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Should prenatal chromosomal microarray analysis be offered for isolated fetal growth restriction? A French multicenter study

Isabelle MONIER^{1,2}, RM, PhD, Aline RECEVEUR³, MD, Véronique HOUFFLIN-DEBARGE⁴, MD, PhD, Valérie GOUA⁵, MD, Vanina CASTAIGNE⁶, MD, Jean-Marie JOUANNIC⁷, MD, PhD, Eve MOUSTY⁸, MD, Anne-Hélène SALIOU⁹, MD, Hanane BOUCHGHOUL¹⁰, MD, Thierry ROUSSEAU¹¹, MD, Anne-Sylvie VALAT¹², MD, Marion GROUSSOLLES¹³, MD, Florent FUCHS¹⁴, MD, PhD, Guillaume BENOIST¹⁵, MD, PhD, Sophie DEGRE¹⁶, MD, Jérôme MASSARDIER¹⁷, MD, Vassilis TSATSARIS¹⁸, MD, PhD, Pascale KLEINFINGER¹⁹, MD, Jennifer ZEITLIN¹, MA, DSc, Alexandra BENACHI², MD, PhD, for the French Federation of Fetal Medicine Centers

Affiliations

1 Obstetrical, Perinatal and Pediatric Epidemiology Research Team (EPOPé), Université de Paris, Epidemiology and Statistics Research Center (CRESS), Institut national de la santé et de la recherche médicale (INSERM), Institut national de la recherche agronomique (INRA), Paris, France

2 Department of Obstetrics and Gynaecology, Antoine Bécclère Hospital, AP-HP, Paris Saclay University, Clamart, France

3 Department of Cytogenetics and Reproductive Biology, Antoine Bécclère Hospital, AP-HP, Paris Saclay University, Clamart, France

4 Department of Obstetrics and Gynaecology, Jeanne de Flandres University Hospital, Lille, France

5 Department of Obstetrics and Gynaecology, Poitiers University Hospital, Poitiers, France

6 Department of Obstetrics and Gynaecology, Centre Hospitalier Intercommunal de Créteil, Créteil, France

7 Fetal Medicine Department, Armand-Trousseau Hospital, AP-HP, Sorbonne University, Paris, France

8 Department of Gynaecology and Obstetrics, Nîmes University Hospital, Nîmes, France

9 Department of Obstetrics and Gynaecology, Brest University Hospital, Brest, France

10 Department of Obstetrics and Gynaecology, Bicêtre Hospital, AP-HP, Paris Saclay University, Le Kremlin Bicêtre, France

11 Department of Obstetrics and Gynaecology, Dijon University Hospital, Dijon, France

12 Department of Obstetrics and Gynaecology, Lens Hospital, Lens, France

13 Department of Obstetrics and Gynecology, Paule de Viguier Hospital, Toulouse University Hospital, Toulouse, France

14 Department of Obstetrics and Gynecology, Montpellier University Hospital Center, Montpellier, France

15 Department of Obstetrics and Gynecology, Caen University Hospital Center, Caen, France

16 Department of Obstetrics and Gynecology, Le Havre University Hospital Center, Le Havre, France

17 Department of Obstetrics and Gynecology, Hospices Civils de Lyon, Bron, France

18 Department of Obstetrics and Gynecology, Cochin Hospital, AP-HP, Paris-Descartes University, Paris, France

19 Laboratoire CERBA, 7/11 Rue de l'Équerre, Saint-Ouen-l'Aumône, France

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Corresponding Author

Isabelle MONIER

Inserm UMR 1153, Obstetrical, Perinatal and Pediatric Epidemiology Research Team
Port Royal Maternity Unit, 53 Avenue de l'Observatoire, 75014 Paris, France

Tel: +33 (0)1 42 34 55 86

Fax: +33 (0)1 43 26 89 79

E-mail: isabelle.monier@inserm.fr

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Condensation

Chromosomal microarray analysis should be offered in addition to karyotype in fetuses diagnosed with isolated fetal growth restriction

Short Title

Chromosomal microarray analysis for isolated fetal growth restriction

AJOG at a Glance

A. Why was the study conducted?

This study aimed to investigate the diagnostic yield of chromosomal microarray analysis over karyotype in the detection of genetic anomalies in fetuses diagnosed with isolated fetal growth restriction.

B. What are the key findings?

Among fetuses diagnosed with isolated fetal growth restriction and a normal karyotype, chromosomal microarray analysis identified clinically significant findings in 3.6% of fetuses and variants of unknown significance in 2.1%.

C. What does this study add to what is already known?

Chromosomal microarray analysis improves the detection of genetic anomalies compared to karyotype alone in fetuses diagnosed with isolated fetal growth restriction. Findings from this study support offering chromosomal microarray analysis in addition to karyotype in cases of isolated fetal growth restriction.

Keywords: fetal growth restriction, prenatal diagnosis, chromosomal microarray, copy number variants, karyotype

Abstract

Background: Chromosomal microarray analysis (CMA) improves the detection of genetic anomalies over standard karyotype and is thus recommended in many prenatal indications. However, evidence is still lacking on the clinical utility of CMA in cases of isolated fetal growth restriction (FGR).

Objective: To estimate the proportion of copy number variants (CNVs) detected by CMA and the incremental yield of CMA over karyotype in the detection of genetic abnormalities in fetuses with isolated FGR.

Study design: This retrospective study included all singleton fetuses diagnosed with FGR and no structural ultrasound anomalies and referred to 13 French fetal medicine centers over a one-year period in 2016. FGR was defined as an estimated fetal weight <10th percentile for gestational age identified in ultrasound reports. For this analysis, we selected fetuses who underwent invasive genetic testing with karyotype and CMA results. Data were obtained from medical records and ultrasound databases as well as post-mortem and placental examination reports in case of spontaneous stillbirths and terminations of pregnancy. Following the American College of Medical Genetics and Genomics guidelines, CNVs were classified into 5 groups as following: pathogenic, likely pathogenic, variant of unknown significance (VOUS), likely benign and benign.

Results: Of 682 referred fetuses diagnosed with isolated FGR, both karyotype and CMA were performed in 146 fetuses. Overall, the detection rate of genetic anomalies found by CMA was estimated to be 7.5% (11/146, CI95%: 3.3,11.8) including 10 CNVs classified as pathogenic and one as likely pathogenic. Among the 139 fetuses with normal karyotype, 5 fetuses were detected with pathogenic and likely pathogenic CNVs resulting in an incremental yield of 3.6% (CI95%: 0.5-6.6) of CMA over

karyotype. All fetuses detected with pathogenic or likely pathogenic CNVs resulted in terminations of pregnancy. In addition, 3 fetuses with normal karyotype were detected with VOUS (2.1%). Of the 7 fetuses with abnormal karyotype, CMA did not detect one fetus with trisomy 18 mosaicism.

Conclusion: Our study found that CMA improves the detection of genetic anomalies over karyotype in fetuses diagnosed with isolated FGR. These results support the use of CMA in addition to karyotype for isolated FGR.

Introduction

Fetal growth restriction (FGR) is associated with high risks of perinatal mortality, neurodevelopmental impairments and other neonatal morbidities that can be related to risks of vascular disease in adulthood.¹⁻⁵ The diagnosis of FGR is most often based on an estimated fetal weight <10th percentile for gestational age.⁶⁻⁸ There are several pathological mechanisms leading to FGR and identifying these is required for optimal management of the pregnancy. One cause can be the presence of chromosomal abnormalities associated with structural anomalies which account for between 7% and 30% of FGR cases.⁹⁻¹¹ However, chromosomal anomalies can also be present when no structural anomalies associated with FGR are detected on the ultrasound,^{9,12} and therefore prenatal invasive genetic testing may be offered in cases of isolated FGR in order to exclude genetic disorders.

Chromosomal microarray analysis (CMA) is known to improve the detection of genomic abnormalities compared to prenatal karyotype¹³⁻¹⁷ but the clinical utility of CMA in fetuses with isolated FGR is still debated. While the Society for Maternal-Fetal Medicine recently recommended that CMA should be offered in cases of isolated FGR diagnosed before 32 weeks of gestation,¹⁸ other societies do not have guidelines for best practice on the use of CMA for this indication.¹⁹⁻²³ This uncertainty highlights, in part, the absence of high-quality empirical data on the impact of CMA in cases of isolated FGR, as reflected by the assessment of the Society for Maternal-Fetal Medicine that the evidence for their recommendation was of low-quality (grade C).¹⁸ There is one meta-analysis of 9 observational studies that showed an increase of 4% in detection of genomic abnormalities by CMA in fetuses diagnosed with isolated FGR with a normal karyotype.²⁴ However, most studies included in this meta-analysis were single-center studies using small samples with inconsistencies between studies in the

definition of FGR. More generally, there is heterogeneity across studies in the method used to estimate the incremental yield of CMA over karyotype leading to uncertainties in the relevance of performing CMA for this indication and to confusion when interpreting and comparing results from different studies.

This study aimed to estimate the proportion and the type of copy number variants (CNVs) detected by CMA and the diagnosis yield of CMA over karyotype in the detection of genomic abnormalities in fetuses with isolated FGR.

Materials and Methods

Data come from a retrospective observational study including all singleton fetuses diagnosed with FGR without apparent structural ultrasound anomalies regardless of gestational age at diagnosis and referred to 13 French fetal medicine centers between January 1st and December 31st, 2016. Exclusion criteria were multiple pregnancy, imprecise pregnancy dating as assessed by no first trimester ultrasound between 11+0 and 13+6 weeks of gestational age,⁶ maternal age <18 years old, short femoral length as the only criteria for suspicion of FGR and congenital malformation detected at ultrasound at the time of referral. FGR associated with congenital infections such as Toxoplasmosis or Cytomegalovirus were not excluded if there was no structural ultrasound anomaly associated with. This study received ethics approval from the National Data Protection Authority (National Commission on Informatics and Liberty no. 917163) and Ethical Committee (approval granted April 02, 2017, reference CEROG OBS 2016-11-05). The study was funded by the French Biomedicine Agency. The study population included fetuses diagnosed with isolated FGR who underwent invasive genetic testing with both karyotype and CMA results. According to the

204 American College of Obstetricians and Gynecologists (ACOG) recommendations,⁸
205 FGR was defined as fetuses with an estimated fetal weight (EFW) <10th percentile for
206 gestational age noted in ultrasound reports by expert sonographers and identified in
207 the electronic ultrasound databases (ViewPoint) used in all participating centers. In
208 France, gestational age is determined using the measurement of crown-rump length
209 at the first trimester ultrasound and French growth references are recommended for
210 calculating EFW percentiles.^{6,25} Structural anomalies associated with FGR included
211 and excluded from the study were independently selected by three experts in fetal
212 medicine based on the list of minor anomalies developed by the European surveillance
213 of congenital anomalies (EUROCAT) network (Table S1).²⁶ Discordant entries were
214 adjudicated between the experts (AB, VT, JM). Soft markers such as intestinal
215 hyperechogenicity and amniotic fluid abnormalities, are not structural anomalies and
216 were thus included.

217 Data were obtained from ultrasound electronic database and medical charts using a
218 standardized form by two midwives (IM and CV) trained for the study. Maternal
219 characteristics included maternal age, parity, height and weight before pregnancy and
220 smoking status; clinical characteristics included prenatal care, management of the
221 pregnancy in fetal medicine centers, pregnancy complications and mode of delivery.
222 We also collected ultrasound characteristics of the ultrasound when FGR was
223 diagnosed; this included gestational age at ultrasound, EFW expressed in grams and
224 in percentiles according to French growth references²⁵ and results of uterine and
225 umbilical Dopplers. Information on gestational age at birth, birthweight, neonatal
226 resuscitation and admission in a neonatal unit was collected to describe the infant's
227 health. We used intrauterine growth curves adapted to the French population for
228 calculating birthweight percentile.²⁷ Fetus autopsy (or postmortem) and placental

examination reports were also reviewed in case of spontaneous stillbirth or terminations of pregnancy if these tests were accepted by parents.

The genomic study was performed using DNA extracted from native amniotic fluid or cultured amniotic cells. According to cytogenetics laboratories, comparative genomic hybridization (CGH) or single-nucleotide polymorphism (SNP) microarrays were used. Hybridization strategies included hybridization against fetal sex reference and trio-hybridization patients against parent's DNA. For array-CGH, chip design were most often PrecytoNEM which is a specific enriched design for prenatal diagnosis assessment (resolution: 105K genome-wide randomized probes with specific enrichment, Agilent) and SurePrint G3 Human which is a basic design (resolution: 60K/180 K genome-wide randomized probes, Agilent); for SNP, Omniexpress 24 design was used (resolution: 130/400 million respectively of genome-wide randomized clusters, Illumina). DNA extracted from parental blood samples were also often analyzed in case of CMA findings for heritability assessment or during trios analyses.

In case of CMA findings, parental DNA were studied using both CMA and quantitative fluorescence polymerase chain reaction to confirm CNVs. For CNV >200 kb, rapid aneuploidy detection by quantitative fluorescence polymerase chain reaction was performed on circulating lymphocytes isolated from parental blood samples.

All CMA were retrospectively reviewed and CNVs were classified using the data available at the time of the study period by two cytogeneticists (AR, PK) based on their size, their genomic position and the involved genes based on the published literature (PubMed) and the following genetic databases: Database of Genomic Variants (DGV), Clinical Genome Resource consortium (ClinGen), Database of genomic variation and Phenotype in Human using Ensembl Resources (DECIPHER, v10.2), Achropuce French consortium database and ClinVar. According to joint

consensus recommendations of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen), CNVs were classified into 5 groups:^{28,29} pathogenic, likely pathogenic, variant of unknown significance (VOUS), likely benign and benign. CNVs documented as likely benign or a benign variant, which corresponds to a normal result, were not reported as recommended in French cytogenetic guidelines.³⁰ CMA and karyotype results were reported using ISCN nomenclature (ISCN 2016).

We first described the maternal, clinical and neonatal characteristics of the study population. Characteristics of the study population were compared to fetuses who did not undergo genetic invasive testing and to with only karyotype. The detection rate of clinically significant findings found by CMA was estimated among all fetuses irrespectively of karyotype results. The diagnostic yield of CMA over karyotype was calculated as the proportion of fetuses detected with pathogenic and likely pathogenic CNVs among fetuses with normal karyotype. Details of cases detected with clinically significant findings found by CMA were reported according to karyotype results.

To compare our results with the existing evidence, we tabulated the results of the 9 studies included in the meta-analysis conducted from January 2009 to November 2016²⁴ and added studies conducted from January 2017 through January 2021. Studies in English language were included if CMA and karyotype were performed in fetuses diagnosed with isolated FGR. These were identified using the PudMed database and the following search terms: 'fetal growth restriction', 'intrauterine growth restriction', 'small-for-gestational age', 'chromosomal microarray analysis', 'copy number variants' and 'karyotype'. We also looked for additional studies in the reference lists of selected articles. This comparison took into consideration the different estimation methods for the incremental yield of CMA over karyotype and the studies'

inclusion of VOUS. Descriptive analyses were performed using STATA software (version 15.0; StataCorp LP, College Station, TX).

Results

The study included 682 singleton fetuses diagnosed with isolated FGR without structural anomalies and referred to one of the 13 fetal medicine centers participating to the study between January 1st and December 31st, 2016. (Figure 1) Invasive testing was performed in 245 fetuses (35.9%). Of these, the study population included 146 fetuses with both karyotype and CMA results. There was only one case of CMV infection and no case of Toxoplasmosis infection.

Maternal and neonatal characteristics of the study population are described in Table 1. Mean maternal age was 30.7 (standard deviation (SD): 6.1) and almost all women performed first or second trimester serum tests for Down's syndrome screening (96.5%). Among all cases, 83.0% were live births, 4.2% were spontaneous stillbirths and 12.8% were terminations of pregnancy. Mean gestational age at FGR diagnosis was 24.7 (SD: 4.1) with an EFW <3rd percentile for 79% of fetuses. Because information on intrauterine growth charts used by ultrasonographers was not available, EFW percentiles were calculating using French College of Fetal Ultrasonography references and 6 fetuses had an EFW >10th percentile. Mean gestational age at birth was 34.7 (SD: 4.8) and 68.6% of infants had birthweights <3rd percentile. As expected, fetuses who did not undergo invasive genetic testing were diagnosed later with FGR and had a higher EFW compared to those where karyotype and CMA were performed. (Table S2) Compared to the study population, fetuses with karyotype only were more often stillbirths or terminations of pregnancy with delivery before 26 weeks of gestation.

(Table S3) All genetic samples were obtained from amniocentesis. Pathogenic and likely pathogenic CNVs were found in 11 of the 146 fetuses resulting in an overall detection rate of genetic anomalies by CMA estimated to be 7.5% (CI95%: 3.3,11.8). Among the 139 fetuses with normal karyotype, 4 fetuses were detected with pathogenic CNVs and one fetus with likely pathogenic CNV resulting in an incremental yield of CMA over normal karyotype estimated to be 3.6% (CI95%: 0.5-6.6). (Table 2) Among pathogenic findings by CMA, there was one uniparental disomy related to Prader-Willi syndrome and non-recurrent microdeletions at 19q12q13.12, 12q13.13 and 19p13.2p13.11loci. All these cases resulted in terminations of pregnancy with fetuses having birthweights <3rd percentile, except one fetus who had a birthweight between the 3rd and the 10th percentile. One fetus was found with polyhydramnios (case 4) and one fetus was diagnosed with FGR after 32 weeks of gestation (case 5). In this last case, a nulliparous 27 year-old woman with a low-risk pregnancy had normal first and second trimester ultrasounds, but fetal weight was estimated to be 1508g (<3rd percentile) with normal amniotic fluid and Doppler at the third trimester ultrasound at 32 weeks and 5 days gestation. The karyotype was normal but a pathogenic CNV was found by CMA; the pregnancy resulted in a late termination of pregnancy. Finally, 3 fetuses (2.1%) were detected with CNVs classified as VOUS at the time of the study period.

Seven fetuses were found with abnormal karyotype (4.8%, CI95%: 1.3-8.3). Clinical characteristics and genetic results of fetuses with abnormal karyotype are presented in Table 3. Abnormal karyotype was related to syndromes in four cases: 4p (Wolf Hirschhorn syndrome, case 9), 47XXY (Klinefelter syndrome, case 11), 45, X (Turner syndrome, case 14) and Trisomy 18 (Edwards syndrome, case 13). The Klinefelter syndrome (case 11) may be considered as an incidental finding since the phenotype

is not associated with FGR. For the Edwards syndrome (case 13), CMA did not detect anomalies although the karyotype was abnormal; this case had rapid prenatal FISH testing performed from uncultured amniotic fluid at 34 weeks and 4 days. It revealed a mosaic trisomy 18 in 13% of interphasic cells, confirmed on cultured cells. The case 15 found with abnormal karyotype and CNV classified as pathogenic was associated with echogenic bowel. Other 11 cases associated with echogenic bowel had normal karyotype and no CMA finding.

Overall, 5 studies were identified in addition to the 9 existing studies included in the meta-analysis of Borrell et al. (Table 4) The incremental yield of CMA over normal karyotype ranged between 0% to 16.7% for all studies and from 2.6% to 6.1% among the studies with samples over 100. Four of the five new studies reported results falling within the 95% confidence intervals of Borrell's pooled estimate. VOUS were found in 0.8% to 7.2% of fetuses with normal karyotype, but this information was not reported in all studies. Some studies included VOUS in their reported estimates of the incremental yield of CMA which had a large impact on the results.

Comment

Principal findings

Our study found that CMA had an added value in the detection of genetic anomalies compared to karyotype in cases of isolated FGR with no structural anomalies. In fetuses with normal karyotype, CMA detected additional clinically significant findings in 3.6% of cases and VOUS in 2.1%.

Results in context

352 The diagnostic yield of CMA over karyotype in singleton fetuses diagnosed with
353 isolated FGR has varied in past studies. In the meta-analysis of Borrell et al., the overall
354 increase of CMA over karyotype in the detection of genetic anomalies defined as
355 pathogenic CNVs was estimated to be 4% (CI95%: 1-6) with a wide range of rates
356 between 0% and 17% across studies.²⁴ The more recent literature has provided
357 detection rates in line with this pooled estimate of this meta-analysis ranging between
358 0% and 5.9%. In our study, we found a similar detection rate of 3.6% (CI95%: 0.5-6.6).
359 When interpreting results from studies, it is important to compare the definitions of FGR
360 and isolated FGR as well as array-based techniques. Further, some studies classify
361 VOUS as genomic abnormalities³¹ although VOUS may be benign CNVs and therefore
362 cannot be considered as abnormalities. Our study found that 2.1% of isolated FGR
363 fetuses were detected with VOUS. Previous studies using large samples found a
364 similar prevalence of VOUS ranging between 2% and 4% in fetuses with ultrasound
365 anomalies including FGR^{15,17,32} and also in those from mothers with low-risk
366 pregnancies.³³ In contrast, 7.2% of VOUS were detected in a recent retrospective
367 study including 428 FGR fetuses with early-onset FGR or FGR associated with
368 ultrasound anomalies,³⁴

369 Further, few studies have considered fetuses found with abnormal karyotype when
370 investigating the benefits and limitations of CMA. However, when comparing with
371 karyotype and rapid aneuploidy detection, one main limitation of CMA using CGH array
372 analysis is the non-detection of low-level mosaicism <10/15% or triploidies, both
373 associated with FGR.³⁵ The non-detection of lower level of mosaicism by CMA when
374 comparing with karyotype is debated because it depends on the DNA extraction's
375 quality and the possibility to avoid a culture selection of the normal cell line. Moreover,
376 triploidies could be detected if SNP array testing is used. In a systematic review of 14

observational studies (N=874), the detection rate of abnormal karyotype in isolated FGR fetuses was estimated to 6.4% with rates ranged between 0% and 26%.¹² In our sample, 4.8% (CI95%: 1.3-8.3) of fetuses had abnormal karyotype. Among the 7 fetuses with abnormal karyotype, one fetus had a mosaic trisomy 18 with 13% of interphasic cells which was not detected using CGH microarray analysis. As opposed to CGH based arrays, the SNP microarray analysis enables the detection of uniparental disomy, consanguinity, triploidy and parental origin. However, the choice between SNP and CGH techniques may depend of their availability in the center and there is currently no recommendation in favor on one of these array-techniques.

Clinical and research implications

Recommendations on the use of CMA for cases of isolated FGR differ between professional societies. Several societies have no rules for performing CMA in case of suboptimal fetal growth.²² In Canada, CMA is recommended for all pregnancies suspected with FGR²⁰ while the Italian Society of Human Genetics called for more investigation of the utility of CMA in cases of FGR.¹⁹ In 2020, the Society Maternal-Fetal Medicine recommended offering CMA in pregnancies diagnosed with isolated FGR before 32 weeks of gestation, although they graded evidence as low-quality.¹⁸ The French National College of Obstetricians and Gynecologists also recommends genetic tests in case of early-onset FGR diagnosed before the third trimester of pregnancy, increase of amniotic fluid, suspicion of congenital malformation or no cause of FGR (i.e. normal doppler, no maternal vascular disorders).³⁶ However, there is no restriction of gestational age for offering genetic invasive testing in France. In addition, French cytogenetic guidelines recommend performing CMA and karyotype in fetuses with an EFW <3rd percentile.³⁰ In line with these recommendations, we found that

almost all fetuses included in our analysis had birthweights <3rd percentile. Further, most fetuses were suspected with FGR before 32 weeks of gestation. Our study provides evidence to support current guidelines in offering CMA in addition to karyotype in fetuses with isolated FGR. However, more studies are needed to investigate the utility of offering CMA after 32 weeks of gestation. Further, VOUS are inconstantly reported in studies, however, CNVs are classified as VOUS at the time of reporting and their interpretation evolves over time as more information becomes available on outcomes. Therefore, information on infants detected with VOUS should be reported systematically and followed-up to strengthen the knowledge base about potentially pathogenic variants.

Strengths and limitations

Strengths of this study are collection of data from 13 French referral fetal medicine centers using a common protocol and pre-definition of fetuses diagnosed with isolated FGR. Two midwives trained for this study collected data in all participating centers using a standardized form and all CMA were reviewed by two cytogeneticists. Further, our sample was large compared to studies in our review, where only 5 out of 14 had more than 100 inclusions and only one had a sample size exceeding ours. Our study also has several limitations. We used a retrospective study design leading to lower quality of data compared to prospective studies; however, most previous studies have used retrospective designs because these ensure the availability of all genetic results at the time of data collection. Despite the French recommendations,³⁶ CMA was not performed in all fetuses with an EFW <3rd percentile because of the availability of CMA in some centers at the time of the study and varying criteria established by each center for performing CMA. Another limit results from differences between centers regarding

CMA resolution and array techniques used, however, this reflects real-life variation in clinical practice. There was also variability in the use of various platforms and VQSO reporting; for this reason, all CMA were re-assessed by 2 cytogenetics based on American professional standards.²⁹ In addition, the external validity of our results should be confirmed in other contexts including in populations with different uptake of invasive genetic testing, especially since the introduction of cell-free DNA screening or where other EFW charts or abdominal circumference <10th percentile are used to diagnose FGR. Finally, our study population was based on pregnancies suspected with FGR and referred to fetal medicine centers; we were not able to estimate the proportion of pregnancies suspected with FGR who were not referred to fetal medicine centers when they should have been.

Conclusions

In summary, CMA provides substantial additional genetic information compared to karyotype and should be performed in case of isolated FGR. More studies using large sample of isolated FGR fetuses should be conducted to confirm the clinical utility of CMA in this population in balance with risks of detecting variants of unknown significance that can impact on prenatal counselling and increase parental anxiety.

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Table 1. Maternal and neonatal characteristics of the study population

Total N=146	n/N (%) or mean \pm sd
Maternal characteristics	
Maternal age (years) (n=146)	30.7 \pm 6.1
Nulliparous	82/146 (56.2)
Smoking during pregnancy	31/141 (22.0)
Clinical characteristics	
Maternal serum screening for Down's syndrome	139/144 (96.5)
Pre-eclampsia	20/139 (14.4)
Caesarean birth	62/139 (44.6)
Ultrasound characteristics	
GA at suspicion of FGR (weeks) (n=145)	24.7 \pm 4.1
Estimated fetal weight (grams) (n=138)	630.7 \pm 404
Estimated fetal weight percentile ^a (n=138)	3.5 \pm 7.5
<3 rd percentile	109 (79.0)
3 rd -10 th percentile	23 (16.7)
10 th percentile+	6 (4.3)
Abnormal uterine Doppler	51/110 (46.4)
Abnormal umbilical Doppler	12/113 (10.6)
Neonatal characteristics	
Vital status at birth	
Live birth	117/141 (83.0)
Spontaneous stillbirths	6/141 (4.2)
Termination of pregnancy	18/141 (12.8)
Male sex	65/141 (46.1)
GA at birth (weeks of gestation)	34.7 \pm 4.8
<26	12/141 (8.5)
27-31	22/141 (15.6)
32-36	34/141 (24.1)
37+	73/141 (51.8)
Birthweight (grams) (n=140)	1835.5 \pm 845
Birthweight percentile ^b	5.8 \pm 12.1

<3 rd percentile	96/140 (68.6)
3 rd -10 th percentile	27/140 (19.3)
10 th percentile+	17/140 (12.1)

Sd: standard deviation; GA: gestational age

a Based on an intrauterine growth charts adapted to French population²⁷ ; b According to French growth references²⁵

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650 Table 2. Type of abnormalities detected and clinical relevant characteristics in fetuses with normal karyotype and CMA findings

Case	WG at suspicion of FGR	Sampling method	CNV position	Type of CNV	Interpretation	Parental study	Outcome	Sex Birthweight (percentile*)
1	21+6	AC	arr[hg19] 16p12.2(21951379_22645765)x3 dn	Likely pathogenic	Duplication at 16p12.2	<i>de novo</i>	TOP 28+6 WG	Male 840g (<3 rd)
2	21+5	AC	upd(15)mat.arr[hg19](15q11.2q26.3)x2 htz	Pathogenic	Prader-Willi syndrome	Maternal inherited	TOP 32+5 WG	Female 1610g (<3 rd)
3	18+3	AC	arr[hg19] 19q12q13.12(30091373_35646245)x1 dn	Pathogenic	Deletion at 19q12q13.12	<i>de novo</i>	TOP 26+0 WG	Male 670g (<10 th)
4	24+1	AC	arr[hg19] 2q21.1(131513263_132098109)x3 dn	VOUS	Duplication at 2q21.1	<i>de novo</i>	Live birth 31+4 WG	Female 1070g (<3 rd)
5	32+5	AC	arr[hg19] 12q13.13(53617806_54463057)x1 dn	Pathogenic	Deletion at 12q13.13	<i>de novo</i>	TOP 37+5 WG	Female 2070g (<3 rd)
6	25+5	AC	arr[hg19] 19p13.2p13.11(13802749_16346160)x1dn	Pathogenic	Deletion at 19p13.2p13.11	<i>de novo</i>	TOP 27+1 WG	Male 730g (<3 rd)
7	21+1	AC	arr[hg19] 7q11.22(69773947_70115001)x1 pat	VOUS	Deletion at 7q11.22	Paternal inherited	TOP 35+4 WG	Female 1835g (<3 rd)
8	18+0	AC	arr[hg19] Xp22.33(907346_1608329)x3	VOUS	Duplication at Xp22.33	Missing	TOP (HELLP syndrome) 26+5 WG	Male 435g (<3 rd)

AC: amniocentesis; WG: weeks of gestational age; TOP: termination of pregnancy
 * according to a French intrauterine growth charts adjusted on infant sex (EPOPé M1)²⁷

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652 Table 3. Type of abnormalities detected and clinical relevant characteristics in fetuses with abnormal karyotype

Case	WG at suspicion of FGR	Sampling method	Cytogenetic result (karyotype or FISH)	CNV position	Type of CNV	Interpretation	Outcome	Sex Birthweight (percentile ^a)
9	24+5	AC	46,XX,rec(4)dup(4q)inv(4)(p14q35)pat. nuc ish(WHSCRx1,4qterx3)	arr[hg19] 4p16.3p15.31(71552_19694060)x1,4q35.1q35.2(186154276_191154276)x3	Pathogenic	Wolf Hirschhorn syndrome	TOP 27+4 WG	Female 610g (<3 rd)
10	23+0	AC	46,XY,del(8)(p11.23p22)	arr[hg19] 8p22p11.23(14921564_36502963)x1	Pathogenic	Deletion at 8p22p11.23	TOP 33+1 WG	Male 1740g (<10 th)
11	27+3	AC	47, XXY nuc ish(DXZ1x2,DYZ3x1,D18Z1x2),(RB1x2,DSCR4x2)[50]	arr[hg19](X)x2,(Y)x1	Pathogenic	Klinefelter syndrome	Live birth 37+2 WG	Male 1810g (<3 rd)
12	22+5	AC	46,XY,r(15)(p10qter)	arr[hg19] 15q26.3(98664117_102465355)x1	Pathogenic	Deletion at 15q26.[3]	TOP 26+5 WG	Male 640g (<3 rd)
13	31+0	AC	nuc ish(DXZ1x1,DYZ3x1,D18Z1x3)[40/300]	arr[hg19](1-22)x2,(X,Y)x1	No finding ^b	Trisomy 18 in mosaicism	TOP 36+6 WG	Male 2150g (<3 rd)
14	25+0	AC	45,X[46]/46,XX[4]	arr(X)x1[0,9]	Pathogenic	Turner syndrome	Live birth 39+1 WG	Female 1920g (<3 rd)
15	18+5	AC	46,XX,del(8)(p11.2).ish del(8)(wcp8+,D8S504-,VIJ2yRM2053+).nuc ish(D8S504x1,VIJ2yRM2053x2)	arr[hg19] 8p23.3p11.21(221611_41559138)x1	Pathogenic	Deletion at 8p11.21p23.3	TOP 24+2 WG	Female 505g (<10 th)

AC: amniocentesis; WG: weeks of gestational age; TOP: termination of pregnancy
a according to a French intrauterine growth charts adjusted on infant sex (EPOPé M1)²⁷
b low mosaicism, not detectable using CMA

659 Table 4. Summary of studies assessing the incremental yield of CMA over karyotype in fetuses with isolated fetal growth restriction

First author	Study design	Year Country	Total	Among normal karyotype				Among abnormal karyotype		
				Number of normal karyotype	Pathogenic and likely pathogenic CNVs	VOUS	Pathogenic + likely pathogenic + VOUS	Number of abnormal karyotype	Pathogenic and likely pathogenic CNVs	VOUS
Zhu ^{*31}	Retrospective Single-center	2013-2015 China	53	45	3 (6.7%)	2 (4.4%)	5 (11.1%)	8	5	0
Borrell ^{*37}	Prospective Multicenter	2015 Spain	114	114	7 (6.1%)	0	7 (6.1%)	Not included	-	-
De Witt ^{*38}	Retrospective Multicenter	2011-2015 Netherlands	68	65	1 (1.5%)	Not included	-	3	No CMA performed	No CMA performed
Schaffer ^{*32}	Retrospective Single-center	2004-2011 US	115	115	3 (2.6%)	Not included	-	Not included	-	-
Gruchy ^{*39}	Retrospective Single-center	2009-2010 France	9	9	1 (11.1%)	0	1 (11.1%)	Not included	-	-
Van den Veyver ^{*40}	Retrospective <i>Not specified</i>	2005-2008 US	3	3	0 (0%)	0	0%	0	0	0
Kleeman ^{*41}	Prospective Multicenter	2007-2008 US	3	3	0 (0%)	0	0%	Not included	-	-
Lovrecic ^{*42}	Retrospective Single-center	2012-2015 Slovenia	16	16	0 (0%)	1 (6.2%)	1 (6.2%)	Not reported	-	-
Oneda ^{*43}	Retrospective <i>Not specified</i>	2010-2013 Switzerland	6	6	1 (16.7%)	0	1 (16.7%)	Not included	-	-
An ⁴⁴	Prospective Multicenter	2015-2018 China	127	123	7 (5.7%)	1 (0.8%)	8 (6.5%)	4	3	1
Sagi ¹²	Retrospective Multicenter	2013-2017 Israel	428	427	12 (2.8%)	31 (7.2%)	43 (10.1%)	1	1	0
Peng ⁴⁵	Retrospective Single-center	2013-2017 China	126	120	4 (3.3%)	6 (5.0%)	10 (8.3%)	6	Not reported	Not reported
Brun ⁴⁶	Retrospective Single-center	2012-2017 France	83	78	0 (0%)	3 (3.8%)	3 (3.8%)	5	Not reported	Not reported
Hui ⁴⁷	Retrospective Single-center	2007-2017 China	34	34	2 (5.9%)	Not included	-	Not included	-	-
Present study	Retrospective Multicenter	2016 France	146	139	5 (3.6%)	3 (2.1%)	8 (5.8%)	7	6	0

* Studies included in the meta-analysis of Borrell et al.; Data are n or n (%)

661 **Figure Legend**

662

663 Figure 1. Flow-chart

664 The study population included 146 singleton fetuses diagnosed with isolated FGR who
665 underwent invasive genetic testing with both karyotype and CMA results

Figure 1. Flow diagram of the study population

