CORRESPONDENCE

Author's reply to Grati and Benn

We thank Dr. Grati and Dr. Benn for their interest in our article.[1] We welcome the opportunity to provide clarification and further stimulate the discussion on this important topic.

The authors raised concerns regarding the design of our study that, in their opinion, precludes accurate determination of analytic sensitivity, because karyotyping from apparently normal live-borns has not been performed. The reason for their criticism is related to the fact that several chromosomal abnormalities, including sex chromosome aneuploidies, some segmental imbalances and low level autosomal mosaic aneuploidies are not necessarily apparent at newborn physical examination.

We may agree with Dr. Grati and Dr. Benn that, to confidently exclude the presence of chromosomal abnormalities, the ideal clinical follow-up should involve karyotyping results from the newborns, and this limitation was clearly recognized in our paper. However, such a study design is very difficult to finalize, because of the related cost and the high proportion of patients that would be lost to follow-up. Even if we cannot rule out that chromosomal abnormalities, such as very small chromosomal rearrangements or low-level mosaicism occurrences, may have remained unnoticed, the possibility of genome-wide analysis false results going undetected remain low.

Dr. Grati and Dr. Benn also raised concerns on the low number of abnormal pregnancies detected in our study cohort. In their opinion, these finding are suggestive of under-ascertainment of chromosomal abnormalities and, consequently, lower test sensitivity.

Specifically, they remarked upon the lower number of rare autosomal trisomies (RATs) identified in our study, as compared with that expected from chorionic villus specimens or that reported in another study evaluating expanded NIPT.[2]

However, the authors start from the assumption that the outcome data from chorionic villus sampling (CVS) positive cases should be the same for the cases ascertained by genome-wide cfDNA screening, which is not necessarily correct. This was properly pointed out by Prof. Bianchi in the 'open the kimono' commentary,[3] and may explain the difference in confined placental mosaicism (CPM) rate between our study and CVS data.

There are multiple reasons supporting the latter hypothesis. First of all, there is likely more complete ascertainment by cfDNA sequencing. In fact, cfDNA likely derives from all placenta sites and has the potential to provide a more accurate representation of placenta heterogeneity. On the contrary, the biopsy material represents only a small fraction of the placenta and may only give a 'snapshot' of one portion of the tissue. Secondly, CVS is only performed on pregnant women who are already at high risk for fetal aneuploidy, while cfDNA screening is being performed on women with both high and general obstetrical risks for aneuploidy.

In addition, the different number of RATs reported in our study could be explained by the method used to determine the fetal fraction (FF).[4] At the time of testing, we performed two independent FF measurements: the first, based on sequencing data from different genomic regions, is independent of the aneuploidy status of the fetus; the second, applied only in cases where an aneuploidy has been detected, measures the fraction of aneuploid cfDNA calculating the sequence counts of that specific aneuploid region. The comparison of the two different FF estimates (whole genome vs aneuploid chromosome) represents a useful tool to predict the mosaic status of the placenta.[5,6] In fact, in this case, the aneuploid fraction value will be markedly smaller than the whole genome estimate. In our study, the samples involving not concordant FF estimates were not scored as RAT and may represent the reason of the lower incidence of RATs as compared with other studies.[2]

Finally, the specific characteristics of the cohort of patients studied may also justify the above differences.

Dr. Grati and Dr. Benn also concerned on the low numbers of abnormal cases with small segmental imbalances identified in our study, suggesting an under-ascertainment of pregnancies with such kind of chromosomal abnormalities. Although the genome-wide cfDNA screening test described in our paper has demonstrated able to detect small chromosome segments, it was designed to identify only copy-number variations (CNVs) larger than 7 Mb (the typical level of resolution reported for G-banded karyotyping), to assure high analytical sensitivity and minimize interpretation challenges.

Smaller events were reported only when associated with a larger segmental deletion or duplication (e.g. Cases 2 and 3), or with a clinically relevant genomic region, limited to samples involving a high (>8%) FF (e.g. Case 6). In fact, testing for small clinically relevant CNV (~2–3 Mb) would require a much

increased sequencing depth in order to reliably detect such rearrangements at 2% FF level. Finally, similarly to standard cfDNA screening, the test is not designed to detect low level mosaicism occurrences or triploidy.

We noticed that, unfortunately, the authors may have missed the main message resulting from our study, that certainly was not to claim 100% sensitivity for genome-wide cfDNA screening nor that the test aims to replace invasive prenatal diagnosis. When performing conventional cfDNA screening by massively parallel sequencing (MPS) protocols, instead of wasting the majority of information achieved from sequencing by limiting the bioinformatic analysis to the common chromosomes, it could be very useful for the patients to extend screening including also RATs and structural chromosome anomalies. Such level of testing provides a significant higher detection yield compared to conventional cfDNA screening, improving overall pregnancy management. This can be achieved without extra sequencing costs, simply using data already obtained from routine NIPT for common fetal aneuploidies, and without sacrificing the specificity of the test, that resulted very similar to the standard screening.

We certainly agree with Dr. Grati and Dr. Benn that the most comprehensive information can only be obtained by microarray analysis of fetal DNA, especially for pregnancies with abnormal ultrasound findings, and that no cfDNA screening can replace invasive prenatal diagnostics. However, we should also take into consideration the willingness of patients to undergo invasive prenatal diagnosis. In our view, any decision should be rendered on a case-by-case basis by the pregnant woman, in close communication with her gynecologist, to truly enable personalized care and to respect her autonomy (ACOG).[6,7]

Dr. Grati and Dr. Benn expressed their legitimate difference of opinion on the clinical utility of genome-wide cfDNA screening. In contrast, there are a number of papers where the authors find clinically useful the detection of RATs or segmental imbalances.[2,6,8–11] Our view in this discussion is strongly on the side of using the extra information provided by genome-wide analysis, because performing screening only for common aneuploidies may substantially limit the potential of NIPT, since a significant proportion of CNVs that can cause serious disability are not detected. The most relevant point in relation to this argument relates to the 12 (7.4%) fetal conditions, potentially resulting in the birth of chromosomally abnormal babies, that would have remained overlooked if only a conventional cfDNA screening had been performed.

We appreciated that the authors recognized that genome-wide cfDNA analysis affords the opportunity to screen for serious genetic conditions and could potentially identify those women at high risk for pregnancy complications. This can be achieved without extra testing cost, simply analyzing already existing sequencing data, with the ultimate goal of improved prenatal screening and a lower risk of giving birth to a chromosomally abnormal neonate.

Further prospective studies will certainly further enhance understanding of the clinical utility of genome-wide screening. In this view, we will welcome the contribution from Dr. Grati and Dr. Benn, in performing a study with the optimal design they proposed.

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