Prevalence of recurrent pathogenic microdeletions and microduplications in over 9500 pregnancies

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9CGC Genetics, Laboratory of Cytogenetics, Madrid, Spain
10University Federico II, Department of Molecular Medicine and Medical Biotechnology, Naples, Italy
11Hospital General de México Eduardo Liceaga-Facultada de Medicina UNAM, NanoLab, Mexico, Mexico
12UPCG, UVSQ, Versailles, France
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ABSTRACT

Objectives The implementation of chromosomal microarray analysis (CMA) in prenatal testing for all patients has not achieved a consensus. Technical alternatives such as Prenatal BACs-on-Beads™ (PNBoBs™) have thus been applied. The aim of this study was to provide the frequencies of the submicroscopic defects detectable by PNBoBs™ under different prenatal indications.

Methods A total of 9648 prenatal samples were prospectively analyzed by karyotyping plus PNBoBs™ and classified by prenatal indication. The frequencies of the genomic defects and their 95%CIs were calculated for each indication.

Results The overall incidence of cryptic imbalances was 0.7%. The majority involved the DiGeorge syndrome critical region (DGS). The additional diagnostic yield of PNBoBs™ in the population with a low a priori risk was 1/298. The prevalences of DGS microdeletion and microduplication in the low-risk population were 1/992 and 1/850, respectively.

Conclusions The constant a priori risk for common pathogenic cryptic imbalances detected by this technology is estimated to be ~0.3%. A prevalence higher than that previously estimated was found for the 22q11.2 microdeletion. Their frequencies were independent of maternal age. These data have implications for cell-free DNA screening tests design and justify prenatal screening for 22q11 deletion, as early recognition of DGS improves its prognosis. © 2015 John Wiley & Sons, Ltd.

INTRODUCTION

Recent technological advances such as chromosomal microarray analysis (CMA) have expanded the range of genomic defects that are possible to detect with a single assay of invasively collected fetal cells. The added benefit of a diagnosis of a plausible cause for ultrasound-identified fetal defects has been reported to occur in 6.1% to 13.3% of fetuses with normal karyotypes. Although with a smaller frequency (1 to 2%), such added benefits have also been reported in fetuses with normal karyotypes for whom the indication was a factor other than abnormal ultrasound anatomy (e.g. advanced maternal age, maternal anxiety and serum screening high-risk reports). Such results led to the, currently consensual, recommendation of the implementation of this technology, in...
the clinical prenatal testing realm, upon the observation of fetal defects in ultrasound exams. It should even probably be offered to all patients undergoing invasive procedures. However some arguments are sometimes invoked against such an expansion, such as counseling challenges created by the identification of so-called Variants of Unknown Significance (VOUS) or of anomalies with variable expressivity and heterogeneity of clinical features with poorly quantifiable chance of an abnormal phenotype if found in prenatal settings. However, most likely, the main argument against this expansion may be related to the costs involved, especially when weighed against what some may perceive as a relatively smaller benefit for the ‘lower risk’ indications and in settings where healthcare is mainly funded by government.

Lower cost, albeit less comprehensive but also less challenging, technical alternatives have thus been proposed, in the past, by cost-conscious providers and payers, as ‘add-on tests’ to be offered to women with the ‘lower risk’ type of indication (non-ultrasound fetal anomaly-based).

Still, there are some proponents of offering these ‘low-cost’ tests even for ‘high-risk’ indications in settings of limited resources.

A technique proposed, in a recent past, as such an alternative, has already been clinically validated as a screening tool,7,8 has been CE-IVD-marked and is a currently commercially available test, marketed under the registered trademark name of Prenatal BACs-on-Beads™ (PNBoBs™). This is a multi-probe hybridization assay that quantifies the relative dosage of whole chromosomes (21, 18, 13, X and Y) and nine genomic critical regions (CR) that, as per the knowledge available at the time, were selected by the developers because they were associated with recurrent, dominant, relatively well-characterized microdeletion syndromes, each perceived as having reasonably well-known genotype-phenotype correlations and associated with relatively smaller challenges in terms of genetic counseling.9

We previously reported a 1-year prospective experience with this technique, collected from five different European laboratories.8

The broad availability of this technology led to its adoption by an increasing number of laboratories and to the resulting increase in the size of the data generated. Because we think that there is value in this enlargement of the data, we decided to report the, now, 3-year experience of the PNBoBs™ assay in 12 different laboratories located worldwide. We intend to provide more accurate predictions of the submicroscopic defects detectable by this technology under different indications. This, in association with data reported elsewhere about other technologies, will support clinical utility assessment, policy and/or patient decision-making. Although above the objectives of this report, these data will also permit the quantitation of the predicted added benefits of each strategy over others and weigh those benefits against the respective accrued costs. In addition, we believe that the results of this study will also support the prediction of the potential added benefits of expanding the current targets of cell-free maternal plasma DNA testing to any of the targets of PNBoBs™.

MATERIALS AND METHODS
This is a retrospective descriptive study of an anonymized cohort. The data was anonymized and analyzed only after all tests considered clinically relevant, including follow-up paternal exams, had been performed. IRB approval was obtained at the institution where the anonymized data analysis took place (TOMA laboratory #IRB protocol 0000006).

Sample description
Chorionic villous (CVS) and amniotic fluid (AF) samples collected for fetal karyotype analysis that were received from May 15th, 2010 to December 31st, 2013 in several participating laboratories were prospectively analyzed as described below and included in the study. We also included fetal or chorionic villi biopsies collected from miscarriages and intrauterine fetal death cases and sent for karyotyping during the same period. Only 17% of these samples were analyzed in the previously published report.7,8 All patients gave consent for the analysis.

Participant laboratories in this study included both private (TOMA Laboratory, United Medix Laboratories Ltd., Iovimics and CGC Genetics) and government-managed centers (CHI Poissy St Germain-Poissy, Jean Verdier Hospital-Paris, University Federico II-Naples, Ospedale San Pietro Fatebenefratelli, CHU de Clermont Ferrand, Chinese University of Hong Kong, University of Medicine Pomeranian, and Hospital General de México).

Sample classification
The samples were first classified by indication for prenatal diagnosis as ultrasound-related (US), advanced maternal age (AMA), increased risk for Down syndrome as per maternal serum screening (IMSS-DS), maternal anxiety (MA), miscarriage/intrauterine fetal death (MS), previous fetus/child with aneuploidy (PFA), parent carrier of a chromosome abnormality (PCCA) and other indications (Other), which include increased risk for monogenic Mendelian inheritance disorders, risk for fetal viral infection or pregnancies initiated by artificial reproductive techniques. For some samples, the indication was not provided (Unknown). Patients younger than 35 years of age with no known additional risk factors were classified as MA, whereas AMA referred to women ≥35 years. IMSS-DS included patients both <35 years and ≥35 years who had a refinement of her age-related risk by maternal serum and/or ultrasound screening techniques. The ultrasound-related group was subdivided into low risk for submicroscopic copy number abnormalities (US-LR), which included nuchal translucency <4.5 mm, kidney abnormalities, cord defects, isolated arm and leg defects, bowel abnormalities, hypoplastic nasal bone and alterations of amniotic fluid volume; all remaining ultrasound findings were considered high risk (US-HR). There was also a small number of samples in which the actual fetal defect was not reported. These samples were classified as US-Unknown (US-Unk).

Secondarily, each indication was further classified into two major groups: those with a high a priori risk for submicroscopic copy number anomalies (PCCA, MS and US-HR) and those with a low a priori risk for submicroscopic copy number abnormalities (AMA, IMSS-DS, MA, PFA, US-LR and Other). The samples classified as US-Unk or Unknown were excluded from these two major groups.
Laboratory procedures

Conventional karyotyping and PNBoBs™ (PerkinElmer Wallac, Turku, Finland) analyses were performed in all samples. For all cases in which a microdeletion or microduplication was identified by PNBoBs™, FISH analysis was performed to confirm the diagnosis and assess the cytogenetic mechanism generating the imbalance. When parents were available, the inheritance of the submicroscopic imbalance was investigated with the same method.

DNA extraction for PNBoBs™ was performed using an automated system in two of the labs (MagnaPure, Roche Applied Science, Indianapolis, IN, USA; Prepito, Perkin, Wallac, Turku, Finland) or manually (QIAamp DNA Mini Kit, Qiagen, Inc., Chatsworth, CA, USA) in the other 10 laboratories.7,8

The Prenatal BoBs™ technology and sample analysis were previously described.7–10

During the recruitment period, the PNBoBs™ protocol was subjected to improvements: at the beginning of the study in 2010, each sample was analyzed in duplicate; from 2012 onwards, the manufacturer licensed a certified variation of the protocol with the sample analysis performed in singlicate and, consequently, an increased number of beads counted per well.

Data analysis

The frequencies of several types of genomic defects were calculated for each class of samples. Ninety-five percent confidence intervals were calculated for each ratio using the Wilson score method without continuity correction.11 The statistical significance of comparisons between ratios was demonstrated by the absence of overlap between the 95%CI of the odds ratio with 1.

RESULTS

Over the period of the study, a total of 9648 diagnostic samples were enrolled (Table 1). The indication for prenatal diagnosis was reported by the source laboratory in 8959 out of 9648 cases (92.86%), and results (karyotyping and PNBoBs™) were obtained from 9327 of the 9648 enrolled samples (96.7%). No result (PNBoBs™) was obtained in 321 cases (3.3%).

The indications for prenatal diagnosis in the 9648 received samples are presented in Figure 1 and Table 2. Based on our risk level classification, 64% of the received samples (6146/9648) were considered to have a low a priori risk for submicroscopic copy number abnormalities (AMA, IMSS-DS, MA, PFA, US-LR and other indications), 25% (2407/9648) were considered to be at high risk (US-HR, MS, PCCA) and, for 11% (1095/9648) of the received samples, insufficient information was provided for their classification.

The absolute frequency of false negatives for the targeted chromosomal aneuploidies covered by the assay was 0.13% (12/9327; 95%CI: 0.07–0.22). They were identified by the concurrent karyotype and were mainly attributable to low-level mosaics (mostly on CVS); at the time of paper submission (~1 year after the recruitment closure), we have not received any report of false-negative cases for the targeted microdeletions/duplications, although, to our knowledge, no further testing was performed on the newborns. The absolute frequency of false positives was 0.19% (18/9327; 95%CI: 0.12–0.30); all of these corresponded to submicroscopic anomalies identified by PNBoBs™ that were not confirmed by FISH (Table 3).

Overall, 9.2% of all successfully processed samples (855/9327) were determined to have one defect (Tables 2 and 3 and Figure 2a). Chromosome abnormality frequency by indication ranged from 18.8% (360/1922; 95%CI: 17.1–20.6) in the US-HR group to 1.2% (9/771; 95%CI: 0.6–2.2) in the MA group.

Of the 855 samples that were reported as abnormal, 68 were found to have clinically relevant autosomal submicroscopic copy gains and losses (Table 2 and Figure 2b). Of the 68 clinically relevant identified anomalies, 66 (66/101, 65.3%) corresponded

![Figure 1](image)

**Table 1** Prospective analysis: characteristics of the cohort

<table>
<thead>
<tr>
<th>Samples</th>
<th>Direct AF</th>
<th>CVS</th>
<th>Other</th>
<th>Total</th>
<th>Total enrolled (n)</th>
<th>Failed samples (n)</th>
<th>Total analyzed (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average gestational week</td>
<td>21 + 5</td>
<td>13 + 0</td>
<td>20 + 5</td>
<td>/</td>
<td>5010</td>
<td>161</td>
<td>4849</td>
</tr>
<tr>
<td>Failed samples</td>
<td>3.21</td>
<td>3.24</td>
<td>5.05</td>
<td>3.33</td>
<td>9327</td>
<td>321</td>
<td>9648</td>
</tr>
<tr>
<td>Total</td>
<td>4.31</td>
<td>3.25</td>
<td>5.08</td>
<td>3.33</td>
<td>9327</td>
<td>321</td>
<td>9648</td>
</tr>
</tbody>
</table>

AF, amniotic fluid; CVS, chorionic villous sample

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to one of the nine targeted types of imbalance identified by the PNBoBs assay as per their standard analyses. The other two cases had a partial deletion involving one or two consecutive beads of chromosome 21 (Table 4). As previously suggested,7 the case with only one deflected bead was re-analyzed by CMA, while not enough quality of DNA was available to re-ex to CMA from the second case. This corresponded to a fetus with an ultrasound diagnosis of holoprosencephaly and IUGR. CMA, performed in combination with a parental study, detected the presence of the following deletion: arr[hg19] 21q22.2q22.3 (40,510,386–48,090,317)x1 dn. The deletion comprised part of the Holoprosencephaly 1 critical region (%236 100), whose candidate genes are TRAPPC10 (602103) and PWP2 (601475), both located in the deleted tract.

Table 2
Overall results of the prospective study on 9648 prenatal samples stratified by indication for prenatal diagnosis

<table>
<thead>
<tr>
<th>Indication for prenatal diagnosis</th>
<th>Enrolled cases (n)</th>
<th>Analyzed cases (n)</th>
<th>Overall abnormal results (aneuploidies + partial unbalances)</th>
<th>Total partial unbalances detectable by karyotype (n)</th>
<th>oriented FISH (n)</th>
<th>PNBoBs only (n)</th>
<th>%b (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US-LR</td>
<td>1062</td>
<td>1012</td>
<td>10.9 (9.1–12.9)</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>0.69 (0.34–1.42)</td>
</tr>
<tr>
<td>US-HR</td>
<td>2002</td>
<td>1922</td>
<td>18.7 (13.1–20.6)</td>
<td>8</td>
<td>21</td>
<td>16</td>
<td>0.83 (0.51–1.35)</td>
</tr>
<tr>
<td>US-Unk</td>
<td>406</td>
<td>381</td>
<td>16.5 (13.1–20.6)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.52 (0.14–1.89)</td>
</tr>
<tr>
<td>IMSSDS</td>
<td>2142</td>
<td>2054</td>
<td>7.4 (6.4–8.7)</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>0.29 (0.13–0.64)</td>
</tr>
<tr>
<td>AMA</td>
<td>1683</td>
<td>1667</td>
<td>3.5 (2.8–4.5)</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0.18 (0.06–0.53)</td>
</tr>
<tr>
<td>MA</td>
<td>780</td>
<td>771</td>
<td>1.2 (0.6–2.2)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.13 (0.02–0.73)</td>
</tr>
<tr>
<td>MS</td>
<td>318</td>
<td>304</td>
<td>11.5 (8.4–15.6)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>1.64 (0.7–3.79)</td>
</tr>
<tr>
<td>PFA</td>
<td>239</td>
<td>226</td>
<td>2.2 (0.9–5.1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.00 (0.00–1.67)</td>
</tr>
<tr>
<td>PCCA</td>
<td>87</td>
<td>85</td>
<td>7.1 (3.3–14.6)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0.00 (0.00–4.32)</td>
</tr>
<tr>
<td>Other</td>
<td>240</td>
<td>223</td>
<td>6.7 (4.1–10.8)</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>1.35 (0.46–3.88)</td>
</tr>
<tr>
<td>Unknown</td>
<td>689</td>
<td>682</td>
<td>5.9 (4.3–7.9)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.15 (0.03–0.83)</td>
</tr>
<tr>
<td>Total number of cases</td>
<td>9648</td>
<td>9327</td>
<td>9.2 (8.6–9.8)</td>
<td>25</td>
<td>24</td>
<td>44</td>
<td>0.47 (0.35–0.63)</td>
</tr>
</tbody>
</table>

US-LR, Low Risk Ultrasound abnormality; US-HR, Low Risk Ultrasound abnormality; US-Unk, Unspecified ultrasound abnormality; IMSSDS, increased maternal serum screening for Down syndrome; AMA, advanced maternal age; MA, maternal anxiety; MS, miscarriage; PFA, previous fetus/child with aneuploidy; PCCA, parent carrier of a chromosome abnormality; Other, other indications; PNBoBs, Prenatal BoBs

a% of total analyzed.
b% of abnormal for each indication.

Table 3
Overall results of the prospective analyzed cohort

<table>
<thead>
<tr>
<th>Samples</th>
<th>Direct AF</th>
<th>CVS</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total analyzed</td>
<td>4849</td>
<td>3970</td>
<td>508</td>
<td>9327</td>
</tr>
<tr>
<td>Normal results</td>
<td>4498</td>
<td>3524</td>
<td>449</td>
<td>8471</td>
</tr>
<tr>
<td>Abnormal results</td>
<td>351</td>
<td>445</td>
<td>59</td>
<td>855</td>
</tr>
<tr>
<td>%a</td>
<td>7.24</td>
<td>11.2</td>
<td>11.6</td>
<td>9.17</td>
</tr>
<tr>
<td>False positive</td>
<td>11</td>
<td>4</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>%b</td>
<td>0.23</td>
<td>0.10</td>
<td>0.59</td>
<td>0.19</td>
</tr>
<tr>
<td>False negative</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>%c</td>
<td>0.02</td>
<td>0.28</td>
<td>0.22</td>
<td>0.14</td>
</tr>
</tbody>
</table>

AF, amniotic fluid; CVS, chorionic villous sample
% of total analyzed of the same column.
remaining five abnormal reports were cases of intrauterine fetal death (Table 2 and Figure 2c).

Another relevant finding is the absence of statistically significant differences between the lowest-risk indications—MA, AMA and IMSS-DS.

The overall incidence of autosomal microdeletions/duplications in our cohort was 0.7% (66/9327; 95%CI: 0.6–0.9). The majority (47/66; 71.2%) involved the DiGeorge syndrome critical region (DGS-CR; deletions: n = 32 (0.3%; 95%CI: 0.2–0.5); duplications; n = 15 (0.2; 95%CI: 0.1–0.3)) (Table 4). PCCA and US-HR seem to be the main risk factors for this defect (not common in MS, the other high-risk group) (Figure 2d). The remaining cryptic imbalances showed an incidence of <1:1000.

In the low a priori risk population, 20 cryptic imbalances were detected (Table 2). The additional diagnostic yield of PNBoBs™ in this group of pregnancies was 0.3% (20/5953; 95%CI: 0.2–0.5). The imbalanced cryptic rearrangements in the low-risk population again mainly involved 22q11.2 (7 losses and 6 gains) but also the 15q11.2 (3 gains), 7q11.23 (2 losses), 4p16.3 (1 loss) and 17p11 critical regions (1 gain) (Table 4).

DISCUSSION

In assessing the frequency of the defects specifically detected by PNBoBs™ in several clinical settings an additional relevant question has to do with the added benefit/information provided by this technique when added as a routine test to current practices. That, of course, depends on the definition of ‘current practices’ because there is broad variation between countries and even individual clinical practices. We decided to select a few comparisons related to the current practices found among the countries contributing data to this study. We further decided to classify those comparisons into two main groups, depending on the indication for the invasive testing and the classification as high or low risk. This risk classification was based on the previously predicted risk for the additional genomic anomalies detectable by PNBoBs™, considering that any of the genomic defects (copy gains and losses) that can be identified by PNBoBs™ have clinical value, with the exception of 22q11 and 15q11 duplication syndromes, which have incomplete penetrance and variable expressivity.12

First, we wanted to assess the benefit of adding PNBoBs™ as a standard laboratory procedure to all high-risk invasive procedure indications, as per comparison with karyotype only. An overall incidence of 1.9% or 1 out 51 (45/2311; 95%CI: 1.5–2.6) was detected in samples classified as high risk. However, because it has been, for quite some time, standard practice to screen for 22q11 deletions in fetuses found to have cardiac defects, we wanted to determine the added benefit after...
### Table 4 Summary of the 66 partial imbalances involving the 9 targeted critical regions

<table>
<thead>
<tr>
<th>Indication for prenatal diagnosis</th>
<th>Analyzed cases (n)</th>
<th>Loss</th>
<th>Gain</th>
<th>Loss</th>
<th>Gain</th>
<th>Loss</th>
<th>Gain</th>
<th>Loss</th>
<th>Gain</th>
<th>Loss</th>
<th>Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(95%CI)</td>
<td></td>
<td>(95%CI)</td>
<td></td>
<td>(95%CI)</td>
<td></td>
<td>(95%CI)</td>
<td></td>
<td>(95%CI)</td>
<td></td>
</tr>
<tr>
<td><strong>22q11.21</strong></td>
<td><strong>304</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0</td>
</tr>
<tr>
<td><strong>15q11.2q12</strong></td>
<td><strong>15q11.2q12</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0</td>
</tr>
<tr>
<td><strong>7q11.23</strong></td>
<td><strong>4p16.3</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0</td>
</tr>
<tr>
<td><strong>17p13</strong></td>
<td><strong>17p11</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0</td>
</tr>
<tr>
<td><strong>US</strong></td>
<td><strong>Total HR</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0</td>
</tr>
<tr>
<td><strong>PCCA</strong></td>
<td><strong>IMSS-DS</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0.05</td>
<td>(0.01–0.28)</td>
<td>0</td>
</tr>
<tr>
<td><strong>US-HR</strong></td>
<td><strong>AMA</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
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<tr>
<td><strong>TOTAL LR</strong></td>
<td><strong>Others</strong></td>
<td>0.33</td>
<td>(0.06–1.84)</td>
<td>0.33</td>
<td>(0.06–1.84)</td>
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<td>0.05</td>
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<td><strong>Total LR</strong></td>
<td>0.33</td>
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excluding those fetuses found to have this copy loss. The number of PNBoBs™-only detectable defects was reduced to less than half – 21 cases – thus reducing the added benefit of PNBoBs™ in this population to 0.9% or 1/110 (95%CI: 0.6–1.4). Furthermore, current professional recommendations actually recommend CMA as a first- or second-tier test (after karyotype or RAD tests) in these cases.2,3 As practically all of the defects detectable by PNBoBs™ are also detectable by microarrays, the much smaller benefit conferred by PNBoBs™ would have to make its recommendation, in this high risk population, limited to settings without access to such technology, because of costs and challenging tech transfer.

We next wanted to know what would be the added benefit of performing PNBoBs™ on all invasively collected samples in our group of low-risk patients. Although there is an increasing tendency to recommend routine CMA also for this group of patients, given published reports of added detectable clinically relevant conditions of 1.7% or 1/60 (95%CI: 1.3–2.2),2,3 this recommendation is not as widely consensual as the previous one. Out of the 5953 successfully tested samples, 20 had a defect detected by PNBoBs™, corresponding to 0.3% or 1/298 (95%CI: 0.2–0.5). This is also much less than the predicted potential added benefit of CMA in this population (OR: 0.20, 95%CI: 0.12–0.34). Interestingly, analyzing the detailed data made available in the above referenced recent report,2 the frequency of common recurrent pathogenic CNVs in the comparable low-risk population (MA, AMA and IMSS-DS) appeared to be not statistically significantly different from that detected by PNBoBs™: 5 out of 3067, corresponding to 0.16% or 1/613 (95%CI: 0.12–0.34). Interestingly, the frequency of the submicroscopic defects targeted by PNBoBs™, estimated to be ~0.16% from the present study, was not significantly different among maternal ages less (MA) or greater than 35 (AMA), and there was no difference for women with positive screening for Down syndrome (IMSS-DS). These findings demonstrate that submicroscopic partial imbalances are not maternal age dependent and are not associated with maternal serum or ultrasound markers for DS.

Our main objective was, however, to assess the frequencies of the genomic defects identifiable by PNBoBs™ and to compare them with the estimates based on pediatric data. Because PNBoBs™ detect the targeted microdeletions and microduplications in a non-biased fashion and long before the appearance of a clearly suggestive phenotype, the method provides the opportunity to better understand the phenotypic spectrum.

Copy number gains and losses of the 22q11.2 critical region (CR) accounted for approximately 70% of the detected submicroscopic imbalances (47/66), with an incidence of 0.2% (13/5953; 95%CI: 0.1–0.4) in the low-risk population and of 1.4% (32/2311; 95%CI: 1–2) in the high-risk population, with more
deletions (25) than duplications (7) in this group. However, in the low-risk set of samples, there were as many deletions as duplications (6 deletions and 7 duplications). This corresponds to a frequency of 1/992 for the deletion in low-risk pregnancies (6/5953; 95%CI: 1/455–1/2164), not different from previously published microarray data (3/3067) but unexpectedly higher than previously estimated from postnatal series (~1:4000–1:6000).14–17 This discrepancy might be explained by the variable expressivity of this syndrome18 leading to the underestimation of its prevalence, likely because of its clinically based diagnosis still frequently relying on the presence of a cardiac defect. Interestingly, the incidence of 22q11 deletion in the intellectually disabled population has been reported to be 1:110,19 which is similar to its incidence in our high-risk cohort of 1:92 (95%CI: 1/63–1/136).20 Regarding the complementary 22q11.2 microduplication,3 it was less prevalent (~1:330; 7/2311; 95%CI: 1/160–1/681) than the deletion in the high-risk population. This also does not differ from its incidence in the intellectual disabled population (1/370–1/700).21,22 In the low-risk samples, the prevalence of the microduplication decreases to ~1:850 (7/5953; 95%CI: 1/412–1/1755), not differing from the prevalence of the reciprocal microdeletion of 1/992 found in the present study. Interestingly, these prevalences are also similar to that reported in the asymptomatic adult population (5/8329 = 1/1666, 95%CI: 1/3899–1/712).12

The 22q11 microdeletion occurs de novo in the majority of patients,18,20 while the 22q11 microduplication is, in the majority of cases, inherited from a clinically asymptomatic parent.21,23 This opposite behavior is likely attributable to clinical and technical biases of ascertainment, i.e. wide phenotypic variability of the 22q11 microduplication (with an absence of any clinical findings in the majority of the cases),22,23 22q11 microdeletion being investigated mainly in the presence of a suggestive cardiac defect and traditional FISH analysis having low resolution for tandem duplications. In the present study, because the dosage of the 22q11 region was investigated in an unbiased fashion in the low-risk samples, the prevalences of the 22q11 copy gain and loss were demonstrated to be quite similar, as expected by the molecular mechanism generating the two complementary imbalances.24

The second most frequently imbalanced CR is the duplication of 15q11.2q12: 1/1984 (95%CI: 1/675–1/5834) in the low-risk and 1/1156 (95%CI: 1/317–1/4213) in the high-risk samples (not significantly different). This suggests a relatively low penetrance for defects detectable by ultrasound for this microduplication; the highest penetrance is for symptoms not identifiable prenatally.12 The frequency in the intellectually disabled population is ~1/600.5

The prenatal phenotypes of Williams–Beuren syndrome and its reciprocal duplication have recently been described,25 but their prevalences were still unknown. In the present study, we found an incidence of 1:1156 (2/2311; 95%CI: 1/317–1/4213) for the microdeletion and of 1/2311 (95%CI: 1/409–1/13091) for the microduplication in the high-risk population. These values are lower than those reported in the developmental delay population, i.e. 1/375 (42/15 767; 95%CI: 1/253–1/557) and 1/985 (16/15 767; 95%CI: 1/523–1/1856), respectively, although not statistically significantly different.12 However, the rarity of the condition may cause a power problem for these comparisons. In the low-risk samples, only the duplication was found, with a frequency of 1:2977 (2/5953; 95%CI: 1/817–1/10 853), quite similar to the high-risk frequency.

Finally, Wolf Hirschhorn CR imbalances were identified in four cases, all microdeletions, for a frequency of ~1/770 (95%CI: 1/262–1/2265) in the high-risk and of 1/5953 (95%CI: 1/1052–1/33 723) in the low-risk samples.

DiGeorge II, Langer Giedion and Cri-du-Chat syndromes were not detected in our series, confirming their rarity. A design assay update should thus be considered, including regions found to be more common as per microarray studies and also associated with known recurrent microdeletion/duplication syndromes.2

Because PNBosSTM targets not only a selected number of submicroscopic genomic defects but also the common aneuploidies, another of the proposed uses for this test is as an alternative to QF-PCR or FISH for Rapid Aneuploidy Detection (RAD). PNBosSTM do indeed have advantages over those two techniques for that purpose. Because of its high sample throughput of 44 samples/run,1 there is a relevant decrease of hands-on time compared with FISH on uncultured amniocytes, although not in comparison with QF-PCR. The other advantage is the additional information on microdeletion syndrome CRs (above all 22q11 CR) provided by PNBosSTM.28 However, it is important to consider the disadvantages as well. There is a slightly higher test failure rate of 3.3% (vs 2.4% for rapid FISH and 1.3% for QF-PCR)7,8 and a slightly higher frequency of false negative (FN) and false positive (FP) results of 0.13% and 0.19%, respectively (QF-PCR FN: 0.003% for autosomes and 0.02% for sex chromosomes; rapid FISH FN: 0.06%; QF-PCR FP: none reported; rapid FISH FP: 0.01%).27,28

The present study, providing the frequencies of some of the most common pathogenic copy number variations in a low-risk prenatal population, can have important practical implications for cfDNA testing assay design development. For instance the maternal age independent ~1/1000 frequency of 22q11.2 deletion may be considered high, this deletion is associated with significant morbidity, its broad clinical variability may delay early diagnosis and some 22q11 haploinsufficient infants may benefit from early therapeutic intervention. Prenatal detection of this condition by cfDNA testing assays and/or by tests on invasively collected products is thus likely to decrease morbidity and even neonatal mortality, with potential implications for the long term outcome of affected newborns.29–32

However, although still to be demonstrated by appropriately powered clinical prospective studies, the addition of smaller genomic unbalances to the panel of anomalies screened by cfDNA testing is likely to increase its false positive rate (FPR), probably differently for the several testing methods, with the consequent decrease in the positive predictive value (PPV). If such FPR increase is really demonstrated, it will jeopardize the biggest advantage of this type of screening technology. Because the Prenatal BoBsSTM design at 22q11.2 targets the smallest overlapping region of the typical and atypical deletions/ duplications, it can reliably detect >99% of the microdeletions causing DiGeorge and 22q11 duplication syndromes.33 Prenatal BoBsSTM CE-IVD marked can thus also be considered the most affordable and robust confirmatory rapid test after a high risk cfDNA test result for the 22q11.2 region copy gain/loss.
WHAT’S ALREADY KNOWN ABOUT THIS TOPIC?

• Common pathogenic microdeletion/microduplication syndromes detectable by PNB0sTM have an estimated prevalence of 1/250 in low-risk pregnancies.
• The discovery in the maternal circulation of cell-free placental DNA (cfDNA) revolutionized non-invasive prenatal screening for common aneuploidies. However, microdeletions/duplications may result in disabilities that can be more severe than certain aneuploidies. Consistent results about the prevalences of common pathogenic cryptic imbalances in low-risk populations are lacking.

WHAT DOES THIS STUDY ADD?

• The frequencies of submicroscopic defects (including 22q11.2 microdeletions and microduplications) under different prenatal indications, particularly in the low-risk prenatal population, are provided.
• The a priori risk for common pathogenic cryptic imbalances was estimated to be ~0.3%.
• The assumption that submicroscopic genomic imbalances are not maternal age dependent is demonstrated.
• Implications for the development of cfDNA-based screening are discussed.

REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web site.