Application of a Rapid Assay to Detect Targeted Numerical and Structural Anomalies in Patient with Congenital Malformations

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ABSTRACT

Bacterial artificial chromosomes (BACs)-on-Beads (BoBs) is one of the novel and rapid technologies that has been a part of recent advances in genomic technologies. BACs-on-Beads technology that assists in speedy detection of copy number changes (CNVs) in targeted genomic regions from minimal amount of DNA. We compared this molecular multiplex, bead-based suspension array that is used in prenatal invasive testing, with conventional cytogenetic and G-banded karyotype techniques. We present the initial BoBs analysis data of 4 patients referred to CABRI with congenital malformations. As per the manufacture’s information the targeted region covers at least 4-5 bacs for each region. The selected loci represent the relatively common chromosomal syndromes associated with deletions that can be missed by karyotype analysis. The syndromes are known with definable phenotype and deletion as the major means giving rise to the syndrome. In addition to this the BAC’s for the common aneuploidies of chromosomes 13, 18, 21, X, and Y are also present. The method not only detected the known trisomy 21 but also identified a deletion on the long arm of chromosome 7 at q11.2 region that represents the Williams – Beuran Syndrome (WBS) critical region in a patient with suspected trisomy 21. BoBs is potentially a very useful first row test for aneuploidy detection because of its lower cost and rapid detection with minimal amount of DNA especially in newborns with suspected congenital malformations. The results suggest that it is a reliable technique to detect common microdeletions that get missed out by conventional chromosomal analysis.

Keywords: Bacterial artificial chromosomes (BACs)-on-Beads, FISH, WBS critical region, Microarray.
INTRODUCTION

Birth defects are accountable for most cases of infant fatality and morbidity all over the world\textsuperscript{1,2}, with 7\% of all neonatal deaths due to congenital malformations\textsuperscript{2,3}. It is estimated that six percent of these defects are due to aneuploidies and nearly one in 200 newborns is affected\textsuperscript{2,4}.

The cause for congenital malformations is due to chromosomal aneuploidies or monogenic or due to environmental causes such as fetal infections, environmental teratogens, and could be by the low percentages for these factors or most of these defects are due to the combined effects of environmental and genetic factors\textsuperscript{2,4}.

The quick success of results for prenatal testing was crucial in these disorders. The recognition that common aneuploidies could be analyzed quickly came from the use of fluorescence in situ hybridization (FISH) that was offered as clinical testing for aneuploidy FISH on uncultured cells\textsuperscript{5,6}. The FISH testing uses probes that can identify aneuploidies and additional markers for particular chromosomes, but it is limited by its inability to detect abnormalities in chromosomal regions that are not included in the testing panel.

Although FISH can be performed on uncultured cells, chromosome analysis requires culturing to obtain metaphase chromosomes where resolution of the chromosome does not permit the identification of most microdeletion syndromes. The chromosome analysis can be helpful to detect aneuploidies and large structural rearrangements, but cannot identify most alterations smaller than 10 Mb in size\textsuperscript{7}.

Based on what is known, chromosome analysis is limited by resolution and FISH is limited to the regions represented by the probes in the testing panel. Introduction of microarrays has overcome these limitations in identifying cytogenetic anomalies in postnatal\textsuperscript{7} and prenatal testing in on-going and ended pregnancies\textsuperscript{8-12}. The density of the probes has increased with the advancement of technology and array designs were based on targeted approach or genome wide with their own advantages and disadvantages.

A targeted approach has the advantages of detecting gains and losses of only those loci that have been well characterized. The disadvantage in this approach is that the regions of the genome that may have clinical relevance in an unbalanced state would go undetected. Increased coverage throughout the genome may allow the identification of additional clinically relevant imbalances but will also lead to the identification of gains or losses of unknown or unclear clinical significance\textsuperscript{13}.

An assay was recently developed for pregnancies with a low risk of chromosome abnormalities (e.g. normal ultrasound examinations) that is broader than current FISH aneuploidy screening but without the possibility of unclear results that can be observed in microarray testing. It is a suspension array-based (Luminex Corp, Austin, TX, USA) assay, also known as BACs-on-Beads\textsuperscript{6}. The Prenatal BobS kit used in the present study consists of selected syndromes that are relatively common among chromosomal disorders; the deletions can be missed, by chromosome analysis. The syndromes result in a known, definable phenotype or outcome that is relatively severe; the syndrome does not usually present with abnormal ultrasound findings; and deletion is the major mechanism giving rise to the syndrome. In addition to these criteria, the common aneuploidies of chromosomes 13, 18, 21, X, and Y were also included\textsuperscript{6}. It has been used in several labs as an alternate to FISH in prenatal testing. Here we used this in our pilot study on the samples we obtained from the newborns referred with congenital malformations.
MATERIALS AND METHODS

Peripheral blood samples from 4 new born children diagnosed with congenital malformations (GMC hospital and research center) were submitted initially for chromosome analysis. DNA Extraction from EDTA Blood included several steps starting with blood lysis denaturation followed by deproteinization and DNA precipitation and re-suspension. The pellet was dissolved in 300µl of TE buffer, pH 7.5 at 55°C. Labeling of genomic DNA along with male and female reference DNA was performed that involved 60-90 min incubation in the thermo cycler at 37°C. It was followed by Purification of the labeled DNA sample that was transferred into NucleoFast 96 PCR purification plate that involved treatment with TE buffer and plated on incubator shaker followed by measurement of DNA concentration. The purified DNA which is adjusted to 150-200ng/µl was subjected to hybridization using hybridization bead mix provided by the manufacturer in the labeling kit. The procedure is followed by DNA washing and Reporter binding as per manufacturer protocol. Next the Assay Measurement was done using the Luminex instrument. The measurement data was analyzed using BoBs analysis software provided by Perkin Elemer.

RESULTS

We performed BOBs assay analysis on 4 patients referred with congenital malformations. We found one patient with a trisomy of chromosome 21 that was also chromosomally abnormal with trisomy 21 another patient that was chromosomally normal showed a deletion of the Williams –Beuran Syndrome critical region (WBSCR) on chromosome 7q11.2. Other two samples analyzed were normal. The samples are run along with the normal male and female references DNA (Figure 1). The deletion noticed was about 1.04Mb that included 5 selected BAC’s with co-ordinates of the deleted region range from 72,598,000 – 73,639,000 (Figure 2) as per the manufactures kit information which is about 1.04Mb of the 5.1 Mb of the WBSCR that includes several genes responsible for WBS. The important genes being CLIP2, ELN, GTF2I, GTF2IRD1 and LIMK1 are among the genes that are typically deleted in patients with William’s syndrome and the FISH testing is usually done to look for ELN gene deletion. Most of these genes are well characterized and the loss of the ELN gene is found in most of these patients with this disease.

DISCUSSION

Our pilot study using BoBs prenatal testing kit on 4 patients with congenital anomalies revealed loss of the WBSC region in patient 2a (Figure 3) with and another patient 3a with a gain of extra copy of chromosome 21(Figure 4). All the four patients were first analyzed chromosomally with patient 3a showing an extra copy of chromosome 21and rest of them were normal. The BoBs Prenatal kit used is usually dedicated to the prenatal samples like amniotic fluid or chorionic villi or products of conception. The loss of the WBS region (25 %) noticed in one of the 4 patients with the abnormality suggest that it is promising assay to study patients who appear chromosomally normal with congenital abnormalities.

The patient 2a with the deletion was born to a 38 yrs. old mother (G4P3) who is the fourth child. All the other 3 elderly children were normal. The patients 2a had
coarse facial features, systolic murmur 3/6 echocardiography with large intact VSD 1.2 cm. The other features included periorbital edema with puffiness of the eyes, flattened nasal bridge with small nose with upturned nose. The epicantthic folds and prominent nasolabial folds were present. The mouth was wide open with long prominent upper lip and small chin. The rubella IgG 19.7 IU/ml (> 10 reactive) and the neonatal screening profile were normal so as chromosome analysis showing 46, XX karyotype. The BoBs assay picked a deletion of the WBSC region but full size of the deletion may not be represented as the assay will detect only copy gain or loss within the regions represented by BACs in the assay. The same patient was also checked on Affymetrix Cytoscan array which confirmed the deletion with definite breakpoints as well as the mosaicism noticed in the BoBs analysis (Fig 5 & Table 2).

The assay can be used as an alternative to current prenatal testing and testing the newborns with normal karyotypes with suspected phenotypes. Most of the microdeletions selected in the array are flanked by segmental duplications (such as Prader-Willi/Angelman and Williams syndromes) and will most often have consistent-sized deletions but sometimes with variable-sized deletions. The size of the deletion cannot be determined by this assay and the size of the deletion sometimes is important to decide the gene content and possible phenotypic consequences, cases with positive results for these regions should be followed up with microarray testing to size the deletion as per previous studies. The BoBs assay has some advantages and a disadvantage when compared to microarray analysis, this assay is limited to the syndromes with known outcomes and won’t detect alterations of unknown clinical significance. The advantage of this assay is that it utilizes two to six beads with unique sequences per locus, which increases the confidence in abnormal calls compared to FISH, which utilizes a single probe for each target locus. The BoBs testing can be done in 24 h from the sample received in the laboratory to the result reporting. The turnaround time is same as the FISH aneuploidy testing performed on uncultured cells, but BoBs has additional features to detect known microdeletions. Another advantage is that it requires a small amount of input DNA (~150–240 ng) when compared to the DNA used in a microarray assay. So the assay can be considered an intermediate one while comparing with FISH and microarray and can be very useful for families who are not willing to go for expensive microarray testing.

Table 1: The median ratios recorded in two patients with abnormalities which are compared with normal male and female reference DNA.
Figure 1: Karyotypic changes detected by Prenatal™ BoBs™ assay in male and female reference DNA.

![QC Ratios Table](Image)

Figure 2: The WBS critical region as seen in the UCSC web browser with refseq gene content. The red bar represents the area where the BAC’s in assay are selected.

![UCSC Genome Browser](Image)
Figure 3: Karyotypic changes detected by Prenatal™ BoBs™ assay in patient 2a who showed loss of 7q11.2 region representing Williams-Beuran syndrome.

Figure 4: Karyotypic changes detected by Prenatal™ BoBs™ assay in patient 3a who had an extra copy of chromosome 21 representing Down’s syndrome.

Table 2: Microarray results of the patient with Williams’s syndrome with definite breakpoints with candidate genes highlighted in red.

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CONCLUSIONS

BoBs results show that 2 of 4 patients (50%) analyzed had structural and numerical abnormalities. Based on our preliminary result, we accept that this assay will prove to be a sensitive and rapid assay on prenatal and postnatal patient samples referred with congenital malformations. The assay is fast, precise, and cost-effective for molecular karyotyping that can be performed within 24 hours with minimal amount of DNA without culturing of the cells or tissues. The assay simultaneously detects several targeted regions that have been proved to be chromosomally associated disorders.

REFERENCES


