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Original article

Prenatal microarray comparative genomic hybridization: Experience from the two first years of activity at the Lyon university-hospital

L. Pons [a,*,b], M. Till [a], E. Alix [a], C. Abel [a], D. Boggio [a], A. Bordes [c], J. Caloone [d], F.C. Raskin [e], N. Chatron [a,b,f], M.-P. Cordier [a], A. Fichez [d], A. Labalme [a], C. Lajeunesse [c], É. Liaras [e], M. Massoud [c], J. Miribel [c], E. Ollagnon [a], C. Schluth-Bolard [a,b,f], A. Vichier-Cerf [a], P. Edery [a,b,f], J. Attia [b,e], C. Huissoud [d], R.C. Rudigoz [b,d], J. Massardier [c], P. Gaucherand [b,c], D. Sanlaville [a,b,f]

[a] *Service de génétique, groupement hospitalier Est, HCL, 59, boulevard Pinel, 69677 Bron, France*
[b] *Université Claude-Bernard Lyon 1, 69008 Lyon, France*
[c] *Département d'obstétrique et de gynécologie, groupement hospitalier Est, HCL, 69500 Bron, France*
[d] *Département d'obstétrique et de gynécologie, centre hospitalier de la Croix-Rousse, HCL, 69004 Lyon, France*
[e] *Département d'obstétrique et de gynécologie, centre hospitalier Lyon Sud, HCL, 69310 Pierre-Bénite, France*
[f] *Équipe Gendev, CNRS UMR 5292, Inserm U1028, centre de recherche en neuroscience de Lyon, 69500 Bron, France*

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ABSTRACT

Objectives. – This study aims to describe how microarray comparative genomic hybridization (aCGH) has shifted to become a prenatal diagnosis tool at the Lyon university-hospital.
Materials and methods. – This retrospective study included all patients who were referred in the 3 pluridisciplinary centers for prenatal diagnosis of the Lyon university-hospital and who received a prenatal aCGH between June 2013 and June 2015. aCGH was systematically performed in parallel with a karyotype, using the PréCytoNEM array design.
Results. – A total of 260 microarrays were performed for the following indications: 249 abnormal ultrasounds (95.8%), 7 characterizations of chromosomal rearrangements (2.7%), and 4 twins with no abnormal ultrasounds (1.5%). With a resolution of 1 mega base, we found 235 normal results (90.4%), 23 abnormal results (8.8%) and 2 non-returns (0.8%). For the chromosomal rearrangements visible on the karyotype, aCGH identified all of the 12 unbalanced rearrangements and did not identify the 2 balanced rearrangements. Among the fetuses with normal karyotypes, 11 showed abnormal microarray results, corresponding to unbalanced cryptic chromosomal rearrangements (4.2%).
Conclusion. – Transferring aCGH to a prenatal diagnosis at the Lyon university-hospital has increased the detection rate of chromosomal abnormalities by 4.2% compared to the single karyotype.

Introduction

In France, the prenatal diagnosis was defined by the bioethics law of July 29, 1994. It corresponds to "all medical practices that aim to detect *in utero* a particularly severe affection in embryos or in fetuses" (article L. 2131-1 from the French Public Health Code – *Code de la Santé publique*). Approximately 800 000 children are born in France each year. Almost 3% of living children carry a genetic disorder, a chromosomal abnormality or a major congenital malformation. For stillborn fetuses, this rate reaches 20% (*Agence de la biomédecine*, 2014).

The fetal karyotype is the reference test used to diagnose numerical and structural chromosomal abnormalities, both balanced and unbalanced, with a resolution that can detect anomalies ≥ 5–10 mega bases (Mb) in size. Conventional cytogenetics went through a transformation in the years 2000 with the arrival of microarray comparative genomic hybridization (aCGH), a new pangenomic analysis that could highlight unbalanced chromosomal abnormalities known as copy number variations (CNVs). These anomalies are identified based on the patient's DNA at an average resolution of a 100 kilo bases (kb), a resolution level

Abbreviations: aCGH, Microarray comparative genomic hybridization; CNV, Copy number variation; CPDPN, Pluridisciplinary centers for prenatal diagnosis (for *centres pluridisciplinaires de diagnostic prénatal*); DNA, Deoxyribonucleic acid; FISH, Fluorescence *in situ* hybridization; IUGR, Intra-uterine growth retardation; kb, kilo base; Mb, Mega base; MTOP, Medical termination of pregnancy; qPCR, quantitative polymerase chain reaction; VOUS, Variant of unknown significance.

* Corresponding author. Service de génétique, groupement hospitalier Est, HCL, 69500 Bron, France.
E-mail address: linda.pons@chu-lyon.fr (L. Pons).

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that is a hundred times greater than a karyotype. aCGH therefore emerged as a first-line test when conducting the postnatal diagnosis of intellectual disabilities and congenital disorders, with a 10% increase in the diagnosis rate compared to the standard karyotype [1].

However, aCGH does have limits. It can't detect balanced chromosomal abnormalities, triploidies, low mosaics < 20% and genic anomalies. Furthermore, as in any pangenomic analysis, and given the state of current knowledge, variants of unknown significance (VOUS) can be found (between 5 to 10% of postnatal cases). Unexpected anomalies that are not linked to the indication can also be discovered (incidental findings).

As the technique and interpretation has been refined, aCGH analysis has progressively been transferred to the prenatal period. Since 2006, many international publications have demonstrated its feasibility and usefulness in this sphere [2–17].

This study's aim is to give a retrospective report on the first 2 years of operation of aCGH prescribed in the 3 pluridisciplinary centers for prenatal diagnosis (CPDPN for *Centres pluridisciplinaires de diagnostic prénatal*) of the Lyon university-hospital, from June 2013 to June 2015.

Patients and methods

Patients

This descriptive retrospective study includes all of the patients who were referred in the 3 CPDPNs of the Lyon university-hospital and who benefitted from aCGH analysis in the prenatal period, from June 2013 to June 2015.

The requirements retained for this test were consistent with the French recommendations in the French best practices guide for prenatal aCGH [18]. On the one hand, it consisted in the characterization of chromosomal rearrangements (chromosomal markers; apparently balanced chromosomal rearrangements *de novo* or with abnormal ultrasounds; unbalanced chromosomal rearrangements to be specified). On the other hand, it also consisted in abnormal ultrasounds (increased nuchal translucency [NT] ≥ 3.5 mm; intra-uterine growth retardations (IUGR) < 3rd percentile without an etiology; malformative syndromes). "Soft markers" needs to be discussed on a case by case basis within the CPDPNs. To analyze the data, abnormal ultrasounds were divided into two categories according to the Shaffer et al. classification [19]: structural anomalies (malformations of the central nervous system, of the face, of the heart, of the respiratory tract, of the digestive tract, of the body wall, of the genitourinary tract, of the musculoskeletal system, of the neck and body fluids) and non-structural anomalies (amniotic fluid anomaly, fetal growth anomaly and "soft markers"). Three groups were therefore set up: single malformations (structural anomalies of a single system); polymalformative syndromes (structural anomalies of a single system with non-structural anomalies, structural anomalies of several systems with or without non-structural anomalies); other anomalies (non-structural, isolated or multiple anomalies). In accordance with the Best practice guide, the following factors were excluded from the indications: combined screening test (with a nuchal translucency < 3.5 mm), maternal screening serum alone, advanced maternal age, and maternal anxiety.

Methods

A well-informed member of the CPDPN systematically explained the aCGH procedure to the couple, and a specific informational sheet was handed out before the microarray test was conducted. This sheet is available in the annex of the Best practice guide [18]. If the pregnant woman agreed to the test, she had to sign an informed consent form in which it was written that she had acquainted herself with the content of the informational sheet (Fig. 1).

Karyotype

A standard karyotype was systematically conducted on cultured amniocytes using GTG banding and RHG banding techniques, in parallel with the microarray.

Fluorescence in situ *hybridization (FISH)*

A FISH technique on non-cultured amniocytes was systematically conducted before any microarray analysis, in order to detect

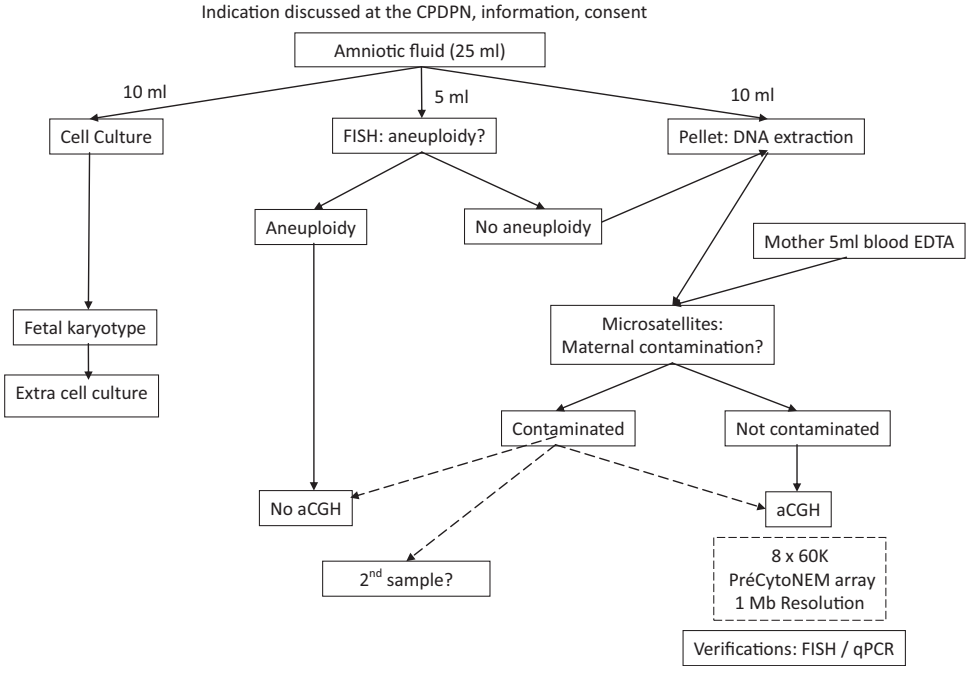

Fig. 1. aCGH practical realization at the Lyon university-hospital.

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