maternal mutations in plasma cfDNA, yet it was impossible to determine if fetus inherited the maternal mutation due to maternal background. Thus, the fetuses of H170 and X391 were only classified as risky of β-thalassemia. Sanger sequencing of amniotic fluid cells confirmed that the fetus of H170 carried both paternal and maternal

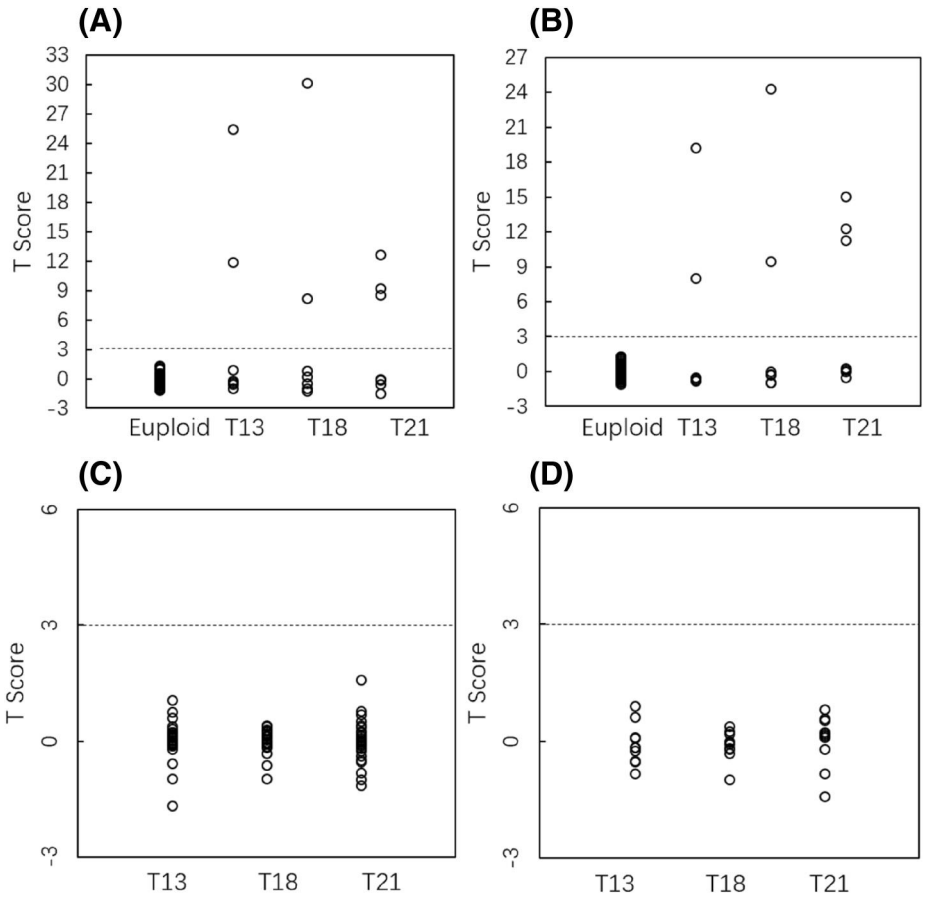

FIGURE 3 Fetal chromosome analysis results using Targeted And Genome-wide simultaneous sequencing (TAGs-seq) data and conventional noninvasive prenatal testing (NIPT) data. (A) T-Scores of conventional NIPT in seven cases of fetal aneuploidy and 26 cases of normal pregnancies; (B) T-Scores of TAGs-seq in seven cases of fetal aneuploidy and 26 cases of normal pregnancies; (C) T-Scores of TAGs-seq in seven cases of fetal achondroplasia/thanatophoric dysplasia (ACH/TD) and 19 cases of pregnancies with ACH/TD-like ultrasound phenotypes; and (D) T-Scores of TAGs-seq in 10 case of pregnancies with fetal risk of β-thalassemia

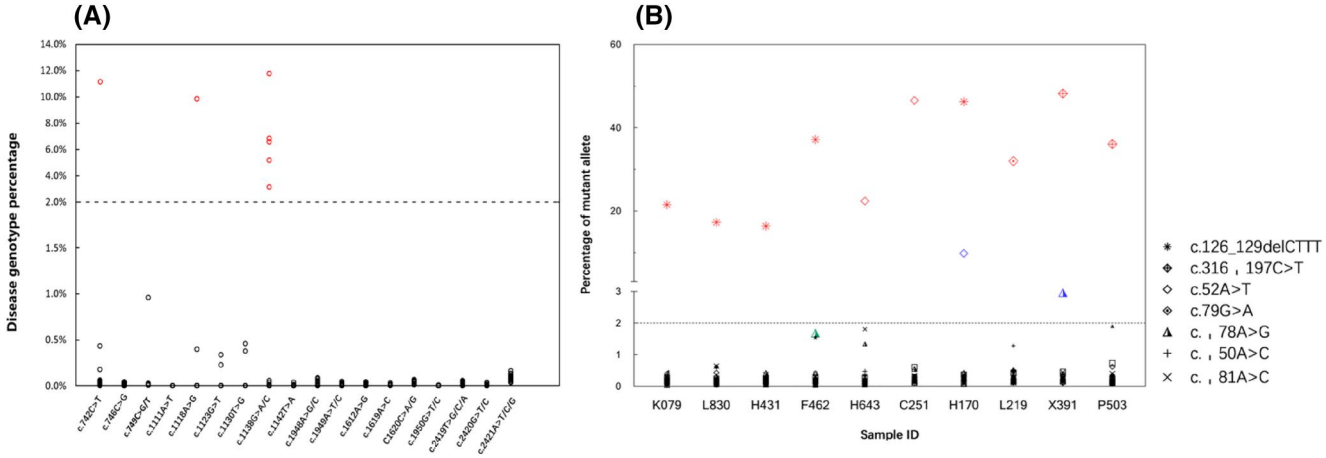

FIGURE 4 Detection of *de novo* mutations causing ACH and TD and paternal mutations of β-thalassemia using reads on the selected genes by Targeted And Genome-wide simultaneous sequencing (TAGs-seq). (A) The minor allelic frequency (MAF) of 18 alleles in 18 pregnancies with achondroplasia/thanatophoric dysplasia (ACH/TD)-like ultrasound phenotypes and seven pregnancies with fetal ACH or TD. (B) The MAF of 26 alleles known cause to β-thalassemia in 10 pregnancies with risk of fetal β-thalassemia and high-frequency alleles are showed in legend. The red dots in the picture are mutants from mother, the blue dots are mutants from father and green dot marked a false-negative case [Colour figure can be viewed at wileyonlinelibrary.com]

mutations, while the fetus of X391 only carried the paternal mutation (Table S7).

Eight samples were detected by TAGs-seq to only contain maternal mutation in plasma cfDNA with the MAF ranging from 17.6% to 46.6% and the fetal fraction from 5.1% to 10.4% (Figure 4B). Among these eight samples, seven (L830, H431, F462, H643, C251, L219, and P503) were couples carrying different *HBB* mutations (Table S7). Hence, the fetuses of these seven pregnancies were determined as low-risk of β-thalassemia due to the lack of paternal mutations. In sample K079, both parents carry the same HBB mutation (c.126_129delCTTT), and the possibility of inheriting the paternal mutation in fetus could not be ruled out. Thus, the fetal risk of β-thalassemia could not be determined by TAGs-seq in K079. Sanger sequencing of amniotic fluid in these eight pregnancies confirmed the low risk of β-thalassemia (Table S7). However, the Sanger sequencing result in F462 revealed the existence of paternal mutation c.-78A > G in fetus, of which the plasma cfDNA sequencing by TAGs-seq identified an MAF of 1.7%, close to but not beyond the 2% cut-off (Figure 4B and Table S7). Thus, although the fetus of F462 was not affected by β-thalassemia, it contained a paternal mutation missed by TAGs-seq.

3.4 | Detection performance

All 68 pregnancies were analyzed for fetal aneuploidy using the TAGs-seq based NIPT, in which seven aneuploidies were correctly identified with no false-positive and false-negative results. Thus, the sensitivity and specificity of detecting aneuploidies by TAGs-seq were 100% (56%–100%, 95% CI) and 100% (93%–100%, 95% CI), respectively. Fifty-eight pregnancies were analyzed for *FGFR3 de novo* mutations using the TAGs-seq method, and seven positive cases were correctly identified with no false-positive and false-negative results. Thus, the sensitivity and specificity of detecting *FGFR3 de novo* mutations were 100% (56%–100%, 95% CI) and 100% (91%–100%, 95% CI), respectively. In 10 pregnancies with fetal risk of β-thalassemia, three carried the paternal mutations of *HBB*, in which two were correctly identified by TAGs-seq. Thus, the accuracy was 66%.

4 | DISCUSSION

In this study, the TAGs-seq method could amplify plasma cfDNA to simultaneously obtain the low-pass reads on whole genome and high-depth reads on selective genes, which allowed the analysis of chromosome aneuploidy and point mutations, respectively. Among 68 tested plasma samples, high sensitivities and specificities were obtained to identify 7 cases of fetal aneuploidy and 7 cases of ACH/TD. Paternal mutations of β-thalassemia could also be detected, although one false-negative result was obtained, resulting in 66% of accuracy.

Several techniques have been described to noninvasively detect monogenic diseases by using droplet digital PCR,[8,21–25] targeted sequencing,[26] COLD-PCR and microarray,[27] allele specific real-time PCR,[28,29] and cSMART.[30,31] However, these methods require experiments that cannot cope with conventional NIPT method for chromosome analysis. Recently, three studies reported targeted capture NIPT to screen for chromosome aneuploidy, copy number variation, and monogenic disease.[32–34] However, their methods were time-consuming due to 16–72 hours of hybridization capture after the preparation of cfDNA sequencing library. In contrast, TAGs-seq could accomplish the analysis of chromosome aneuploidy and point mutations without dramatically increasing the amount of sequencing reads and changing the current NIPT protocol. Thus, the TAGs-seq based NIPT may have the ease to provide flexible medical choices for prenatal screening for genetically dominant disorders such as ACH/TD caused by de novo mutations, as they contribute to nearly 60% of severe postnatal monogenic diseases.[26]

With low-pass sequencing depth, TAGs-seq amplified the whole-genome region of cfDNA comparable to conventional NIPT, as confirmed by the consistent CV and chromosome distribution percentage between the TAGs-seq and conventional NIPT. As a result, the algorithm in the conventional NIPT[20] could be easily transferred for chromosomal analysis using the TAGs-seq data and achieved similar sensitivity and specificity. Interestingly, we observed an increased fetal fraction for about 1.2 folds in the TAGs-seq data, possibly because the linear amplification of multiplex PCR preferably amplified shorter fragments of cfDNA. This might help reduce false-positive or false-negative results in samples with low fetal fractions such as early pregnancies or certain aneuploidies.[2,,35]

A recent study has demonstrated the importance of prenatal screening for genetically dominant disorders caused by *de novo* mutations.[26] In other studies, the clinical utility of noninvasively testing for ACH caused by *de novo* mutations on *FGFR3* has been demonstrated.[7,23,36,37] In this study, the specific amplification of target genes by TAGs-seq resulted in high coverage (close to 100%) and high depth (more than 60,000X on average) among all samples, which allowed the accurate calculation of MAF on *FGFR3* and *HBB* loci associating with monogenic diseases. Thus, it is possible to use the TAG-seq based NIPT to identify single nucleotide variants, especially the *de novo* mutations with clear clinical significance, at early pregnancy when chromosome screening is routinely conducted. Due to the presence of maternal cfDNA in plasma, for autosomal recessive diseases, the TAGs-seq could not directly determine the presence of maternal mutation in fetus. Nonetheless, the TAGs-seq allowed the noninvasive detection of paternal mutation, which reduce the need for diagnostic testing in half of at-risk couples. [38]

Currently, we used the MAF>2% as the cut-off value to call for de novo or paternal mutations in plasma cfDNA, which might be interfered by low fetal fraction and biased amplification preference, leading to false-positive and false-negative results. There is the possibility of false-positive or false-negative results using the 2% of MAF cut-off at low fetal fraction, as being suggested previously.[39] Lower mapping quality of nonreference allele variant, biased amplification preference, or sequencing errors may also be responsible for false-negative result.

Our study had the limitation of a small sample size and very few positive cases of disease. This makes it difficult to evaluate the clinical performance, resulting in large ranges of confidence intervals and low sensitivity compared to other studies.[39,40] Although

our method was less complicated comparing with previous methods,[41,42] our method could not provide a full fetal genotype interpretation, and consequently was only suitable for analyzing *de novo* mutations and paternal alleles. Nonetheless, as a proof-of-concept study, the data collected from 68 pregnancies demonstrated the stability and repeatability of TAGs-seq in simultaneously generating low-pass whole-genome data and high-depth data of target genes. Furthermore, the TAGs-seq based NIPT demonstrated high sensitivities and specificities in detecting fetal aneuploidy and *de novo* mutations causing ACH and TD. Thus, the TAGs-seq demonstrated good clinical potentials, and further evaluation with larger samples is still required to determine the clinical utility of TAGs-seq based NIPT.

5 | CONCLUSION

This study described a novel NIPT method to simultaneously generate low-coverage genome-wide reads for analyzing chromosome aneuploidy, and high-depth targeted reads for detecting *de novo* mutations and paternal mutations of monogenic diseases. With no false positive results, this method correctly identified all the fetal aneuploidy and mutations on *FGFR3* in plasma cfDNA, showing good potential for clinical use.

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CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

All TAGs-seq sequencing data of this study have been deposited in the CNSA (https://db.cngb.org/cnsa/) of China National GeneBank DataBase (CNGBdb).

ETHICS STATEMENT

This study was approved by the Ethics Committee of the Shenzhen People's Hospital (no. 20170710), the Ethics Committee of the PLA General Hospital (no. 20151101) and the Institute Review Board of BGI-Shenzhen (BGI-IRB 17151).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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