NEXT GENERATION SEQUENCING-BASED PRENATAL DIAGNOSIS USING CELL-FREE FETAL DNA IN AMNIOTIC FLUID.

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INTRODUCTION

Amniotic fluid supernatant (AFS) is a source of cell-free fetal DNA (cffDNA) that is usually discarded (1). cffDNA in this type of samples has the advantages of being free from maternal and trophoblast derived DNA (2), the amount of cffDNA is about 100-200 times greater than in maternal blood plasma (1), the quality of the DNA may be better than those obtained from amniocytes without culture (2), and it is possible to obtain timely and informative results even for problematic grossly bloody and otherwise compromised AF samples or culture failures (3). Several studies showed good quality results by using cffDNA in Quantitative fluorescence Polymerase Chain Reaction (QF-PCR) and Chromosomal Microarrays (CMA) testing (2, 4), but scarce knowledge is available regarding analysis performed by Next Generation Sequencing (NGS) (5,6).

The aim of this study was to assess the concordance between the results regarding aneuploidy detection between the classic approach (analysis of DNA obtained from amniocytes (amniocytes-DNA)) and those obtained of the analysis of cffDNA in AFS by NGS.

MATHERIAL AND METHODS

cffDNA was obtained from 1 ml of AFS from 30 high-risk pregnancies (assessed by non-invasive prenatal test based on the analysis of cffDNA in maternal blood (NIPT) or by first trimester combined screening). Samples of cffDNA in AFS were analysed with a low-pass whole genome sequencing platform, strictly following the NIPT procedure as described by the manufacturer (Illumina Inc., San Diego, CA, USA). Follow-up information regarding the invasive genetic testing performed in amniocytes by QF-PCR, Fluorescent in situ hybridization (FISH), karyotype or CMA was recorded for all the 30 cases.

RESULTS

Results for the prenatal diagnostic in amniocytes by means of QF-PCR, FISH, karyotype or CMA showed the presence of a trisomy 21 in 5 samples, a trisomy 18 in 4 samples and aneuploidies for the sex chromosomes in 4 samples (two with a trisomy XXY and two with trisomy XYY). The other 17 samples showed no anomaly detected. The concordance of these results with those obtained by NGS was of 96.7%. The only case with discordant results was a pregnancy classified as high-risk for trisomy 18 by NIPT, in which the analysis of cffDNA in amniotic fluid supernatant revealed a mosaic trisomy 18, with a mosaicism degree of approximately 20%. The analysis with QF-PCR of this sample didn't find any anomaly, probably due to limit of resolution of this technique. Although requested, no additional follow-up was obtained, neither regarding pregnancy outcome nor any other genetic testing (pre or postnatally). Detailed results for all the samples are shown in the following table.

Patient	Amniocytes-DNA analysis method	Results of amniocytes-DNA analysis	Results of AFS analysis
1	QF-PCR	NAD	NAD
2	FISH	NAD	NAD
3	QF-PCR	NAD	NAD
4	QF-PCR	NAD	18% Mosaic T18
5	QF-PCR	T21	T21
6	FISH	XYY	XYY
7	FISH	NAD	NAD
8	QF-PCR	T21	T21
9	FISH	XXY	XXY
10	Karyotype	NAD	NAD
11	FISH	NAD	NAD
12	Karyotype	T21	T21
13	Karyotype	T18	T18
14	Karyotype	T18	T18
15	QF-PCR	T21	T21

Patient	Amniocytes-DNA analysis Method	Results of amniocytes-DNA analysis	Results of AFS analysis
16	QF-PCR	T18	T18
17	FISH	XXY	XXY
18	FISH and karyotype	XYY	XYY
19	QF-PCR	T21	T21
20	QF-PCR	T18	T18
21	Karyotype and CMA	NAD	NAD
22	Karyotype and CMA	NAD	NAD
23	Karyotype and CMA	NAD	NAD
24	Karyotype and CMA	NAD	NAD
25	Karyotype and CMA	NAD	NAD
26	Karyotype and CMA	NAD	NAD
27	Karyotype and CMA	NAD	NAD
28	Karyotype and CMA	NAD	NAD
29	Karyotype and CMA	NAD	NAD
30	Karyotype	NAD	NAD

NAD: No Aneuploidy detected; T: Trisomy

CONCLUSION

cffDNA in AFS for NGS testing is a good alternative to the commonly used amniocytes-DNA samples. One of the advantages is the fact that the turn around time for results is significatively faster the one required for a karyotype, and the information obtained is more comprehensive than the one obtained with the QF-PCR, in terms of aneuploidy calling. Furthermore, it could be a good back-up for the conventional techniques of analysis in cases with maternal cell contamination, low cellularity of an amniotic fluid and failure in the culture of amniocytes.

However, some limitations must be taken into account. This limitations are that, alike other molecular testing techniques, NGS fails in detecting polyploidy and balanced chromosomal rearrangements. Regarding Copy Number Variations analysis, new algorithms need to be improved, since currently it is not possible to detect them below a certain size.

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