Genomic Microarray in Fetuses with Early Growth Restriction: A Multicenter Study

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Keywords

Genomic microarray · Fetal growth restriction · Prenatal diagnosis · Copy number variants

Abstract

Background: Little information is available about the risk of microdeletion and microduplication syndromes in fetal growth restriction (FGR) with a normal karyotype. **Objective:** To assess the incremental yield of genomic microarray over conventional karyotyping in fetuses with early growth restriction. **Study Design:** Genomic microarray was prospectively performed in fetuses with early growth restriction defined as a fetal weight below the 3rd percentile estimated before 32 weeks of pregnancy, and a normal quantitative fluorescent polymerase chain reaction result. The incremental yield of genomic microarray was defined by the rate of fetuses presenting with a pathogenic copy number variant below 10 Mb. **Results:** Among 133 fetuses with early FGR, a 6.8% (95% CI: 2.5–11.0) incremental yield of genomic microarray over karyotyping was observed. This incremental yield was 4.8% (95% CI: 0.2–9.3) in isolated FGR, 10%

(95% CI: 0–20.7) in FGR with nonstructural anomalies, and 10.5% (95% CI: 0–24.3) in FGR with structural anomalies.

Conclusion: Our multicenter study reveals that 6.8% of fetuses with early growth restriction present with submicroscopic anomalies after common aneuploidies were excluded. Even when FGR is observed as an isolated finding, genomic microarray analysis should be considered after or instead of karyotyping, due to its 4.8% incremental yield.

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Introduction

Chromosomal anomalies have been reported to account for up to 19% of fetuses presenting fetal growth restriction (FGR), with triploidy being the most common anomaly in fetuses at <26 weeks, and trisomy 18 at ≥26 weeks of gestation [1]. However, the risk of genetic syndromes in FGR with a normal conventional karyotype is not well established. A classic dysmorphology textbook classified postnatal growth restriction as a feature of several microdeletion syndromes such as Wolf-Hirschhorn

Table 1. Means or proportions (with 95% confidence intervals) of pregnancy demographic characteristics and pregnancy outcomes of the 3 FGR groups

	Isolated FGR (<i>n</i> = 84)	FGR+ nonstructural anomalies (<i>n</i> = 30)	FGR+ structural anomalies (<i>n</i> = 19)
Maternal age, years	34.8 (33.8 to 35.8)	34.1 (32.1 to 36.1)	35.2 (33.1 to 37.4)
Primiparous, %	52 (41 to 63)	63 (46 to 81)	63 (42 to 85)
Smoker, %	15 (7 to 23)	20 (6 to 34)	11 (0 to 24)
Gestational age at procedure, weeks	25.4 (24.4 to 26.5)	26.7 (24.7 to 28.7)	24.4 (21.8 to 27.0)
Weight percentile at procedure	1.5 (1.0 to 1.9)	2.6 (1.3 to 3.9)	1.6 (0.0 to 3.3)
Termination of pregnancy, %	5 (0 to 10)*	13 (1 to 26)	35 (13 to 58)*
Intrauterine demise, %	7 (1 to 12)	13 (1 to 26)	5.9 (0 to 17)
Born alive, %	88 (81 to 95)	73 (58 to 89)	59 (35 to 82)
Gestational age at delivery, weeks	35.6 (34.7 to 36.6)	35.5 (34.8 to 36.4)	35.4 (31.6 to 39.3)
Labor induction, %	60 (48 to 73)	64 (44 to 84)	56 (23 to 88)
Cesarean section, %	65 (54 to 77)	64 (44 to 84)	70 (42 to 98)
Cesarean section for fetal distress, %	15 (7 to 24)	27 (9 to 46)	10 (0 to 29)
Birth weight, g	1,933 (1,727 to 2,140)	1,837 (1,531 to 2,145)	1,989 (1,294 to 2,684)
Weight percentile birth weight	8.2 (5.6 to 10.8)	4.5 (1.9 to 7.0)	8.8 (−2.2 to 19.8)
Neonatal unit admission, %	50 (38 to 62)	55 (34 to 75)	60 (30 to 90)
Neonatal mortality, %	1.5 (0 to 4.5)	9.5 (0 to 22)	20 (0 to 45)

* Statistically different.

syndrome, Rubinstein-Taybi syndrome, Miller-Dieker syndrome, Smith-Magenis syndrome, Di George syndrome, Williams-Beuren syndrome, and of some microduplication syndromes such as the reverse of the Sotos syndrome and XQ distal duplication [2].

Genomic microarray is the genetic technique that can detect microdeletions and microduplications, together with conventional chromosomal anomalies. Submicroscopic DNA gains or losses, undetectable by conventional karyotyping, can be detected with a variable resolution down to 10 kb within the genome. In fetuses with structural anomalies, recent studies have assessed 3–9% excess detection rate in the performance of microarray analysis [3–7], although an FGR group has rarely been considered for analysis. When available, microarray was able to detect pathogenic submicroscopic changes in up to 11% of fetuses with isolated FGR, and in up to 17% of those with FGR associated with fetal structural anomalies [8–11].

In this multicenter study, we present our cohort of 133 fetuses with growth restriction below 32 weeks' gestation undergoing genomic microarray analysis to establish the incremental yield of this technique after common aneuploidies were excluded by quantitative fluorescent polymerase chain reaction (QF-PCR).

Methods

Population

Our study population included consecutive fetuses in which growth restriction was diagnosed before 32 gestational weeks in pregnant women with no signs of preeclampsia, evaluated prospectively with genomic microarray in amniotic fluid at 3 centers of the city of Barcelona, between January 2009 and July 2015. Hospital Universitari Quirón Dexeus (HUQD) participated between January 2009 and December 2015, and the BCNatal team in both Hospital Clínic Barcelona (HCB) and Hospital Sant Joan de Déu (HSJD) participated between January 2013 and December 2015. FGR was defined as an estimated fetal weight below the 3rd percentile according to local reference ranges. Written informed consent was signed from pregnant women before amniocentesis. Information on maternal demographic characteristics, reproductive history, and current pregnancy clinical data at amniocentesis was obtained. QF-PCR was performed after DNA extraction to rule out maternal contamination, to determine the fetal sex (in order to select the reference DNA for microarray analysis), and to exclude maternal contamination and fetal triploidy. Samples of amniotic fluid were first assessed for chromosome 21, 18, 13, X, and Y aneuploidies by QF-PCR [12, 13] and cases with common aneuploidies excluded from further studies.

Genomic Microarray Analysis

The same platform and assays were used to process amniotic fluid samples from the 3 centers by 2 different labs: Hospital Clínic Biomedical Diagnostic Center processed samples from HCB and HSJD, and Labco Diagnostics Barcelona those from HUQD. Test

and reference DNA samples of the same gender were hybridized to whole genome BAC (bacterial artificial chromosome) microarray (CytoChip Focus Constitutional, BlueGnome, Illumina). This microarray has a 1-Mb resolution along the whole genome and 100 kb in 143 constitutional regions associated with pathology. This microarray applies University of California Santa Cruz Genome Browser hg19 (NCBI Build 37, Feb. 2009) as reference assembly. Captured images were quantified and analyzed using the software Bluefuse Multi (BlueGnome, Illumina). Identified copy number variants (CNV) were compared with CNV data available (e.g., Decipher database [http://decipher.sanger.ac.uk], ClinGen database [https://www.clinicalgenome.org/], or ClinVar [http://www.ncbi.nlm.nih.gov/clinvar/]).

Analysis of Results

CNV found at genomic microarray analysis of 10 Mb or more in size were considered detectable by conventional karyotyping, as established by Shaffer et al. [8] and therefore excluded from the study. Incremental yield was calculated as the proportion of the abnormal results nondetectable by karyotyping (CNV <10 Mb at microarray analysis) divided by the total number of cases with an eventual normal karyotype (after excluding CNV ≥10 Mb). Thus, two samples with CNV higher than 10 Mb and normal QF-PCR were excluded from the study. Ninety-five percent confidence intervals (CI) for all ratios were also calculated and when including zero were considered nonsignificant. Statistical analyses were carried out using SPSS 17.0 (Corp.2011, IBM Corp., IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY, USA). FGR was classified into three groups: isolated FGR, FGR with nonstructural anomalies (abnormal amniotic fluid volume, soft markers, minor congenital anomalies), and FGR with structural anomalies (major congenital anomalies). EUROCAT guidelines were used to define major and minor congenital anomalies [14], with the exception of ventricular septal defects (VSD). According to our experience, perimembranous VSD is considered a major birth defect and muscular VSD a minor one [15].

Pregnancy and Fetal Outcome

Pregnancy and fetal and neonatal outcomes were recorded and compared after stratification by FGR groups. Namely, outcomes such as rates of termination of pregnancy, fetal demise, live births, gestational age at delivery, labor induction, cesarean section, cesarean section for fetal distress, birthweight and birthweight percentile, neonatal unit admission, and neonatal mortality were analyzed.

Results

Five hundred thirty-nine fetuses with a suspected FGR before 32 weeks were studied in the 3 participating centers during the study period: 256 at HCB, 125 at HSJD, and 158 at HUQD. Among those, 133 fetuses with an estimated fetal weight below the 3rd percentile diagnosed between 16 and 32 gestational weeks were included in the study. The corresponding rates of early FGR fetuses undergoing microarray analysis at each of the participating

centers were as follows: 23% (60/256) at HCB, 22% (28/125) at HSJD, and 28% (45/158) at HUQD. DNA extraction was performed in amniotic fluid. Cases with abnormal QF-PCR results or CNV greater than 10 Mb were not included in the study. Demographic pregnancy characteristics and pregnancy outcomes are presented in Table 1. Among the 133 fetuses, FGR was an isolated finding in 84 cases, while there were additional ultrasound findings in 49 cases: 30 presented nonstructural anomalies and 19 presented major structural anomalies (Table 2). Among nonstructural anomalies, oligohydramnios, polyhydramnios, cardiomegaly, mild pericardial effusion, and muscular VSD were the most frequent features. On the other hand, ventriculomegaly, hypospadias, and perimembranous VSD were the most frequent major structural defects (Table 2).

Table 2. Fetal nonstructural and structural anomalies associated with FGR

<i>Structural anomalies</i>	
Ventriculomegaly	4
Intracranial cyst	2
Porencephaly	1
Microcephaly	1
Facial clefting	1
Ascites	1
Perimembranous ventricular septal defect	3
Tetralogy of Fallot	1
Cystic malformation of the lungs	1
Hypospadias	3
Bilateral renal dysplasia	1
Total	19
<i>Nonstructural anomalies</i>	
Abnormal amniotic fluid volume	
Oligohydramnios	3
Polyhydramnios	3
Soft markers or minor anomalies	
Prominent cerebral sulci	1
Increased nuchal translucency	1
Retrognathia	1
Cardiomegaly	4
Aberrant right subclavian artery	2
Right cardiac dominance	1
Mild pericardial effusion	3
Muscular ventricular septal defect	3
Periventricular echogenicity	1
Bowel dilatation	1
Intestinal hyperechogenicity	3
Ambiguous genitalia/undescended testes	2
Clubfoot	1
Total	30

Nine pathogenic CNV of less than 10 Mb in size were found, 4 in fetuses with isolated FGR, 3 in nonstructural anomalies, and 2 in malformed fetuses (Table 3). The overall pooled data revealed a 6.8% (95% CI: 2.5–11.0) incremental yield of genomic microarray over conventional karyotyping, namely 4.8% (95% CI: 0.2–9.3) in fetuses with isolated FGR, 10% (95% CI: 0–20.7) in FGR with nonstructural anomalies, and 10.5% (95% CI: 0–24.3) in malformed growth-restricted fetuses. Pathogenic CNV are listed in Table 4. The pathogenic CNV size ranged between 670 kb and 9.9 Mb. No association was observed between the CNV size and specific ultrasound findings. There were 6 microdeletions and 3 microduplications, 5 of them overlapping a known contiguous gene deletion or duplication syndrome region. Four of them have already been described in association with FGR (proximal and distal 22q11.2 microduplication syndrome, 3q29 microdeletion syndrome, and the Williams-Beuren syndrome), whereas the remaining can be considered as an incidental finding in the FGR context. No variants of unknown significance (VOUS) were found in the study group.

The 3 FGR groups showed significant difference outcomes only for termination of pregnancy between iso-

lated FGR and malformed fetuses. Termination of pregnancy was performed in 5% of cases with isolated FGR, in 13% of nonstructural anomalies, and in 35% of FGR cases with structural anomalies. Remarkable but nonsignificant differences were observed in live births, intrauterine demise, and neonatal mortality. With increasing severity of associated anomalies, there was an apparent decrease of live birth from 88% (isolated FGR) to 73% (FGR with nonstructural anomalies) and to 59% (FGR with structural anomalies), and an apparent increase of neonatal mortality from 1.5% (isolated FGR) to 9.5% (FGR with nonstructural anomalies) and to 20% (FGR with structural anomalies).

Discussion

Results derived from this multicenter study indicate that genomic microarray analysis leads to a 6.8% incremental yield over conventional karyotyping in fetuses with early growth restriction, and when stratified according to associated anomalies, this rate increases from 4.8% in isolated FGR to 10% in FGR with nonstructural anomalies and to 10.5% in FGR with major structural anomalies. Since genomic microarray is already recommended in malformed fetuses, the more relevant finding is that there was a 4.8% incremental yield in FGR with no other ultrasound findings.

It is well known that prenatal microarray analysis yields additional clinical value information in malformed fetuses with a normal karyotype. However, most of the microarray studies provide overall detection rates for fetuses with ultrasound anomalies and only a few specify the incremental yield in fetuses with FGR. Thus, published series reported a 6–10% prevalence of pathogenic submicroscopic findings in fetuses with structural anom-

Table 3. Pathogenic CNV rates observed in each of the 3 FGR groups

	Pathogenic CNV	Number of cases	Incremental yield, %
Isolated FGR	4	84	4.8
FGR+ nonstructural anomalies	3	30	10.0
FGR+ structural anomalies	2	19	10.5
Total	9	133	6.8

Table 4. Pathogenic CNVs found in fetuses with early growth restriction

FGR group	Ultrasound findings	Pathogenic CNV	CNV size
Isolated FGR	none	arr 22q11.21(17,662,989x2, 19,172,842-19,843,647x3, 22,691,548x2)	670 kb
Isolated FGR	none	arr Xp22.31(6,223,645x1, 7,239,742-8,153,286x0, 8,227,525x1)	913 kb
Isolated FGR	none	arr 15q26.3(97,368,574x2, 98,678,082-99,607,415x1, 100,187,983x2)	930 kb
Isolated FGR	none	arr 8q21.11q21.3(75,938,201x2, 77,951,651-87,713,030x1, 89,309,494x2)	9.9 Mb
FGR+ nonstructural anomalies	oligohydramnios	arr 3q29(195, 196, 032x2, 195,989, 448-196, 801, 209x1, 197, 225, 550x2)	812 kb
FGR+ nonstructural anomalies	polyhydramnios	arr 22q11.22q11.23(22,691,548x2, 23,146,740-24,319,952x3, 25,254,126x2)	1.17 Mb
FGR+ nonstructural anomalies	polyhydramnios	arr 7q11.23(72,583,171x2, 72,699,013-73,955,362x1, 74,227,093x2)	1.25 Mb
FGR+ structural anomalies	bilateral renal dysplasia	arr 3p26.3(233,708-1,056,880x3,2,299,276x2)	820 kb
FGR+ structural anomalies	hypospadias	arr 2p25.1p24.2(10,064,304x2, 11,291,005-17,228,290x1, 18,714,949x2)	5.9 Mb

alies [3–5]. However, scarce data is available on genomic microarrays applied to FGR. The largest FGR series reported a 2.7% incremental yield in fetuses with isolated growth restriction, 2.4% in FGR with nonstructural anomalies, and 10.3% in FGR with structural anomalies. Interestingly, our series, which is the second biggest cohort, demonstrated a similar rate, 10.5% for malformed fetuses, but nonsignificant higher rates for non-malformed fetuses, 4.8% (isolated FGR) and 10% (FGR+ nonstructural anomalies), which were twofold and fourfold, respectively, compared to those reported by Shaffer et al. [8] ($p = 0.499$ and $p = 0.172$, respectively). The reason for such an apparent difference may be explained by the use of a more astringent inclusion criteria for FGR in our series, considering estimated fetal weight <3rd percentile and gestational age <32 weeks as selection criteria, and not relying on data provided by a diversity of centers in their requisition forms. Two recently reported FGR series, including 45 and 65 fetuses, demonstrated a 2–7% incremental yield in non-malformed FGR fetuses [10, 11]. The remaining published data on FGR included series with less than 10 fetuses and reported incremental yields ranging from 11 to 17% [9].

Regarding the pregnancies with genomic imbalances and associated anomalies, the 3 nonstructural anomalies observed involved an abnormal amniotic fluid amount, either polyhydramnios or oligohydramnios. In relation to the 2 malformed growth-restricted fetuses, both had urogenital anomalies, bilateral renal dysplasia, and hypospadias. Whereas oligohydramnios and hypospadias are common findings in FGR due to placental insufficiency [16], which is the most common cause of FGR, the uncommon association of polyhydramnios with FGR may be a sign of genetic syndromes.

Five of the nine pathogenic imbalances found in this multicenter study have already been described in association with FGR, such as proximal and distal 22q11.2 microduplication syndrome, the 3q29 microdeletion syndrome, the Williams-Beuren syndrome (7q11.23 microdeletion), and the 15q26.3 microdeletion. One of the 22q11.2 region duplications is the reciprocal to the Di George/velocardiofacial syndrome recurrent deletions, while the other one corresponds to distal 22q11.2q11.23 microduplication. Clinical manifestations observed in patients with 22q11 microduplications may range from milder phenotypes to multiple severe defects, sharing some clinical features with the reciprocal microdeletional syndrome, such as intellectual disability, FGR, and cardiac defects [17, 18]. Deletion of chromosome 3q29 region encompasses 22 genes, two of them (*PAK2* and

DGL1) autosomal homologues of known X-linked genes associated with clinical features including intellectual disability, microcephaly, and mild dysmorphisms [19]. The prenatal diagnosis of the Williams-Beuren syndrome is even more challenging because only few phenotypic features can be investigated by fetal ultrasound, although FGR is present in most of them [20]. Finally, the haploinsufficiency due to terminal 15q26.2 deletion encompassing the *IGF1R* gene has been described to result in prenatal and postnatal growth failure, similarly to heterozygous mutation of the *IGF1R* gene [21, 22].

Genomic microarray analysis in FGR pregnancies demonstrated that outcomes differed according to the severity of phenotypic features. Thus, increasing severity of associated anomalies to FGR was related to a progressively less favorable outcome, although the submicroscopic anomaly rate was only related to the presence or absence of associated findings. Termination of pregnancy rates increased from 5 to 35%, live births decreased from 88 to 59%, and neonatal mortality increased from 1.5 to 20% (Table 1). It appears that if FGR is diagnosed without structural anomalies before 32 weeks and chromosomal and submicroscopic anomalies can be ruled out, the prognosis is favorable. In our series, stillbirth occurred in 6–13% of cases, and when not performed prenatally, genomic microarray analysis appears to be particularly useful to provide a genetic diagnosis, since cytogenetic analysis, requiring cell culture, presents high failure rates [23]. Furthermore, nowadays postmortem studies are increasingly not agreed by parents and when performed, may easily overlook the association between FGR and submicroscopic abnormalities.

It may appear that the major limitation of our multicenter study is that microarray was only offered and performed in the most severe cases, and this fact may result in a selection bias leading to an overestimated incremental yield. However, the use of the 3rd percentile of the estimated fetal weight as a selection criterion to define FGR is in accordance with the expert consensus reported recently [24]. Hence, among 539 fetuses with an estimated fetal weight below the 10th percentile, microarray was only performed in the 133 FGR fetuses presenting below the 3rd percentile in absence of a clear clinical context of a preeclampsia. On the contrary, the use of low-resolution array CGH may have resulted in underestimated rates of microdeletion syndromes. In many clinical prenatal settings, low-resolution array CGH is preferred to high-resolution array CGH or SNP arrays, in order to limit the number of VOUS, which may increase maternal anxiety due to uncertain results that cannot be supported

by a postnatal neurodevelopmental evaluation. In fact, no VOUS were found in our study. It is obvious that array CGH cannot diagnose all genetic syndromes. If other approaches, such as SNP arrays, or methylation analysis were used, uniparental disomy and methylation changes, causing Silver-Russell syndrome for instance, could be diagnosed. In addition, exome sequencing may be applied to diagnose monogenic syndromes caused by genomic sequence mutations and can be of additional value to improve the diagnostic yield, as suggested by a recent study in which only a subset of postnatally syndromic phenotypes could be explained by a chromosomal abnormality [11]. Another limitation of the study is the limited postnatal follow-up, restricted to the neonatal period. The main strength of our study was that our series includes the biggest number of fetuses with isolated early FGR assess-

ing the incremental yield of genomic microarray over the karyotype, which is the most clinically relevant group, given that the finding of a malformed fetus is already a well-established indication for genomic microarray analysis.

In summary, this multicenter study demonstrates a 6.8% incremental yield over karyotyping. Even in isolated FGR, 4.8% of those fetuses revealed to be associated with microdeletion or microduplication syndromes, such as 22q11.2 microduplication syndrome. Therefore, genomic microarray analysis should be considered in FGR after exclusion of the most common aneuploidies.

Disclosure Statement

The authors report no conflict of interest.

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