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Original Articles/นิพนธ์ต้นฉบับ

Validation of Molecular Karyotyping Techniques for Rapid Prenatal Diagnosis of Common Aneuploidies

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Abstract

BACs-on-Beads (BoBs) technology and Quantitative fluorescent PCR (QF-PCR) are recent molecular karyotyping methods which have been used for prenatal diagnosis of the most common aneuploidies. Both of them are rapid, cost-effective and suitable for automation and can detect most abnormalities diagnosed by conventional karyotyping. The objective of this study was to evaluate the performance of both molecular-based techniques for the detection of chromosomes 13, 18, 21, X and Y. The results obtained from 22 prenatal samples in which BACs-on-Beads technology (KaryoLite™ BoBs and Prenatal™ BoBs), QF-PCR and conventional karyotype had been performed. We found that concordant KaryoLite™ BoBs, Prenatal™ BoBs, QF-PCR and karyotype results were obtained in 95.5% (21/22) of the common aneuploidies. Only a 49,XXXXX sample could not be detected by BoBs assay and QF-PCR. In conclusions, BoBs technology and QF-PCR are the reliable methods to detect common aneuploidies and should replace conventional cytogenetic analysis whenever prenatal testing is performed solely because of an increased risk of chromosomes 13, 18, 21, X and Y. Cytogenetic follow-up of molecular karyotyping findings is recommended to rule out mosaicism, maternal cell contamination, balanced rearrangement and polyploidy.

Keywords: BACs-on-Beads, QF-PCR, common aneuploidies

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Introduction

Conventional karyotyping is a gold standard technique used for prenatal diagnosis of chromosomal abnormalities, because it is highly reliable for the detection of aneuploidy and structural rearrangement which are the most frequent abnormalities identified in prenatal diagnosis[1,2]. However, it carries a number of disadvantages, including the need for cell culture, being labor intensive, time consuming and requires great technical expertise[2]. The ability to rapidly detect aneuploidy and identify small structural abnormalities of fetal chromosomes has been greatly enhanced by the use of molecular cytogenetic technologies. These techniques include fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), multiplex ligation dependent probe amplification (MLPA), quantitative fluorescence polymerase chain reaction (QF-PCR), and BACs-on-beads (BoBs) technology[3-7].

QF-PCR is a rapid, simple and accurate prenatal diagnostic test. The inclusion of markers on chromosomes X, Y, 21, 18 and 13 allows the detection of the great majority of clinically significant chromosome abnormalities in a few hours after sampling. QF-PCR analysis includes amplification, detection and analysis of chromosome-specific DNA sequences known as genetic markers or small tandem repeats (STRs). Fluorescently labeled marker specific primers are used for PCR amplification of individual markers and the copy number of each marker is indicative of the copy number of the chromosome. The resulting PCR products may be analyzed and quantified using an automated genetic analyzer.[4,5]

For prenatal diagnostic purposes, two types of BoBs assays are available: Prenatal BoBs™ and KaryoLite BoBs™. The Prenatal BoBs™ kit is a multiplex bead-based assay designed to detect gains and losses of chromosomes 13, 18, 21, X, Y and nine targeted microdeletion regions. The KaryoLite BoBs™ assay provides dosage information about the proximal and terminal regions of each chromosome arm. By immobilizing bacterial artificial chromosome derived DNA probes onto fluorescently coded beads, BoBs enable rapid detection of copy number changes in targeted genomic regions from a minute amount of DNA. BoBs technology supports high throughput molecular karyotyping in a microplate well which in turn, can lead to greater laboratory efficiency and better use of resources.

The objective of this study was to evaluate the performance of both molecular-based techniques for the detection of common aneuploidies: chromosomes 13, 18, 21, X and Y.

Materials and Methods

Sample collection

A total of 22 amniocentesis samples were collected from the remaining specimen from routine service in Human genetics laboratory, Department of Pathology, Ramathibodi Hospital, Mahidol University.

QF-PCR assay

DNA from amniotic fluids were extracted using QAIamp DNA blood mini kit (Qiagen, Germany). DNA amplification was carried out for a total of 26 primer pairs specific for STR marker on common aneuploidies, Chromosomes 13, 18, 21, X and Y (Devyser kit, Sweden). The PCR products were detected by ABI3130 genetic analyzer (Applied Biosystems, USA) and analyzed by GeneMapper software v3.2 (Applied Biosystems, USA). The interpretation was performed according to the professional guidelines for clinical cytogenetics and clinical molecular genetics QF-PCR for the diagnosis of aneuploidies best practice guidelines (2012) v3.01.

Table 1 Comparison of the result of the amniocentesis samples detected by conventional karyotyping and molecular methods; QF-PCR, Prenatal BoBs™ (P-BoBs) and KaryoLite BoBs™ (KL-BoBs)

		BoBs	
Karyotyping	**QF-PCR**	**P-BoBs**	**KL-BoBs**
47,XY,+13	XY,+13	XY,+13	XY,+13
47,XX,+18	XX,+18	XX,+18	XX,+18
47,XY,+18	XY+18	XY+18	XY+18
47,XX,+18	XX,+18	XX,+18	XX,+18
47,XY,+18	XY,+18	XY,+18	XY,+18
47,XY,+18	XX,+18	XX,+18	XX,+18
47,XY,+21	XY,+21	XY,+21	XY,+21
47,XY,+21	XY,+21	XY,+21	XY,+21
45,X	monosomy X	monosomy X	monosomy X
45,X	monosomy X	monosomy X	monosomy X
49,XXXXX	XXX	XXXX	XXXX
mos 47,XXY [17]/46,XY [13]	XXY	XXY	XXY
46,XY	XY	XY	XY
46,XY	XY	XY	XY
46,XY	XY	XY	XY
46,XY	XY	XY	XY
46,XY	XY	XY	XY
46,XY	XY	XY	XY
46,XX	XX	XX	XX
46,XX	XX	XX	XX
46,XX	XX	XX	XX
46,XX	XX	XX	XX

BACs-on-beads assay

DNA from amniotic fluids were extracted using QAIamp DNA blood mini kit (Qiagen, Germany). DNA quantification and quality evaluation were carried out with the spectrophotometric evaluation using Nanodrop spectrophotometer (Delaware, USA). The purification of the labeled DNAs was performed using the NucleoFast 96PCR plate (Macherey-Nagel GmbH & Co., KG D-52313, Düren, Germany) (French and Australian laboratories). Samples were run on a Luminex 200™ instrument and the initial data processing was performed using Luminex100 IS software v2.3182 (Luminex Corp., Austin, TX). The data were analyzed using a software program (BoBsoft™ v1.1, PerkinElmer, Waltham, MA). The results were interpreted following the interpretation guidelines of the manufacturer (PerkinElmer, Finland).

Results

We evaluated and compared the results obtained from 22 amniocentesis samples in which BACs-on-beads technology, QF-PCR and conventional karyotype had been performed. As shown in Table 1, out of 22 samples, abnormal results were detected in 12

Table 2 Detection rate of QF-PCR, Prenatal BoBs™ (P-BoBs) and KaryoLite BoBs™ (KL-BoBs)

		Detection rate (%)		
Karyotype	**N**	**QF-PCR**	**P-BOBs**	**KL-BOBs**
Aneuploidies	8	8/8 (100)	8/8 (100)	8/8 (100)
46,XX	6	6/6 (100)	6/6 (100)	6/6 (100)
46,XY	4	4/4 (100)	4/4 (100)	4/4 (100)
45,X	2	2/2 (100)	2/2 (100)	2/2 (100)
mos 47,XXY [17]/46,XY [13]	1	1/1 (100)	1/1 (100)	1/1 (100)
49,XXXXX	1	0/1 (0)	0/1 (0)	0/1 (0)
Total	**22**	**21/22 (95.5)**	**21/22 (95.5)**	**21/22 (95.5)**

samples. There were 8 of aneuploidies (1, 5 and 3 of trisomy 13, 18, 21, respectively), 2 of monosomy X(45,X), one of mos 47,XXY and one of 49,XXXXX. Normal female and male were found in 10 samples (6 and 4 samples, respectively). Concordant KaryoLite BoBs™, Prenatal BoBs™, QF-PCR and karyotyping results were obtained in 95.5% (21/22) of the common aneuploidies and sex chromosome abnormalities. Detection rate of KaryoLite BoBs™, Prenatal BoBs™, QF-PCR in aneuploidies, monosomy (45,X), mos 47,XXY, normal male and female were 100%. However, In the case of 49,XXXXX, QF-PCR result was reported as triple X, while KaryoLite BoBs™ and Prenatal BoBs™ were reported as XXXX (Table 2).

Discussion

In this study, the performance of both molecular-based techniques for the detection of common aneuploidies: chromosomes 13, 18, 21, X and Y was evaluated and compared with conventional cytogenetic analysis. The sensitivity of both QF-PCR and BoBs was 95.5% and the specificity was 100%. Our finding was similar to the previous studies in which the sensitivity of both molecular methods was ranging from 95 to 98% and the specificity was 100%[2,7,8].

The area in which the BoBs assay loses out to QF-PCR is in diagnosing polyploidies and maternal contamination. Previous observations regarding the performance of BoBs technology were confirmed that polyploidy and balanced translocation cannot be unequivocally diagnosed by BoBs and chromosome mosaicism can be detected at trisomy rate of >30%[1]. Donaghue et al. had reported that the QF-PCR assay could be detected when 15% abnormal cells were present[10]. In our case, the percentage of mosaicism was about 57% in karyotyping (mos 47,XXY [17]/46,XY[13]). Therefore, it can be detected by both techniques.

However, it's important to realize that BoBs assay is superior to QF-PCR or other rapid aneuploidy testing, because it can identify known microdeletion syndromes or additional structural chromosomal abnormalities undetected by current rapid aneuploidy testing that targets only five chromosomes[13]. It is cheaper than the chromosome microarray method and without the worry of creating the dilemma of finding variants of unknown significance. Moreover, BoBs technology and QF-PCR are well-established methods for investigating the genetic content of product of conceptions (POCs) because

of the high rate of culture failure and maternal cell contamination[13].

The limitation of both BoBs and QF-PCR is its inability to detect tetrasomy, pentasomy [S5]or balanced rearrangement. It may not reflect the fetal chromosome constitution in case of confined placental mosaicism or in samples contaminated with maternal cells[2,11,13]. Therefore, in our study, both QF-PCR and BoBs could detect gain of X chromosome in 49,XXXXX case but could not report the exact copy number of chromosome X.

In conclusion, BACs-on-beads technology and QF-PCR are the reliable methods to detect common aneuploidies and should replace conventional cytogenetic analysis whenever prenatal testing is performed in case of an increased risk of common aneuploidy in chromosomes 13, 18, 21 and sex chromosome abnormalities. Cytogenetic follow-up of molecular karyotyping findings is recommended to rule out mosaicism, maternal cell contamination, balanced rearrangement and polyploidy.

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Original Articles/นิพนธ์ต้นฉบับ

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บทคัดย่อ

เทคนิค BACs-on-Beads (BoBs) และ Quantitative fluorescent PCR (QF-PCR) เป็นเทคนิคระดับโมเลกุลสมัยใหม่ที่ถูกนำมาใช้ในการตรวจวินิจฉัยก่อนคลอดที่มีความผิดปกติของจำนวนโครโมโซม โดยทั้ง 2 วิธีมีความรวดเร็ว คุ้มค่า และสามารถใช้เครื่องตรวจวิเคราะห์อัตโนมัติช่วยในการทดสอบได้ ซึ่งผลที่ได้จากการตรวจวิเคราะห์สามารถตรวจพบความผิดปกติส่วนใหญ่ที่ตรวจพบได้จากการตรวจโครโมโซม จุดประสงค์ของการศึกษานี้เพื่อประเมินประสิทธิภาพของการตรวจโดยเทคนิคระดับโมเลกุลในการตรวจหาความผิดปกติของโครโมโซมแท่งที่ 13, 8, 21 และโครโมโซมเพศ โดยการเปรียบเทียบผลการตรวจวิเคราะห์ตัวอย่างจากการตรวจวินิจฉัยก่อนคลอด 22 ตัวอย่างด้วยวิธี BACs-on-Beads (KaryoLite™ BoBs และ Prenatal™ BoBs), QF-PCR และการตรวจโครโมโซม ผลการศึกษาพบว่าผลที่ได้สอดคล้องกันทั้ง 3 วิธีคิดเป็นร้อยละ 95.5 (21/22) มีเพียง 1 ตัวอย่างที่ผลเป็น 49,XXXXX ที่ไม่สามารถตรวจวิเคราะห์ได้ด้วยวิธี BoBs และ QF-PCR โดยสรุปเทคนิค BoBs และ QF-PCR เป็นวิธีที่น่าเชื่อถือในการตรวจความผิดปกติของโครโมโซมที่พบบ่อย และควรนำมาใช้แทนการตรวจโครโมโซมในกรณีที่สงสัยความผิดปกติเพียงโครโมโซมแท่งที่ 13, 18, 21 และโครโมโซมเพศเท่านั้น การตรวจติดตามด้วยการตรวจโครโมโซมยังมีความจำเป็นในกรณีที่สงสัยความผิดปกติแบบ mosaicism มีการปนเปื้อนของเซลล์จากมารดา การเรียงตัวใหม่ของโครโมโซมแบบไม่มีการสูญเสียชิ้นส่วนโครโมโซม และการเพิ่มจำนวนชุดของโครโมโซม

คำสำคัญ: BACs-on-Beads, QF-PCR, common aneuploidies

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