OBSTETRICS

Prospective chromosome analysis of 3429 amniocentesis samples in China using copy number variation sequencing

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BACKGROUND: Next-generation sequencing is emerging as a viable alternative to chromosome microarray analysis for the diagnosis of chromosome disease syndromes. One next-generation sequencing methodology, copy number variation sequencing, has been shown to deliver high reliability, accuracy, and reproducibility for detection of fetal copy number variations in prenatal samples. However, its clinical utility as a first-tier diagnostic method has yet to be demonstrated in a large cohort of pregnant women referred for fetal chromosome testing.

OBJECTIVE: We sought to evaluate copy number variation sequencing as a first-tier diagnostic method for detection of fetal chromosome anomalies in a general population of pregnant women with high-risk prenatal indications.

STUDY DESIGN: This was a prospective analysis of 3429 pregnant women referred for amniocentesis and fetal chromosome testing for different risk indications, including advanced maternal age, high-risk maternal serum screening, and positivity for an ultrasound soft marker. Amniocentesis was performed by standard procedures. Amniocyte DNA was analyzed by copy number variation sequencing with a chromosome resolution of 0.1 Mb. Fetal chromosome anomalies including whole chromosome aneuploidy and segmental imbalances were independently confirmed by gold standard cytogenetic and molecular methods and their pathogenicity determined following guidelines of the American College of Medical Genetics for sequence variants.

RESULTS: Clear interpretable copy number variation sequencing results were obtained for all 3429 amniocentesis samples. Copy number variation sequencing identified 3293 samples (96%) with a normal molecular karyotype and 136 samples (4%) with an altered molecular karyotype. A total of 146 fetal chromosome anomalies were detected, comprising 46 whole chromosome aneuploidies (pathogenic), 29 submicroscopic microdeletions/microduplications with known or suspected associations with chromosome disease syndromes (pathogenic), 22 other microdeletions/microduplications (likely pathogenic), and 49 variants of uncertain significance. Overall, the cumulative frequency of pathogenic/likely pathogenic and variants of uncertain significance chromosome anomalies in the patient cohort was 2.83% and 1.43%, respectively. In the 3 high-risk advanced maternal age, high-risk maternal serum screening, and ultrasound soft marker groups, the most common whole chromosome aneuploidy detected was trisomy 21, followed by sex chromosome aneuploidies, trisomy 18, and trisomy 13. Across all clinical indications, there was a similar incidence of submicroscopic copy number variations, with approximately equal proportions of pathogenic/likely pathogenic and variants of uncertain significance copy number variations. If karyotyping had been used as an alternate cytogenetics detection method, copy number variation sequencing would have returned a 1% higher yield of pathogenic or likely pathogenic copy number variations.

CONCLUSION: In a large prospective clinical study, copy number variation sequencing delivered high reliability and accuracy for identifying clinically significant fetal anomalies in prenatal samples. Based on key performance criteria, copy number variation sequencing appears to be a well-suited methodology for first-tier diagnosis of pregnant women in the general population at risk of having a suspected fetal chromosome abnormality.

Key words: amniocentesis, aneuploidy, chromosome anomalies, copy number variation sequencing, invasive prenatal diagnosis, microdeletions, microduplications, variants of uncertain significance

Introduction

For >40 years, prenatal diagnosis has played an important role in management of pregnancies at high risk for a fetal chromosome abnormality.[1] Despite the successful introduction of noninvasive prenatal testing using the circulating cell-free fetal DNA from a blood sample,[2] the mainstream procedure currently undertaken by at-risk patients involves an invasive prenatal diagnosis procedure whereby fetal cells are retrieved by either chorionic villous sampling or amniocentesis and tested for chromosome copy number changes using cytogenetic or molecular karyotyping.[3] High-resolution G-banding karyotyping is still the most commonly used cytogenetics methodology and can identify the majority of fetal chromosome abnormalities, at a resolution of >10 Mb.[4] More recently, the application of high-resolution chromosome microarray analysis (CMA) methods such as array comparative genomic hybridization (CGH) and single nucleotide polymorphism arrays has revolutionized diagnostic testing of prenatal samples, with an increased yield of cryptic copy number variations (CNVs) regardless of the clinical indications for analysis.[5–7] In addition, these advanced array technologies have also been used as a

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AJOG at a Glance

Why was this study conducted?

We sought to evaluate the performance of a whole genome sequencing method called copy number variation (CNV) sequencing (CNV-Seq) for detecting chromosome anomalies in prenatal samples.

Key findings

In a large prospective cohort of 3429 high-risk pregnant women referred for amniocentesis, CNV-Seq detected 97 (2.83%) pathogenic or likely pathogenic chromosome anomalies and 49 variants of uncertain significance (1.43%) and returned a 1% higher yield of CNVs compared to what would have been expected for karyotyping.

What does this add to what is known?

This study demonstrates that CNV-Seq is a suitable first-tier diagnostic test for the identification of clinically significant fetal chromosome anomalies.

complimentary method to confirm karyotyping findings and to provide finer details of a suspected chromosome abnormality.

Next-generation sequencing (NGS) methodologies based on massively parallel sequencing now offers a viable alternative methodology to CMA, with a genomewide resolution down to 0.1 Mb for detecting clinically significant chromosomal abnormalities in prenatal and postnatal samples.[8,9] In several retrospective analyses of amniocentesis and pediatric samples, NGS-based methods have been shown to detect whole and partial chromosome aneuploidies, segmental imbalances such as microdeletions, microduplications, unbalanced translocations, and ring chromosomes.[8−10] Further, NGS has been able to detect small segmental aneuploidies in miscarriage samples that were associated with genes involved in fetal development.[11] Moreover, NGS has been used to identify altered maternal karyotypes and detect low levels of maternal mosaicism in noninvasive prenatal testing discordant samples.[12,13] Apart from proven reliability, accuracy, and reproducibility of NGS for 24-chromsome analysis, several other key laboratory advantages have been demonstrated, including an efficient laboratory work flow, a short turnaround time of <12 hours, results from a starting DNA template as low as 10 ng, scalability for simultaneous sequencing of large sample numbers, and a lower reagent cost compared to CMA. However, some limitations of NGS have been noted such as the inability to detect balanced translocations, uniparental disomy, and polyploidies such as 69,XXX.[14]

Several studies using CMA-[5,15,16] or NGS-based[8,9] approaches have shown that both these methods yield a higher return of chromosomal anomalies than karyotyping, specifically for submicroscopic segmental imbalances associated with syndromic and nonsyndromic CNVs. Currently, CMA is routinely used to detect chromosome anomalies in amniocentesis samples, finding pathogenic segmental imbalances such as microdeletions and microduplications in approximately 6% of pregnancies where a structural fetal abnormality has been detected by ultrasound scanning[17,18] and approximately 1.7% of other high-risk pregnancies without evidence of a fetal structural abnormality.[18,19] However, the clinical utility of using NGS as a first-pass diagnostic method for amniocentesis samples routinely referred for different clinical indications and fetal risk has not been systematically evaluated in a large sample set. In this study, we applied the NGS method CNV sequencing (CNV-Seq) to 3429 amniocentesis samples collected prospectively from pregnant women with different pregnancy risk factors and evaluated the incidence and type of pathogenic fetal chromosomal anomalies by clinical indication.

Materials and Methods

Study patients

A total of 3429 prospective pregnant women referred for amniocentesis and chromosome testing at the Prenatal Diagnosis Center of West China Second University Hospital of Sichuan University from February through August 2017 were recruited to participate in the study. The study was approved by the Medical Ethics Committee of West China Second University Hospital of Sichuan University (medical research 2016-7). All women provided written informed consent for genetic investigation of their pregnancy, including maternal serum screening, ultrasound scanning, as well as amniocentesis for detecting fetal chromosomal anomalies by CNV-Seq. Patients were categorized into 3 different fetal risk groups, namely: advanced maternal age (AMA) ≥35 years, high-risk maternal serum screening (HR-MSS), and positive for ultrasonographic soft markers (USMs). A fourth group was also included and consisted of patients with mixed indications, including inheritable risk of a single gene disease, prior risk of an abnormal pregnancy outcome, an abnormal amniotic fluid volume, and those who voluntarily requested amniocentesis without high-risk factors. Patients with a fetal ultrasound structural abnormality were not included as part of this study, but were offered a diagnosis by CMA as the gold standard.

In this study, AMA was defined as age ≥35 years at the expected date of childbirth. The risks for trisomy 21 and trisomy 18 were determined by measurement of first- or second-trimester serum markers and scores of ≥1 in 270 and ≥1 in 350, respectively, were considered high risk. Scanning for USMs was performed between 16−30 weeks' gestation and included screening for thickened nuchal fold, absence or dysplasia of nasal bone, short long bones, hyperechogenic bowel, mild ventricular expansion, intracardiac echogenic focus, choroid plexus cyst, single umbilical artery, enlarged cisterna