

ARTICLE

Chromosomal microarray analysis as a first-line test in pregnancies with *a priori* low risk for the detection of submicroscopic chromosomal abnormalities

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In this study, we aimed to explore the utility of chromosomal microarray analysis (CMA) in groups of pregnancies with *a priori* low risk for detection of submicroscopic chromosome abnormalities, usually not considered an indication for testing, in order to assess whether CMA improves the detection rate of prenatal chromosomal aberrations. A total of 3000 prenatal samples were processed in parallel using both whole-genome CMA and conventional karyotyping. The indications for prenatal testing included: advanced maternal age, maternal serum screening test abnormality, abnormal ultrasound findings, known abnormal fetal karyotype, parental anxiety, family history of a genetic condition and cell culture failure. The use of CMA resulted in an increased detection rate regardless of the indication for analysis. This was evident in high risk groups (abnormal ultrasound findings and abnormal fetal karyotype), in which the percentage of detection was 5.8% (7/120), and also in low risk groups, such as advanced maternal age (6/1118, 0.5%), and parental anxiety (11/1674, 0.7%). A total of 24 (0.8%) fetal conditions would have remained undiagnosed if only a standard karyotype had been performed. Importantly, 17 (0.6%) of such findings would have otherwise been overlooked if CMA was offered only to high risk pregnancies. The results of this study suggest that more widespread CMA testing of fetuses would result in a higher detection of clinically relevant chromosome abnormalities, even in low risk pregnancies. Our findings provide substantial evidence for the introduction of CMA as a first-line diagnostic test for all pregnant women undergoing invasive prenatal testing, regardless of risk factors.

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INTRODUCTION

The widespread use of postnatal chromosomal microarray analysis (CMA) has shown submicroscopic rearrangements to represent a greater cause of genetic disorders than previously thought and they are currently associated with up to 15% of syndromic and nonsyndromic mental retardation.^{1–3}

Much of the increased detection rate of CMA can be explained by its potential to detect chromosome abnormalities at significantly higher resolution,^{4–7} as compared to conventional cytogenetic methods, which may detect chromosomal anomalies to an average resolution of only ~7–10 Mb in size. CMA allows detection of microscopic and submicroscopic copy number variants (CNVs), as small as 50–100 Kb, ~100 times smaller than the changes that can be identified by traditional karyotyping.^{7–10}

CMA is now recognized as the appropriate first-line test, in place of conventional karyotyping, for the clinical evaluation of postnatal patients with developmental delay/intellectual disability, multiple congenital anomalies and/or autism spectrum disorders.^{3,4,6,8,11–14} This change from conventional karyotyping to CMA in postnatal cytogenetics has generated an increasing interest in determining whether CMA will offer any advantages for the detection of fetal chromosomal abnormalities in prenatal diagnostics.

Although several previously reported retrospective and prospective studies^{15–24} have demonstrated the effectiveness and usefulness of CMA in clinical prenatal diagnosis, only limited conclusions could be drawn due to the small size of the cohorts analyzed. These studies have all provided reassuringly consistent results in terms of analytical validity, clinical validity and clinical utility of the technique applied in the prenatal diagnosis setting, compared to traditional karyotyping. However, their limited data has necessitated undertaking of large-scale prospective clinical trials, with parallel processing of samples for both CMA and conventional cytogenetic analysis, before any firm conclusions can be drawn to recommend the technique as a first-line test in all prenatal samples.²⁵

Recently, a large-scale prospective study²⁶ was carried out by our center, in order to assess the feasibility and clinical advantages of offering CMA as a first-line test in clinical prenatal diagnosis. This study demonstrated the usefulness and benefits of prenatal diagnosis as performed by whole-genome CMA for direct analysis of amniocytes or CVS tissues, without culturing cells. Our study indicated that the CMA approach was robust, with no false positive findings when followed up with different methodologies, or false negative findings when samples were tested in parallel with conventional karyotyping. In addition to our study, other similar

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large cohort prospective trials have been published, all reporting results concordant with our findings.^{27–30}

The above large-scale clinical trials, combined with previously reported small cohorts studies, have shown that the use of CMA in prenatal diagnosis produces a substantial improvement of ~1–3% in detection rate of genetic aberrations compared with conventional karyotyping, without missing potentially pathogenic chromosomal abnormalities and with no appreciable increase in results of unclear clinical significance. These findings provided evidence for the feasibility of introducing CMA into routine prenatal diagnosis practice, indicating that it could be already acceptable to apply CMA as a first-line diagnostic test, at least concurrently with conventional karyotyping.^{26,29} However, at present, it is not yet clear what indication of prenatal diagnosis could benefit from CMA. In a recent meta-analysis of prenatal samples,³¹ the additive value of CMA was reported to be 5.2% when applied to pregnancies with abnormal ultrasound findings. However, the fact that only a minority of the submicroscopic abnormalities that are detected in postnatal patients with developmental delay/intellectual disability are accompanied by congenital abnormalities detectable by prenatal ultrasound, suggests the use of CMA in prenatal diagnostics not only for pregnancies with ultrasound abnormalities.³²

In the absence of specific guidelines and large-scale studies for different categories of indications, it has been suggested that CMA should be offered as an adjunct tool to selected groups of high-risk pregnancies (eg abnormal ultrasound findings and a normal conventional karyotype), using the technique as a second-line test only, after a standard karyotype.²⁵ Recently, the Canadian College of Medical Geneticists (CCMG) has discouraged the use of CMA in pregnancies at low risk for a structural chromosomal abnormality (eg advanced maternal age, positive maternal serum screen, previous pregnancy with a chromosomal abnormality or the presence of 'soft markers' on fetal ultrasound), motivating their position with the fact that CMA performed for the above categories of pregnancies would likely be associated with a low positive predictive value, since the vast majority of fetuses in these situations would be clinically unaffected.³³

In this study, we aimed to explore the usefulness of CMA in groups of pregnancies with *a priori* low risk for the detection of submicroscopic chromosome abnormalities, usually not considered an indication for testing, in order to assess if CMA improves the prenatal detection rate of chromosomal aberrations.

MATERIALS AND METHODS

Experimental design and clinical samples

A large prospective trial was designed, involving analysis of 3000 prenatal samples and processed in parallel for both CMA and conventional cytogenetic analysis. CMA was offered as an option to couples considering an invasive prenatal genetic testing procedure, in addition to conventional karyotyping. Prenatal samples, inclusive of a subset of 1037 cases previously reported,²⁶ were sent to a central karyotyping lab between October 2010 and March 2012 from 167 healthcare providers in Italy. Specimen types submitted included 2650 (88.3%) amniotic fluid, 308 (10.3%) chorionic villus sampling (CVS), 32 (1.1%) cultured amniocytes and 10 (0.3%) DNA extracted by other labs from uncultured amniocytes.

Patients underwent pre-test counseling as described elsewhere,³⁴ during which the issues that are encountered with CMA were discussed. The couples who accepted evaluation by CMA signed an informed consent form containing a summary of the testing process, potential benefits and limitations of testing, and possible testing outcomes. A post-test genetic counseling session was provided in all cases when a genetic alteration was detected by any method. The study was approved by the Institutional Review Board of GENOMA laboratory.

Indications for prenatal diagnosis

The indications for invasive prenatal testing included: increased risk of fetal aneuploidy associated with advanced maternal age, that is 35 years or older at time of conception; parental anxiety, that is patients younger than 35 years with no specific indication for invasive prenatal diagnosis; abnormal results from maternal serum screening tests; abnormal ultrasound findings; a known abnormal fetal karyotype; family history of a genetic condition or chromosome abnormality; and cell culture failure.

CMA and data interpretation

Prenatal samples were processed according to the protocol reported elsewhere.²⁶ Briefly, DNA extracted from amniotic fluid, CVS and cultured amniocytes was first tested for gender determination of the fetus and maternal cell contamination. DNA samples were then processed by CMA using whole-genome bacterial artificial chromosome (BAC) microarrays—CytoChip Focus Constitutional (BlueGnome, Cambridge, UK), according to the manufacturer's protocol. The genomic coverage of this microarray platform is up to 1 Mb resolution across the genome and ~100 kb resolution in 139 regions associated with constitutional disorders (more details available at <http://www.cytochip.com/productfocus>). Data analysis was performed by algorithm fixed settings in BlueFuse Multi Software (BlueGnome, Cambridge, UK). The minimum number of microarray probes used for calling a copy number change was at least 3 contiguous BAC clones. Detected CNVs were compared to known CNVs in publicly available databases (eg Database of Genomic Variants – DGV; Decipher etc.) and in our own database of results, and classified as clinically significant, likely benign or as variants of uncertain clinical significance (VOUS), as reported elsewhere.^{14,26} Briefly, results were reported as 'normal' (no clinically significant CNVs detected, with or without benign CNVs), 'abnormal' (with clinically significant copy-number changes containing dosage-sensitive or disease-causing genes or occurring within a region of the genome known to be involved in syndromes associated with chromosome abnormalities), or with a 'VOUS', defined as an alteration of unclear clinical relevance that has not been previously identified in a laboratory's patient population, not been reported in the medical literature, not been found in publicly available databases or does not contain any known disease-causing genes. The size cut-off for was 0.5 Mb for the backbone and 100 Kb for disease-focused regions. The microarray findings were confirmed as previously described.²⁶

RESULTS

A total of 3000 prenatal samples were processed in parallel using both CMA and G-banding for conventional karyotyping.

The indications for prenatal testing included: advanced maternal age ($n = 1118$; 37.3%), abnormal results from maternal serum screening tests ($n = 29$; 1.0%), abnormal ultrasound findings ($n = 95$; 3.2%), abnormal fetal karyotype ($n = 25$; 0.8%), family history of a genetic condition ($n = 25$; 0.8%), parental anxiety ($n = 1675$; 55.8%) and cell culture failure ($n = 33$; 1.1%). Successful results were obtained by CMA in 3000/3000 (100%) of cases, 98.9% of which was achieved using only uncultured material. The majority of prenatal samples (2905/3000; 96.8%) had normal results, with no copy number changes or only benign CNVs identified. Clinically significant chromosome alterations were identified in 95 (3.2%) samples, 71 (74.7%) of which were also detected by conventional karyotyping.

Numerical chromosome anomalies were detected in 54 (1.8%) fetuses, including 35 with trisomy 21, 9 with trisomy 18, 3 with trisomy 13, 2 with 45,X, 1 with 47,XXY, 2 with 47,XXX and 2 with 47,XYY. Microscopic segmental chromosomal deletions or duplications were diagnosed in 6 (0.2%) fetuses. CMA was also able to detect chromosomal mosaicism in 11 (0.4%) samples, with the lowest abnormal chromosome representation being at the 6% level.

In 24 cases (0.8% of the entire cohort of samples and 25.3% of the clinically relevant findings), CMA identified pathogenic CNVs that

would have remained overlooked if only a conventional karyotype had been performed, 18 of which mapped to regions associated with well-established syndromes. Notably, 6.3% of cases with a normal karyotype and abnormal ultrasound findings had either a microdeletion or duplication of potential or known clinical significance, as did 0.5% of those sampled for maternal age or 0.7% for parental anxiety.

The details of the chromosomal abnormalities detected are listed in Supplementary Material and are presented below in relation to the indications for prenatal diagnosis.

Abnormal ultrasound findings

CMA detected a pathological chromosomal imbalance in 26/95 (27.4%) fetuses with abnormal ultrasound findings: 8 with trisomy 21, 4 with trisomy 18, 1 with trisomy 13, 5 with mosaic aneuploidies and 2 with microscopic segmental chromosomal deletion or duplication. Submicroscopic chromosomal aberrations were detected in 6 (6.3%) fetuses, 2 of which presented with congenital heart disease (tetralogy of Fallot), 2 with abnormal nuchal translucency and 1 with cystic hygroma. In one fetus with single umbilical artery, a 3.4 Mb inherited deletion at 17p12 was detected, consistent with hereditary neuropathy with liability to pressure palsies, a chromosomal abnormality not directly related to the ultrasound findings.

Advanced maternal age

Among the 1118 cases sampled for maternal age indication, CMA detected chromosome anomalies in 34 (3.0%) fetuses: 14 with trisomy 21, 4 with trisomy 18, 1 with trisomy 13, 5 with sex chromosomes aneuploidies and 4 with mosaic aneuploidies. Pathogenic submicroscopic CNVs were detected in 6 (0.5%) fetuses, 5 (cases 6–10, 12) of which mapped to regions associated with well-established syndromes, as described in the Online Mendelian Inheritance in Man (OMIM) database; one (case 11) affecting a male fetus, consisted of a 3.3 Mb inherited (maternal) deletion at Xp11.3p11.23 and was classified as clinically significant CNV, because it was involving a chromosomal region containing clinically relevant genes.

Parental anxiety

Among the 1675 fetuses without a specific prenatal risk, CMA detected numerical chromosome anomalies in 28 (1.7%) fetuses: 11 with trisomy 21, 1 with trisomy 18, 1 with trisomy 13, 2 with sex chromosomes aneuploidies and 2 with segmental duplications, one of which was mosaic. Pathogenic submicroscopic CNVs were detected in 11 (0.7%) fetuses, nine (cases 13–18, 20, 21 and 23) of which mapped to known syndromes. Case 22, was shown to have a *de novo* unbalanced translocation between chromosomes 7 and 9, resulting in a 7.3 Mb duplication at 7q36.1q36.3 and a 9.6 Mb deletion at 9p24.3p23. Case 19 was shown to have a *de novo* 7.5 Mb duplication at 19q13.41q13.43. The above *de novo* aberrations, involving large chromosomal regions and containing clinically relevant genes, were classified as clinically significant CNVs.

Known abnormal fetal karyotype

A total of 25 samples were investigated by CMA because an abnormal fetal karyotype was detected. Eleven samples contained an apparently balanced chromosomal translocation or inversion; in 5 samples *de novo* small supernumerary marker chromosomes (sSMC) were detected, 4 samples were investigated because of a suspected segmental structural chromosomal abnormality and 5 samples were presented because a chromosomal mosaicism was detected following CVS karyotyping. CMA confirmed the suspected segmental chromosomal abnormality in 2 samples and in one sample it was helpful for

correctly scoring of the aberration (a duplication 15q24.1-qter instead of a 5q duplication). In one sample, CMA allowed the identification of the origin of the sSMC. In one sample referred because of an apparently *de novo* balanced chromosomal rearrangement [46,XY,der(6)inv(6)(p12;q14)t(6;8)p21.1;q23)], CMA detected a 5.2 Mb deletion at breakpoint 6q14.3 (Case 24).

Abnormal maternal serum screening test

CMA detected a numerical chromosome abnormality in 3/29 (10.3%) fetuses with abnormal results of maternal serum screening tests: 2 were trisomy 21 and one was a 20% mosaic for trisomy 18. No submicroscopic chromosomal aberrations were detected for this indication.

Family history of a genetic condition or chromosomal abnormality

No genetic imbalances were detected in any of the 25 cases sampled for this indication.

Results of unclear clinical significance

A single CNV of unclear significance, out of 3000 samples tested, was detected in an amniotic fluid sample referred with indication of parental anxiety. It consisted in a 0.34 Mb *de novo* deletion at 7q11.23 [arr 7q11.23 (71 209 746 × 2, 72 049 391–72 418 522 × 1, 72 747 995 × 2)], proximal to the Williams–Beuren syndrome (WBS) critical region. This deletion included the FKBP6 gene, which has been shown to play a role in homologous chromosome pairing in meiosis and male fertility in mouse models.^{35,36} The involvement of the typical WBS loci was excluded by fluorescent *in situ* hybridization (FISH), using a commercially available probe mapping to the Elastin gene, as commonly used for confirmation of the deletion associated with WBS. After post-test counseling, the patient decided to continue with pregnancy.

DISCUSSION

Whether CMA should be used in prenatal diagnosis as a first-line test in routine practice has been widely debated.^{37–39} At present, numerous independent prospective studies,^{17–19,22–24,26–30} involving the use of various strategies and validated with several different array platforms, have demonstrated the effectiveness and usefulness of CMA in prenatal diagnosis compared to conventional karyotyping.

In our large-scale prospective study, a figure of 0.8% represented the average improvement in the detection rate of CMA compared with traditional karyotyping, concordant with those reported by other similar studies.^{27–30} The combined data from published prospective studies represent now a cohort of over 13 000 prenatal samples^{7–19,22–24,26–30} (Table 1). Taken together, their findings clearly indicate that the use of CMA in prenatal diagnosis produces a substantial improvement in the detection rate of pathogenic chromosome abnormalities compared with conventional karyotyping. Reports to date suggest that CMA is a more sensitive diagnostic test and adds incremental value to conventional karyotyping, with the potential to replace karyotyping in the initial cytogenetic evaluation of the fetus.

The results of this study provide particular evidence on the improved diagnostic ability of CMA to detect clinically relevant abnormalities and the utility of bringing CMA into routine prenatal diagnosis practice as a primary diagnostic tool with a number of different indications. Even more clearly, it indicates that offering only CMA as second-line test just to high-risk pregnancies, may substantially limit the diagnostic potential of prenatal diagnosis, since a significant proportion of CNVs that can cause a serious disability will not be detected by traditional karyotyping. The most relevant clinical

Table 1 Comparison of CMA detection rates in prospective prenatal studies

Reference	No. of prenatal samples analyzed	No. of clinically significant submicroscopic aberrations (%)	No. of CNVs of unclear significance (%)
Sahoo <i>et al</i> ¹⁷	98	0 (0.0)	2 (2.0)
Shaffer <i>et al</i> ¹⁸	151	2 (1.3)	1 (0.7)
Kleeman <i>et al</i> ²¹	50	1 (2.0)	0 (0.0)
Coppinger <i>et al</i> ²²	244	5 (2.0)	1 (0.4)
Van de Veyver <i>et al</i> ²³	300	2 (0.7)	3 (1.0)
Maya <i>et al</i> ²⁴	269	3 (1.1)	0 (0.0)
Park <i>et al</i> ²⁷	4.033	11 (0.3)	NR
Armengol <i>et al</i> ²⁸	906	14 (1.5)	6 (0.7) ^a
Lee <i>et al</i> ²⁹	3.171	34 (1.1)	5 (0.2)
Breman <i>et al</i> ³⁰	1.115	25 (2.2)	18 (1.6)
Current study	3.000	24 (0.8)	1 (0.03)
Total	13.337	121 (0.9)	37 (0.3)

^aonly not inherited CNVs are taken into consideration.

point in regard to this argument relates to the 24 submicroscopic chromosomal abnormalities (0.8% of the entire cohort of samples and 25.3% of the clinically relevant findings) that would have remained undiagnosed if only a conventional karyotype had been performed. Yet more interestingly, 17 (0.6%) of these karyotypically cryptic findings would have been otherwise overlooked following the diagnostic strategy of offering CMA only to high-risk pregnancies. Similar results have also been reported by other independent large-scale prospective studies (Table 1).^{26–30}

A point to note was how CMA was able to robustly detect copy numbers changes in all cases, such as case 2 (in which the G-banded karyotyping failed to detect the chromosomal abnormality) and case 3 (in which a cell culture failure occurred). For these cases, a good quality traditional karyotyping at the 400-band level should have been able to reliably detect the aberrations, but it failed to do so. It is well known the resolution of banding techniques is generally influenced by the quality of the chromosome preparation, thus it is not always possible to achieve the desired resolution in routine prenatal banding analysis. This is all the more true when performing karyotyping from CVS samples, as it was for case 2, where cytogenetic preparations are often technically challenging. Cases 2 and -3 represent clear examples of inherent limitations of the use of traditional karyotyping, but not in CMA.

The evidence presented here makes CMA use in a routine prenatal diagnosis practice a proposition that needs to be considered. In fact, the chromosomal abnormalities that can be exclusively detected by CMA are not always age-related and considering the frequency of these disorders, it could be argued that this technique could be offered to all women undergoing invasive prenatal diagnosis, irrespective of the indication for testing.⁴⁰ Ogilvie *et al*⁴¹ estimated that, using CMA, there is a 1:300–1:600 (0.33–0.16%) chance of finding a CNV that will cause a serious disability, which can not be otherwise detected by traditional karyotyping.

The results of the present study are clearly in favor of the above hypothesis, although it shows that Ogilvie *et al*⁴¹ estimations to be underestimations, as our data shows the use of CMA results in an increased detection rate regardless of the indication for analysis. This becomes especially evident when examining the data from high-risk groups (abnormal ultrasound findings + abnormal fetal karyotype), in which the detection rate is elevated to 5.8% (7/120), but also in groups with *a priori* low risk for the detection of submicroscopic chromosomal abnormalities, such as advanced maternal age (6/1118,

0.5%) and parental anxiety (11/1675, 0.7%) groups, with an average increase in detection rate of 0.6% for low risk pregnancies (Table 2).

In the recent paper from Lee *et al*,²⁹ a figure of about 0.5% represented the average improvement in detection rate in low-risk pregnancies over conventional prenatal cytogenetics; Armengol *et al*,²⁸ instead, reported a figure of 1.1% for advanced maternal age group and 1.7% for parental anxiety group. The combined data from prospective studies represent a cohort of over 8000 prenatal samples, referred for different categories of indication and indicates an average probability of detecting pathogenic submicroscopic CNVs in prenatal samples from low-risk pregnancies of around 0.6% (see Supplementary Material). Furthermore, a large prospective multi-center trial involving analysis of over 4000 samples, sponsored by the National Institute Of Child Health and Human Development (NICHD), investigating the role of CMA as a primary prenatal diagnostic tool, has been recently completed and its anticipated results⁴² report data concordant with our study. This level of increased detection rate may suggest that, instead of restricting the use of CMA only to fetuses with ultrasound anomalies, this technology should be available to all pregnant women undergoing invasive prenatal testing, regardless of risk factors.

There is understandable concern regarding the use of CMA on prenatal samples, mainly related to the potential detection by CMA, of mild or unpredictable phenotypes and/or VOUS.^{37,43} However, it is well described that the differences in the proportion of VOUS detected is mainly related to the array platform used and its resolution. In our study, we have carefully selected a genome wide BAC-array platform specifically developed for prenatal applications, with a balance of increased resolution (~100 kb) in locations of known constitutional disorders as well as less coverage in polymorphic CNVs and in backbone (up to 1Mb resolution), compared with high resolution oligonucleotide or single-nucleotide polymorphism (SNP) microarray platforms. While higher resolution, high resolution microarrays offer the theoretical opportunity to find additional submicroscopic abnormalities compared to BAC arrays and are the default array choice for postnatal cytogenetics. However, the detection rate for high- and low-risk pregnancies found in the present study using a BAC array platform, compares well with other large scale prospective studies^{28,29,31,42} in which high resolution microarrays have been used.

There are clear issues with the use of these platforms for prenatal diagnostics. The high resolution detection potential of oligonucleotide

Table 2 CMA results according to the indication for prenatal diagnosis

Indication	No. of Samples analyzed (%)	No. Samples with a pathogenic CNV (%)				CMA detection rate (%)
		Detected by		Not detected by		
		conventional karyotyping		conventional karyotyping	Total	
Abnormal ultrasound findings	95 (3.2)	20 (76.9)		6 (23.1)	26 (27.4)	6.3
Advanced maternal age	1118 (37.3)	28 (82.4)		6 (17.6)	34 (3.0)	0.5
Parental anxiety	1675 (55.8)	17 (60.7)		11 (39.3)	28 (1.7)	0.7
Abnormal fetal karyotype	25 (0.8)	3 (75.0)		1 (25.0)	4 (16.0)	4.0
Abnormal maternal serum screening test	29 (1.0)	3 (100.0)		0 (0.0)	3 (10.3)	0.0
Family history of a genetic condition	25 (0.8)	0 (0.0)		0 (0.0)	0 (0.0)	0.0
Cell culture failure	33 (1.1)	0 (0.0)		0 (0.0)	0 (0.0)	0.0
High risk pregnancies (abnormal ultrasound findings + abnormal fetal karyotype)	120 (4.0)	23 (76.7)		7 (23.3)	30 (25.0)	5.8
Low risk pregnancies (advanced maternal age + parental anxiety + abnormal maternal serum screening test + family history of a genetic condition + cell culture failure)	2880 (96.0)	48 (73.8)		17 (26.2)	65 (2.3)	0.6
Total	3000	71 (74.7)		24 (25.3)	95 (3.2)	0.8

or SNP microarrays is offset in prenatal cases with more complex aberration calling issues due to the higher noise introduced when analyzing the poorer quality/quantity prenatal samples on these platforms. High resolution platforms also often require DNA extraction from cultured cells, which adds to diagnostic delays/costs and was not the case here for BAC arrays. Most importantly, the BAC array design strategy used here resulted in a substantially lower detection of variants of unclear significance (only a single VOUS occurrence out of 3000 samples analyzed), the central prenatal microarray diagnostic issue, than reported by other studies using high resolution microarrays (Table 1).

In a recent systematic review,³¹ VOUS are reported to occur in ~1% of prenatal samples. Combining the above data with previously reported prospective studies, the average probability of detecting such findings in prenatal samples can be estimated approximately 0.3% (Table 1). It is also well known that the frequency of detection of VOUS increases if parental samples are not available, since the inheritance status of the VOUS cannot be ascertained. However if parental samples can be submitted with the prenatal samples, parental testing can be performed as soon as a fetal chromosomal aberration is identified, thereby alleviating additional timescale related anxiety for the patients and substantially reducing the number of uncertain calls.

The point to debate is surely whether we are able to or willing to deal with such level of VOUS. We believe that this potential uncertainty is not dramatically different from what is observed with routine prenatal cytogenetic karyotype analysis. In fact, results of unclear significance are not unfamiliar in traditional prenatal diagnosis because unclear diagnostic results or findings with unclear clinical consequences are occasionally encountered even with conventional karyotyping. Thus, VOUS identified by prenatal CMA might be approached in a similar manner and managed by providing the patients a thorough pre-test and post-test counselling.³⁴ This will allow patients to achieve the maximum autonomy and right to information from CMA in order to make informed choices about their pregnancies.⁴⁴

Although detection of VOUS cannot be completely excluded with CMA, the growing clinical experience with genome-wide arrays, the increasing availability, sophistication and size of CNV databases (in

house or public) for both phenotypically normal and affected individuals, together with parental analysis, allows classification and interpretation for the majority of the alterations discovered, thus substantially reducing the number of VOUS and making CMA increasingly applicable to clinical prenatal diagnosis practice.

We believe that the improved prenatal diagnosis provided by CMA compared with the traditional karyotype outweighs the potential uncertainties for families. We also think that some patients would prefer to have maximum information and autonomy.⁴⁵

In conclusion, the results of this study suggest that more widespread CMA testing of fetuses would result in a higher detection of clinically relevant chromosome abnormalities, even in low risk pregnancies. Our findings, combined with data from previous studies, provide substantial evidence for the introduction of CMA as a first-line diagnostic test for all pregnant women undergoing invasive prenatal testing, regardless of risk factors. We believe that the diagnostic accuracy of CMA may offer a much more fine-grained tool for prenatal diagnosis than other currently available technologies, allowing patients to achieve an improved prenatal diagnosis, with a lower risk for the newborn to be affected by a chromosomal abnormality. Of course, its introduction into clinical practice should proceed with caution, being offered only by experienced laboratories and after a proper internal validation of the technique showing robust, reliable, reproducible and possibly externally validated results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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