ORIGINAL ARTICLE

Prenatal BACs-on-Beads™: the prospective experience of five prenatal diagnosis laboratories

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ABSTRACT

Objective We previously reported on the validation of Prenatal BACs-on-Beads™ on retrospectively selected and prospective prenatal samples. This bead-based multiplex assay detects chromosome 13, 18, 21 and X/Y aneuploidies and the nine most frequent microdeletion syndromes. We demonstrated that Prenatal BACs-on-Beads™ is a new-generation, prenatal screening tool. Here, we describe the experience of five European prenatal diagnosis laboratories concerning the ongoing use of Prenatal BACs-on-Beads™.

Methods Some 1653 samples were analyzed. All results were confirmed by conventional karyotyping or another appropriate technique. All indications for invasive prenatal diagnosis were included. Amniotic fluid and chorionic villus samples were analyzed in equivalent proportions.

Results The failure rate was 3.3% and the overall abnormality detection rate was ~1/10. Eighty-five percent of the detected abnormalities were common aneuploidies. Eleven microdeletions and duplications were identified, thus giving an overall yield for microdeletion and microduplication detection of 1/145. Compared with QF-PCR, Prenatal BACs-on-Beads™ provides an additional detection rate of ~1/250 for low-risk pregnancies. The false positive and negative rates were both <1%.

Conclusion When associated with conventional karyotyping, the Prenatal BACs-on-Beads™ assay combines a short turnaround time (typical of rapid aneuploidy detection tests) with valuable detection of the most frequent microdeletion syndromes that cannot be detected in cytogenetic analyses. © 2012 John Wiley & Sons, Ltd.

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INTRODUCTION

Conventional karyotyping is the gold standard for invasive prenatal diagnosis. However, over the last two decades, new rapid aneuploidy detection (RAD) tests on uncultured samples have been progressively developed and introduced into clinical practice in some countries.[1] They are applied as 'stand-alone' tests for pregnancies with an indication of advanced maternal age (AMA) or increased risk for Down syndrome after maternal serum screening (MSS) but no ultrasound abnormalities suggestive of chromosomal abnormality.[2] These tests provide results for common aneuploidies for chromosomes 13, 18, 21 and X/Y (accounting for about 65–80% of all fetal chromosome unbalances) in about 48 h.[1,3]

The introduction of array comparative genomic hybridization (aCGH) revealed new microdeletion and microduplication syndromes in 15% to 20% of postnatal cases with unexplained developmental delays and/or intellectual disability, autism spectrum disorders, and multiple congenital anomalies with apparently normal karyotypes. In fact, aCGH has become the 'first-line' test in this group of patients.[4] In prenatal karyotyping, the risk of failure to detect a known, disability-causing cryptic copy number variation (CNV) has been estimated at between 1 in 300 and 1 in 600.[5] Most of these submicroscopic syndromes may go undetected before 26 weeks of gestation. Consequently, the affected pregnancies are classified as being 'low risk' for submicroscopic imbalances (AMA and increased risk after MSS indications) because of the lack of ultrasound

findings. Alternatively, these pregnancies may present with no suggestive ultrasound signs or some unspecific signs (e.g. structural 'soft markers' or growth retardation). This evidence may justify the chromosomal microarray (CMA) testing in all women undergoing invasive prenatal diagnosis. However, the risk of unpredictable phenotypes associated with genome-wide CMA and an unfavorable ratio of CNVs of uncertain significance relative to known disease-causing CNVs,[6] has prompted the development of a new bead-based, targeted assay – Prenatal BACs on Beads™ (BoBs™). In addition to detecting common aneuploidies, Prenatal BoBs™ investigates the nine most frequent microdeletion syndromes with dominant inheritance and a fully penetrant phenotype in apparently low-risk pregnancies without specific pathologic indications for invasive prenatal diagnosis.[7] This assay meets the practice guidelines issued by the American Congress of Obstetricians and Gynecologists[8,9] and European best practice guidelines,[10] which recommend that aCGH should not be routinely applied in prenatal diagnosis (i.e. instead of conventional karyotyping) until more consistent data on the clinical relevance of CNVs are available. The Prenatal BoBs™ technology has received the European conformity mark for *in vitro* diagnostics and has been clinically validated as a screening tool.[7,11] Additional disorders that are well characterized and unambiguous in terms of genetic counseling have been also included.[12] To date, all the published Prenatal BoBs™ data on retrospective cohorts have been consistent.[7,11] Here, we report on the one-year, ongoing laboratory experience of five different European prenatal diagnosis laboratories. The study sought to (i) describe and evaluate the feasibility of Prenatal BoBs™ in routine prenatal diagnosis, (ii) estimate the technique's additional diagnostic yield in the different groups of indications for invasive prenatal diagnosis, (iii) calculate the current false positive and false negative rates, and (iv) evaluate the benefits and limitations of this technology with respect to other rapid molecular tests in prenatal diagnosis.

MATERIALS AND METHODS

The study was performed in private-sector labs (the TOMA laboratory and Innogenetix) and public-sector laboratories (CHI Poissy St Germain, Jean Verdier Hospital, and Federico II University). The TOMA laboratory and CHI Poissy St Germain hospital had already performed a beta-test, retrospective evaluation of the technology based on 616 samples[11]; the other three labs started the prospective laboratory study after each performed in-house validation. A sample collection information form was completed by each laboratory director. The analyzed samples were collected from 15 May 2010 to 30 June 2011. All results were confirmed by conventional karyotyping (performed in parallel) or fluorescence *in situ* hybridization (FISH) analysis (in cases of microdeletion or microduplication and to define the cytogenetic mechanism generating the imbalance). When parents were available, the inheritance of the submicroscopic imbalance was investigated with the same methods. DNA was extracted from 5 mL of amniotic fluid (AF) using an automated system (MagnaPure, Roche Applied Science Indianapolis, IN, USA) or extracted manually from 3–4 mL of AF (QIAamp DNA Mini Kit, Qiagen, Inc., Chatsworth, CA, USA), as described previously.[11] For chorionic villus samples (CVS), DNA was extracted from a microscopically selected, entire native villous tree or from about 3–5 mg of tissue. Similar amounts of DNA were obtained from cultured cells versus other specimens. In the TOMA lab, a microsatellite-based maternal cell contamination (MCC) analysis was performed on normal female DNA from CVS found to be fragmented and suboptimal in terms of morphology (based on a validated, standard scale after an in-house quality assessment). Furthermore, in TOMA lab fresh samples were processed in two sessions per week, with an average turnaround time of 36 h from bar-coding of the sample to electronic signature by the technical and laboratory directors and online reporting of the final result. In the other centers, samples were processed (with or without freezing) once a week, with an average turnaround of time of 7 days; these samples were not screened for MCC. The Prenatal BoBs™ technology and the sample analyses have been described previously.[11] Briefly, Prenatal BoBs™ is a multiplex, bead-based suspension array using microspheres that are internally dyed with two spectrally distinct infrared and red fluorochromes. Each bead is coupled to DNA amplified from bacterial artificial chromosomes (BACs) and is read by two separate lasers in the Luminex® 200 analyzer (Luminex Corp, Austin, Texas) equipped with xPonent® 3.1 software (Perkin Elmer, Turku, Finland). Experiments with acceptable quality control parameters have more than 50 beads/BAC analyzed. In each session, two male and two female samples are included as reference DNAs. Each patient or reference DNA is analyzed in duplicate. The BoBsoft™ software (PerkinElmer Wallac, Turku, Finland) generates a 'Results tab' with a numeric and graphic representation of the probe and group ratios versus the female and male references. A sample is defined as 'normal disomic' when the ratio between the green fluorescence intensities in the test and in male/female reference is about 1.0 for all analyzed loci (Figure 1). A sample is defined as 'duplicated/deleted' in a chromosome locus when the fluorescence in the test is higher/lower than that in the reference (Figure 1). Single copy gains and losses generate ratios ranging from 1.3 to 1.4 and from 0.6 to 0.8, respectively, as initially reported.[11]

RESULTS

A total of 1653 diagnostic samples were analyzed (212 having been described previously[11]), including 783 AF samples with a mean gestational age in weeks (wg) of 21 + 6 (15–36) wg, 838 CVS with a mean gestational age of 13 + 0 (11–15) wg and 34 specimens from other sources (cultured cells, fetal blood and miscarriages) with a mean gestational age of 17 + 1 (8–28) wg (Table 1). The indication for prenatal diagnosis was specified by each laboratory in the great majority of cases (85.2%, 1409 out of 1653): abnormal ultrasound findings: 43.4% (611 out of 1409); AMA: 21.9% (309 out of 1409); an abnormal MSS with an increased risk for Down syndrome: 17.7% (249 out of 1409); maternal anxiety: 10.1% (143 out of 1409); abnormal parental

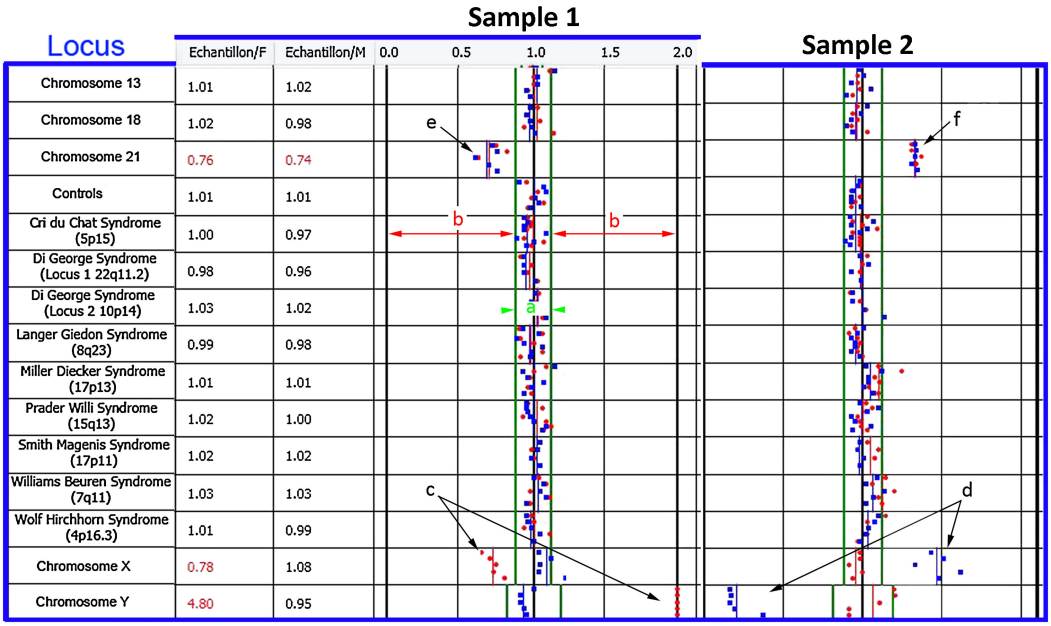

Figure 1 Interpretation of the Prenatal BoBs™ profiles with red spots (the sample to female reference ratio) and blue spots (the sample to male references ratio): (a) normal ratio, (b) abnormal ratio, (c) male gonosome profile, (d) female gonosome profile, (e) 21q22 deletion, and (f) 21q22 duplication

karyotype: 2.3% (33 out of 1409); previous fetus or child with aneuploidy: 2.1% (29 out of 1409); fetal death: 1.0% (14 out of 1403); other indications: 1.5% (21 out of 1403).

Results were obtained from 1599 of the 1653 samples (96.7%). Initially, 87 samples failed the analysis but, after an additional analysis, a result was obtained in a further 33 specimens. The overall technical failure rate was 5.3% (87 out of 1653); when counting only samples with no results, the rate decreased to 3.3% (54 out of 1653).

Failures were due to (i) a low DNA amount (less than 5 ng/µl; $n=11$), (ii) technical issues ($n=23$; mainly because of poorly closed tube caps during overnight incubation), (iii) labeling failure ($n=29$; mainly because of poor DNA quality) and (iv) unknown reasons, which led to uninterpretable results ($n=24$).

Of the 1599 sample results, 153 (9.6%, 153 out of 1599) were abnormal and 1446 were normal (90.4%). The overall detection rate was ~1 in 10. In AF and CV samples, the abnormality detection rates were 7.4% (53 out of 712; ~1 in 13) and 13% (92 out of 702; ~1 in 8), respectively. In the remaining, heterogeneous samples, the abnormality detection rate was 32% (8 out of 25, ~1 in 3) (Table 1). When considering the indication for prenatal diagnosis, the detection rate was around 1 in 6 for abnormal ultrasound findings, 1 in 31 for AMA, 1 in 14 for an abnormal MSS with an increased risk for Down syndrome, 1 in 143 for maternal anxiety and 1 in 6 for the remaining indications.

Table 1 Results of the ongoing laboratory experience, with 1653 prenatal samples

Samples	Direct AF	Direct CV	Other	Total (%)
Total selected	783	838	34	1653
Weeks of gestation (mean)	21+6	13+0	17+1	—
Failed	36	50	1	87
Failed after re-analysis	18	15	0	33 (2)
Total analyzed	765	801	33	1599 (98)
Abnormal result	53	92	8	153
Normal result	712	709	25	1446
Abnormality rate	1 in 13	1 in 8	1 in 3	1 in 10
False positives	0	3	0	3
False negatives	1	2	0	3

After FISH confirmation with specific probes, three false-positive results were observed: two deletions of the Williams Beuren critical region (showing borderline values just outside the normal expected range for a Prenatal BoBs™ analysis) (Figure 2a) and a case with a single bead/clone gain variation (mapping from 6 957 713 bp to 7 136 907 bp in Xp22.31) (Figure 2b). In one of the two borderline copy loss cases, a second Prenatal BoBs™ experiment performed on DNA from a second independent CVS gave the same borderline trend for the Williams Beuren critical region and for two other regions – thus indicating a possible DNA quality and/or quantity bias. A FISH analysis conducted on cytotrophoblast metaphases gave a normal result: ish 7q11.23(ELN,LIMK1, D7S613)x2. In the second case, the first BoBs™ experiment was conducted on DNA from direct CVS; FISH performed on metaphases from cultured CVS gave a normal result. No further Prenatal BoBs™ experiment was performed on this sample. The duplication in the Xp22.31 region was confirmed with a second, independent Prenatal BoBs™ experiment by starting from a second aliquot of DNA extracted from long-term cultured CVS. A FISH analysis on interphase nuclei did not confirmed the observed gain and yielded a result of nuc ish(DXYS130,STS,KAL)x2. This region partially overlaps

Table 2 Type, frequency, and detection strategy for the abnormal cases found in the ongoing laboratory experience

Type of abnormality	Total number (incidence as a % of 1599 samples)	Could be diagnosed by:		
		Conventional cytogenetic approaches	Targeted FISH	Prenatal BoBs™ only
Trisomy 21	76 (4.75)	76	–	–
Trisomy 18	21 (1.31)	21	–	–
Trisomy 13	7 (0.43)	7	–	–
Trisomy 22	2 (0.13)	2	–	–
Trisomy 15	2 (0.13)	2	–	–
Trisomy 7	1 (0.06)	1	–	–
45,X	16 (1.00)	16	–	–
Other sex chr. abnormalities	4 (0.25)	4	–	–
Complex aneuploidies	3 (0.18)	3	–	–
Unbal. structural cytog. abn.	7 (0.43)	7	–	–
Microdeletion/microduplication	11 (0.75)		5	6
Total	150 (9.38)	139	5	6

with a segmental duplication reported in the Database of Genomic Variants (URL: http://projects.tcag.ca/variation/) for the general population (Variation_83312, chrX:6,929,734..6,976,088).

After karyotyping, three false-negative results were observed: one 69,XXX (initially interpreted as a normal, disomic female) and two 69,XXY (initially considered as uninterpretable by the BoBs assay).

Considering the 150 confirmed abnormal results (Table 2), a common aneuploidy in a mosaic ($n=3$) or homogeneous ($n=124$) form was identified in 127 cases (rate 7.9%, ~1 in 13): 76 cases with trisomy 21, 21 with trisomy 18, 7 with trisomy 13, 16 with monosomy X, 4 with other sex chromosome aneuploidies and 3 with complex aneuploidies.

The remaining 23 abnormal results involved the nine investigated microdeletion critical regions. In 12 cases, the abnormality was detected because of the regions covered by the Prenatal BoBs™ and was due to the presence of uncommon chromosome aneuploidies ($n=5$; 2 specimens with trisomy 22, 2 with trisomy 15 and 1 involving trisomy 7 in a mosaic form) and abnormalities caused by a cytogenetically visible unbalanced rearrangement ($n=7$). In particular, we observed a dup(22)(q11.2) because of the presence of a supernumerary marker chromosome associated with cat-eye syndrome in a mosaic form; a del(22)(q11.2) because of the presence of a derivative of a reciprocal 46,XX,+der(13)t(13;22)(q13;q12)mat,–22 translocation; three cases with deletion of the 5p15 region because of the presence of a derivative of a reciprocal der(5)t(1;5)(q41;p14.3) translocation; a mos46,XY,del(5)(p13)[14]/46,XY[11] and a 46,XX,del(5)(p13); one case with duplication of the 5p15 region because of the presence of a complex chromosome rearrangement (46,XY,add(5)(p15.3).ish der(5)(5p13.1–>5p15.3::5p15.3–>5qter) and, lastly, a case with a homogeneous 45,XY,der(13)rob(13;21)t(21,?)(q22;?) karyotype on direct CVS that was not characterized because of MCC in the sample culture.

Five of these 139 results with a cytogenetically visible abnormality were in a mosaic form: the cat-eye syndrome supernumerary marker chromosome in an AF sample (mos47,XX,+mar[1]/46,XX[29]: ~3.3% of abnormal cells), the case with trisomy 7 with a 47,XY,+7 cytotrophoblast and a 46, XY mesenchyme (estimated mosaic level: 50%), one case of trisomy 21 in an AF sample (mos47,XY,+21[20]/46,XY[20]; estimated mosaic level: ~50%), a mos45,X/46,XX karyotype

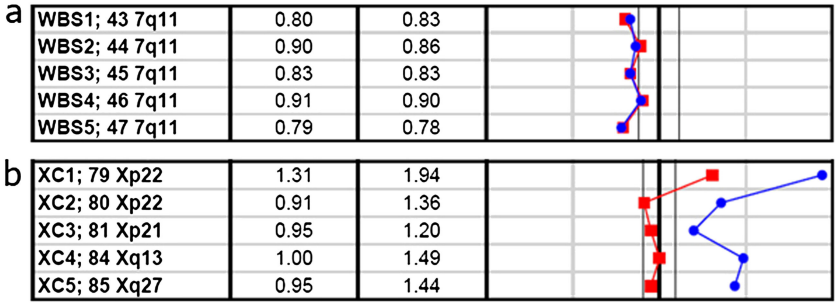

Figure 2 A Prenatal BoBs™ profile of the three false positive cases: (a) a borderline profile just outside the normal expected range of the region 7q11.2, resembling a mosaic haploinsufficiency; (b) duplication of the Xp22 region

with a 45,X cytotrophoblast and a 46,XX mesenchyme (estimated mosaic level: 50%) and a mos46,XY,del(5)(p13)[14]/46,XY[11] (56% of abnormal cells).

Lastly, 11 submicroscopic copy number losses and gains were detected by Prenatal BoBs™ assay, thus giving a detection yield for cryptic imbalances of 1 in 145 (11 out of 1599, 0.7%).

The 11 cases with a copy number loss or gain were as follows:

- Six 22q11.2 deletions: in four of these cases, the fetuses showed the presence of the complex or typical conotruncal cardiac defect frequently associated with Di George syndrome and therefore would have been diagnosed by applying a specific FISH analysis (referred to here as 'targeted FISH'). In one of these four cases, an unbalanced translocation 46,XX,+der(14)t(14;22)(q11;q12)mat,–22 was identified. In the fifth case, the mother carried the same deletion and had Di George syndrome. In the last case, an abnormal MSS with an increased risk for Down syndrome was the indication. All six pregnancies were terminated. The detection rate for the 22q11.2 deletion in our population was 1 in 267 (6 out of 1599; ~0.4%); two of these were inherited.
- One case with a 7q11.23 microdeletion associated with Williams Beuren syndrome: the case presented at 27 weeks of gestation with growth restriction, an interhemispheric cyst and nasal bone hypoplasia. The pregnancy was terminated.[13]
- A duplication of the 17p11 region associated with Potocki Lupski syndrome, with no ultrasound fetal abnormalities (only reduced movements and wrinkled hands) at 25 weeks of gestation.
- One case of 17p13 duplication found in a miscarriage at 14 weeks of gestation, with multiple abnormalities.
- One case of Xp22 deletion in a female fetus with increased nuchal translucency at 12 wg: the FISH analysis showed an interstitial heterozygous deletion of the sequence tagged sites only (ish del(X)(p22p22)(STS-,KAL+,DXZ1+). The mother carried the same deletion. During genetic counseling, she mentioned that her 2-year-old son had skin disturbances and excessive dryness. She also had two maternal male first cousins with ichthyosis. The pregnancy was continued.
- Two cases with duplication of the 22q11.2 region and no abnormal ultrasound findings. The copy number gains were inherited from healthy fathers in both cases. In one case, the indication was isolated diaphragmatic hernia in a previous pregnancy. Both pregnancies were continued, the deliveries were uneventful and the newborns were apparently normal.

Excluding the five cases with a 22q11.2 deletion that could have been detected by FISH analysis (based on clinical suspicion), Prenatal BoBs™ revealed a total of six microdeletions and microduplications that would not have been detected by conventional cytogenetic techniques or molecular cytogenetic assays. The estimated additional diagnostic yield of the Prenatal BoBs™ assay (in combination with conventional cytogenetic analysis) was 1 in 267 (6 out of 1599; ~0.4%).

Five of these six cases were pregnancies at a low risk of submicroscopic imbalances, because they presented with indication of increased MSS/AMA/maternal anxiety ($n=3$) or ultrasound findings without an evident clinical suspicion (with either isolated, increased nuchal translucency or one soft marker, combined with intrauterine growth retardation) ($n=2$). Given that about two thirds of the present population had a low-risk indication for prenatal diagnosis, the specific detection rate for Prenatal BoBs™ in unsuspicious cases is estimated at ~1 in 250.

DISCUSSION

Here, we report on a one-year prospective experience of the Prenatal BoBs™ assay in five European prenatal diagnosis laboratories from three different countries (Italy, France, and Spain), following clinical validation.[11] In agreement with previous results,[7] the failure rate in the present survey was 3.3%; this was mainly due to the low amount of DNA (<3 ng/μl) and/or DNA degradation ruling out interpretable results or preventing efficient DNA amplification during the labeling step. It is noteworthy that technical issues were also initially related to tube cap problems that caused samples to dry out.

In all five laboratories, Prenatal BoBs™ was applied to DNA from uncultured samples in parallel with karyotyping (instead of QF-PCR or rapid FISH for the same purpose).

The additional detection rate of Prenatal BoBs™ (combined with conventional karyotyping) was found to be 1 in 145, including all 11 detected microdeletions and microduplications. When considering only low-risk pregnancies, this value fell to 1 in 250. In these cases, a prenatal 'genotype-first' approach enabled the detection of dominant and fully penetrant submicroscopic copy number aberrations long before discriminatory prenatal signs were visible on detailed ultrasound. As expected, the del(22)(q11.2) associated with Di George syndrome was the most frequently detected submicroscopic abnormality (six cases; incidence: 1 in 267).

Because of the system's ability to analyze up to 44 samples per session, a consistent decrease in the hands-on time (relative to FISH on uncultured amniocytes) was observed, as we previously hypothesized.[11] The same decrease was not observed with respect to QF-PCR. However, a comparable turnaround time was observed in a laboratory setting with two experiments per week – even though QF-PCR was slightly faster than Prenatal BoBs™ (24 h and 36 h from receipt of the samples, respectively). When combined with the additional information provided by Prenatal BoBs™ on copy number losses and gains associated with high rates of neonatal morbidity and/or mortality, this aspect may support the diagnostic use and cost-effectiveness of the BoBs™ assay. This evaluation is much more straightforward in diagnostic settings where QF-PCR is applied as a stand-alone analysis in pregnancies with an elevated risk of trisomy 21 and no ultrasound abnormalities indicative of a chromosomal abnormality that, however, are at risk of submicroscopic copy number aberrations.[2] In our survey, Prenatal BoBs™ was able to detect eight abnormal cases that would have been missed by a stand-alone QF-PCR approach and thus provided an additional diagnostic yield of ~1 in 250 (8 out of 1599).

A diagnostic approach based on the investigation of 23 chromosome regions with an expanded panel of multiplex ligation-dependent probe amplification (MLPA) kits (in combination with conventional karyotyping) has recently been described. It confirmed an incidence of ~1 in 240 of submicroscopic CNVs in pregnancies with an indication of AMA/anxiety and increased MSS.[14] However, because of high sensitivity to DNA fragmentation (which gives noisy MLPA results in AF samples), the MLPA assay was shown to be highly reliable on DNA extracted from cultured amniocytes. Together with the absence of European conformity mark for *in vitro* diagnostics certification for the use of MLPA for microdeletions with reduced penetrance and complementary microduplications with unpredictable clinical significance, this finding might limit the application of this assay as a diagnostic tool.

In the present study, the overall abnormality detection rate is 1 in 10 with more than 40% had an invasive procedure for ultrasound findings. As expected, the diagnostic yield was higher in CVS (1 in 9) than in AF samples (1 in 13). Together with the technique's robustness and versatility, these findings suggest that the technology could be introduced in developing countries where few cytogenetic laboratories are present, giving patients access to aneuploidy diagnosis and enabling detection of the most frequent submicroscopic copy number aberrations.

Previous observations regarding the performance of the Prenatal BoBs™ technology were confirmed: (i) chromosome mosaicism can be detected at trisomy rate of 20–30%; (ii) triploidies and tetraploidies cannot be unequivocally diagnosed by Prenatal BoBs™ (in contrast to the situation with QF-PCR or MLPA) but will always be detected when combined with karyotype analysis (false negative incidence <1%).[11] In contrast to previous retrospective studies, three false positive cases (incidence <1%, all found in CVS) were observed. Two of them showed a borderline trend for clones mapping in the Williams Beuren syndrome and resembling a mosaic haploinsufficiency condition. The third case regarded a single clone deviation in Xp22 that, surprisingly, was not confirmed by FISH probe analysis on nuclei when present as a copy number gain but was detected as a copy number loss in another case. These findings emphasize the need to confirm these particular patterns, as we have already suggested.[11] As a general rule, the use of high-quality laboratory procedures (Cytogenetic Guidelines and Quality Assurance, EQA Permanent Working Group for Cytogenetics and Society) is important in cases that give abnormal or ambiguous results (mostly in CVS), because they might denote the presence of a placental discrepancy or a clonal CNV restricted to placenta. These deviations could also be due to the presence of contaminated DNA (i.e. with RNA or inhibiting factors).

The Prenatal BoBs™ assay was initially designed to screen for microdeletion syndromes. However, as we previously anticipated, the technology can also be used to identify microduplication. If software-based filtration of uncertain results is not applied,[12] the 15q11.2 and 22q11.2 microduplications can be detected. The latter are associated with a susceptibility to autism and to an interfamilial and intrafamilial phenotype variability ranging from normality to mental retardation /congenital abnormalities, respectively.[15,16] However, a recent, comprehensive study on the CNV morbidity map for developmental delay reported full (value = 1.00) or nearly full (value = 0.91) penetrance for 15q11.2 and 22q11.2 duplications, respectively – suggesting that these duplications may predispose to mental retardation or are pathogenic.[17] In the series reported here, four microduplications were identified. Although no 15q11.2 duplications were identified, two 22q11.2 duplications were diagnosed. Both were inherited from an unaffected father and were not associated with abnormal ultrasound findings. The pregnancies were continued and two healthy children were born.

Considering all the investigated loci, 22q11.2 stands out as the most frequently rearranged region; 10 of the 18 imbalances involved this locus (or 8 out of 11 when considering only abnormalities that could not be detected by conventional cytogenetics). These data agree with the literature and the diagnostic relevance of the 22q11.2 region and its proneness to genomic rearrangements.[18] However, for dup22q11.2, additional information on clinical correlations from large series of postnatal and prenatal cases are necessary to define a strategy for prenatal investigation and the counseling. Further use of Prenatal BoBs™ would probably help to clarify these points.

In conclusion, the Prenatal BoBs™ assay combines a short turnaround time (typical of rapid aneuploidy detection tests) with valuable detection of the most frequent microdeletion syndromes that cannot be detected in cytogenetic analyses. It has a favorable ratio of CNVs of uncertain significance to known, disease-causing CNVs in apparently low-risk pregnancies. In our experience, Prenatal BoBs™ should nevertheless always be combined with conventional karyotyping – especially for CVS, where the false positive and false negative rates related to placental mosaicisms are non-negligible and the combination of the cytotrophoblast with the mesenchyme cytogenetic results can provide a specific risk of confirmation of the chromosome aberration in the fetus.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- BACs on Beads assay, a new cytogenetic approach, has been recently validated on 3 retrospective works. No prospective studies have been conducted to evaluate its performance and additional diagnostic yield in combination with karyotyping compared with other molecular and cytogenetics assays.

WHAT DOES THIS STUDY ADD?

- We reported a multicentric prospective study on Prenatal BACs on beads assay. Our results indicate that, in addition to the recurrent aneuploidies generally detected by current rapid aneuploidy detection tests, Prenatal BACs on beads assay allows an additional detection rate for the most frequent microdeletion syndromes of ~1/250 in low-risk pregnancies.

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