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Effectiveness of QF-PCR, Karyotyping and Microarray in Detecting Clinically Significant Chromosomal Aberrations of Foetuses with Abnormal Findings on Ultrasound

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Abstract

Objective: The aim of this study was to investigate in our area the clinical utility of QF-PCR, karyotyping and CMA for detecting chromosomal aberrations in fetuses with abnormal findings on first or second trimester ultrasound.

Methods: We performed a retrospective analysis of 139 pregnancies with fetal structural anomaly or ultrasound markers.

Results: Chromosomal abnormalities were identified in 28 patients (20.1% of all cases). Twenty-four of theses abnormalities (17.2% of total) were aneuploidies detected by QF-PCR. The remaining 4 chromosomal abnormalities (2.9% of cases) identified in this study were detected by CMA and/or by karyotyping, and only two genomic aberrations of 28 (1.4%) were identified by CMA but not by QF-PCR and conventional cytogenetics.

Conclusion: QF-PCR must remain as the first-line test in prenatal diagnosis. Further studies with a bigger number of cases are desirable to corroborate the low additional detection rate of CMA analysis in our area.

Keywords: Fetal malformations; Soft markers; Chromosomal aberrations; Cytogenetic and molecular methods; Clinical utility

Introduction

Fetal malformations occur in 2% to 3% of fetuses in industrialized countries. Although advanced maternal age may affect pregnancy outcome adversely, 80% to 90% of congenital malformations occur in the absence of a specific risk factor for parents [1]. A proportion of such anomalies can be explained by chromosomal aberrations (aneuploidy, unbalanced translocation, deletions or duplications) [2]. Nevertheless, others may represent recognizable syndromes with another type of DNA alteration [3]. In addition to fetal structural anomaly, some obstetric ultrasound findings considered variants of normal are sometimes observed. They are noteworthy because they also increase the risk for underlying fetal aneuploidy. Five markers: thickened nuchal fold, echogenic bowel, mild ventriculomegaly, echogenic focus in the heart, and choroid plexus cyst, which are named soft markers, should be evaluated in sonogram because they are associated with an increased risk of fetal aneuploidy and represent indication for fetal cytogenetic studies. Others, as single umbilical artery, enlarged cisterna magna, and pyelectasis do not should represent an indication for further studies when they are seen as isolated findings [4].

To evaluate the presence of chromosomal abnormalities in prenatal samples, several methods have been developed, as quantitative Fluorescent Polymerase Chain Reaction (QF-PCR), conventional karyotyping and chromosomal microarray. QF-PCR offers faster turn-around times, but usually only detects abnormalities of chromosomes X, Y, 13, 18, 21 [5,6]. Conventional cytogenetics enables the examination of genome-wide numerical and structural abnormalities, but with a resolution of 5-10 megabases [7]. Chromosome microarray (CMA) presents the advantage of assessing also whole genome aberrations (gains and losses) but with better resolution than karyotyping [8].

In the present retrospective study, we aimed to evaluate the effectiveness of QF-PCR, karyotyping and CMA in detecting chromosomal aberrations of fetuses with abnormal findings on first or second trimester ultrasound, in order to establish in our area the best diagnostic approach for the genetic analysis in these patients.

Methods

Samples

From January 2013 to December 2017, pregnancies with fetal structural anomaly or ultrasound markers (isolated measurement of nuchal translucency (NT) ≥ 99th centile, choroid plexus cyst, echogenic bowel or mild ventriculomegaly, or the presence of several soft markers) observed in first (up to 14 weeks of pregnancy) or second trimester (14-28 weeks) scan were included in the study. Samples were obtained after an invasive method (amniotic fluid or chorionic villi) and analyzed at Reference Laboratory, in Barcelona (Spain). Written informed consent was obtained from participants. QF-PCR, CMA and karyotyping were performed according to diagram showed in Figure 1.

QF-PCR and karyotyping

Genomic DNA for QF-PCR was extracted from 1 mL from uncultured amniotic fluid or chorionic villi using QIAamp DNA Blood Mini Kit (ID 51104, Qiagen, Hilden, Germany), and for 1 mL from maternal blood to exclude maternal cell contamination. Testing for

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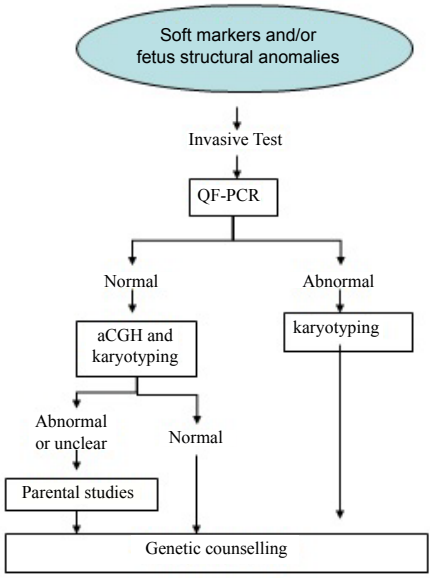

Figure 1: Diagnostic algorithm performed in the samples of the study.

aneuploidies of the chromosomes 13, 18, 21, X or Y was performed using Devyser Compact v3 Kit (Cytogen, Sinn, Germany). Whenever an aneuploidy was detected, conventional karyotyping of the sample was performed to study whether the fetal abnormality could be due to a parental balanced rearrangement. Karyotyping was performed following standard procedures.

CMA

Genomic DNA for array CGH was extracted manually from 8-10 mL from uncultured samples, using QIAamp DNA Mini Kit (ID 51304, Qiagen, Hilden, Germany), following the instructions of the manufacturers. We used the Agilent ISCA design 60 K (Part Number G4827A) array in the SureScan Microarrray Scanner (Agilent Santa Clara, CA, United States). Data were analyzed by CytoGenomics 4.0.3 software (Agilent Santa Clara, CA, United States). The major quality control metrics for Agilent array is the median absolute pairwise difference (MAPD) score. In our diagnostic setting, the values for this parameter needed to be ≤ 0.25 for MAPD.

The identified variants were compared with those recorded in the Database of Genomic Variants (DGV) and in Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER), and classified as pathogenic, probably pathogenic, variants of unknown significance (VOUS), probably benign or benign, as recommend the American College of Medical Genetics standards 9. Genomic formula was assigned according to current ISCN nomenclature. The breakpoint positions of each aberrant region were converted to UCSC hg19 (UCSC Genome Browser, released in February 2009). When clinically relevant or uncertain CNVs was detected additional parental testing was performed to define whether the CNVs had occurred *de novo*, were inherited, or caused by the presence of a parental balanced translocation.

Results

A total of 139 pregnancies were studied. Women age average 32 years-old and gestational age mean was 19 weeks. Twenty-eight patients (20.1%) were referred in the first trimester of pregnancy. In 80, indication for study was the presence of at least one congenital malformation, being almost half of them heart structural anomalies. In 59 patients, the reason for further analysis was the presence of an isolated ultrasound marker or abnormal growth (Table 1). Thickened nuchal fold was the most frequent marker detected. In the remaining 5 patients, it was not possible to know the indication for study.

Chromosomal abnormalities were identified in 28 patients (20.1% of all cases). NT ≥ 99th centile was also the most frequent alteration detected between fetuses with chromosomal abnormalities (43%) (Table 2). Twenty-four of theses abnormalities (17.2% of total) were aneuploidies detected by QF-PCR. The aneuploidies included trisomy 13 (N=1), trisomy 18 (N=3), trisomy 21 (N=14), XXY aneuploidy (N=1) and triploidy (N=5). Karyotyping confirmed the results of QF-PCR in all cases. Except the fetus with trisomy 13, which showed a *de novo* unbalanced robertsonian translocation with karyotype 46, XY, der (13;14) (q10;q10),+13, all trisomies were free. The remaining 4 chromosomal abnormalities (2.9% of cases) identified in this study were detected by CMA and/or by karyotyping (Table 3, cases 1-4). In addition, five VOUS (3.6%) were identified by CMA. Parental study showed that they had been inherited (Table 3, cases 5-9) and, as parents were unaffected, were reclassified as probably benign. Three were gains and one loss of genomic material, all of them with sizes < 1.1 Mb.

As we have mentioned previously, an overview of clinical significant CNVs detected by CMA and/or karyotyping is shown in Table 3. Patient 1 showed two genomic aberrations in CMA and 46, XY, add (8) (p23) chromosomal formula in conventional cytogenetics. Parent studies were normal, so it was a *de novo* alteration. Patient 2 presented the most frequent heterozygous deletion on the long arm of chromosome 22 observed in patients with 22q11 deletion syndrome. It had been

Ultrasound Category	Number of Cases (%)
Structutal abnormalities in multiple systems	5 (3.6)
Structutal abnormality in a single system	69 (49.6)
CNS	8 (5.8)
Heart	29 (20.8)
Respiratory	3 (2.2)
Facial features	4 (2.9)
Respiratory	3 (2.2)
Body Wall	11 (7.9)
Genitourinary	4 (2.9)
Neck and/or body fluids	2 (1.4)
NA	5 (3.6)
Structutal abnormality in a single system + soft mark	6 (4.3)
Abnorma growth. isolated	11 (7.9)
Single soft marker. isolated	48 (34.5)
Thickened nuchal fold	35 (25.2)
Echogenic bowel	3 (2.2)
Mild Ventriculomegaly	9 (6.5)
Choroid plexus cyst	1 (0.7)

Table 1: Summary of abnormal findings observed in pregnancies of the present study. Percentage of sub-categories are related to the number of cases of each category.

Ultrasound Finding	Number of Cases
Thickened nuchal fold	12
Cardiopathy	7
CNS anomaly	2
Fetal hydrops	1
Structural abnormalities in VA	2
Unknown	4

Table 2: Sonografic findings in foetuses with abnormal karyotype.

Cases	Indication for study	Size	Clromosomal region	Star-end position (hg 19)	Event	Outcome
1	Thickened nuchal fold	44.2 Mb	7q31.2q36.3	114.831.296- 159.128.556	Gain	TOP 17 w
		120 Kb	8p23.3	191.530-312.016	Loss	
2	Conotruncal heart disease	2.8 Mb	22q11.21	18.661.724-21.505.417	Loss	TOP 22w
3	Thickened nuchal fold	18.5 Mb	13q32.1-q34	96.546.769-115.092.648	Loss	TOP 18+5w
4	Fallot tetralogy. agenesi	4.1 Mb	3q29	193717225_197837049	Gain	TOP 16w
		3.3 Mb	17p13.3-p13.2	24457_ 3339639	Loss	
5	Congenital talipes equinovarus	1.1 Mb	7p14.3	32837125_33955426	Gain	Live birth at 38+4 w. 3.540 kg
6	Thoracic dysplasia	0.77 Mb	3p14.3	Chr3:54953112-55729830	Gain	Live birth at 38+5 w. 2.800 kg
7	Heart structural anomaly	77 Kb	16p13.3	3 704 209-3 776.980	Loss	Live birth at 37+5w. 4.190 kg
8	Ventriculomegaly	915 Kb	9p24.2	2430897_3346702	Gain	Live birth at 37+1 w. 2.540 kg
9	Single pelvic kidney	620 Kb	Yp11.2	6592868_7213772	Gain	Pregnancy continues

Table 3: CNVs detected in CMA studies. [TOP: Termination of Pregnancy. w: weeks].

inherited from his apparently asymptomatic father. After reviewing clinical history, we noticed that he had been diagnosed of hypocalcemia and idiopathic thrombocytopenia purpura several years ago. In patient 3, a terminal deletion of chromosome 13 with breakpoints at 13q32 and 13q34 was observed. Karyotyping showed that it had been produced due to the formation of a ring chromosome-13. Parents had no visible alterations on karyotyping. Genomic aberrations observed in case 4 had occurred due to an unknown and submicroscopic reciprocal translocation in the parent, which had a maternal family history of recurrent pregnant loss. Genomic aberrations observed in patients 1 and 3 were also identified by conventional karyotyping. That is that only two genomic aberrations of 28 (1.4% of cases) were identified by CMA but not by QF-PCR and conventional cytogenetics.

Discussion

Only in a small percentage of fetuses with ultrasound anomalies a genomic alteration is identified. Nowadays, the chance of finding an abnormal fetal karyotype with current methods is low and ranges from ~9% to ~19% [9]. It is possible that next-generation sequencing (NGS), which is becoming an invaluable tool for clinical diagnostics, increase substantially the identification of underlying etiologies. It could improve prenatal diagnostic yield by identifying pathogenic genetic variants that are below the resolution of CMA and karyotyping [10].

The chromosomal abnormalities identified in fetuses with ultrasound anomalies are mainly aneuploidies, specially trisomy 21, which is the most common abnormality [11]. Our study showed similar detection rates for genetic abnormalities (20.1%) and for common aneuploidy (17.2% of the samples). In addition, we observed that QF-PCR detects almost all anomalies identified. Therefore, considering that QF-PCR enables highest detection rate and accurate diagnosis in prenatal samples and is the cheapest test to identify common aneuploidies, it must be used, as have been proposed by other authors as the first-line test in prenatal genetic studies of first and second trimester pregnancies to exclude aneuploidy before performing array analysis [11].

After excluding aneuploidies, the remaining chromosome abnormalities identified are marker chromosomes and large deletions and duplications [12]. CMA used in prenatal diagnosis must identify deletions and duplications > 400kb [13,14]. Several studies have investigated the effectiveness of this methodology in detecting chromosomal aberrations in fetuses with abnormal ultrasound findings and have established that it is a useful and cost-effective diagnostic tool in pregnancy in the context of fetal abnormality [2,13-17]. The percentage of detection of clinical significant CNVs by CMA in patients with normal routine karyotyping is variable among previous reports, ranges from 1-16%. It may be related to differences in ultrasound anomalies selected for indicating the study of CMA or differences in CMA resolution. In our study, the total number of non-aneuploid, abnormal fetal karyotype that is identified by CMA but not by QF-PCR was low (2.9%).

Conventional karyotyping has minor resolution than CMA and detects bigger CNVs (>5 Mb), but also marker chromosomes. Some authors have proposed that conventional cytogenetics be replaced with microarray testing for all pregnancies with one or more structural anomalies identified on an ultrasound scan, after excluding common aneuploidies and triploidies by QF-PCR [11]. Some authors have reported an increased diagnostic yield for CMA of 6% compared to conventional karyotyping [18]. Nevertheless, as we mentioned before, we detected an additional diagnostic yield lower (1.4%) for CMA. In addition, it is also important to consider that CMA is not free for risks and has the drawback that detects more VOUS than karyotyping, which generates parental anxiety. In our study, five VOUS, classified later as probably benign, was detected by CMA, meanwhile none were identified with the karyotype.

Conclusion

We evaluated the effectiveness of different tests (QF-PCR, conventional karyotyping and microarray analysis) in detecting genetic aberrations in foetuses with abnormal findings in ultrasound. Our results showed that QF-PCR must remain as the first-line test in prenatal diagnosis. Nevertheless, further studies with a bigger number of cases are desirable to corroborate the low additional detection rate of CMA analysis in our area.

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