CASE REPORT

Detection of genomic imbalances by comparative genomic hybridization in Chinese fetuses with malformations

Yu Wang [1], Jin-Lai Meng [1], Xie-Tong Wang [1], Yue-Ran Zhao [2], Lai-Cheng Wang [2], Joe Leigh Simpson [3] & Zi-Jiang Chen [1]

1. Department of Obstetrics and Gynecology, Shandong Provincial Hospital of Shandong University, Jinan, Shandong, P. R. China
2. Central Laboratory, Shandong Provincial Hospital, Jinan, Shandong, P. R. China
3. College of Medcine, Florida Internation University, Miami, USA

Correspondence to: Zi-Jiang Chen,
Department of Obstetrics and Gynecology,
Shandong Provincial Hospital of Shandong University,
324 Jing-Wu Road, Jinan 250021, P. R. China
EMAIL: chenzijiang59@sina.com

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Abstract

AIM: The purpose of the study was to evaluate the feasibility and reliability of comparative genomic hybridization (CGH) in the detection of genomic imbalances in Chinese malformed fetuses.

METHODS: Genomic DNA was extracted from umbilical cord blood or fresh amniotic fluid of 9 malformed fetuses and labeled with SpectrumGreen dUTP or SpectrumRed dUTP. A pair of CGH analyses in which the fluorochromes were exchanged was carried out for each sample.

RESULTS: Samples from 9 malformed fetuses were analyzed successfully by CGH. Numerical chromosome aberrations were detected in samples from cases 4, 8 and 9, and were verified by fluorochrome-exchanged CGH. Trisomy 21q was detected in case 4, del 2p24-pter and dup 12p13 was detected in case 8, and del 1p33-pter and del 22q11–12 were detected in case 9.

CONCLUSION: CGH is a reliable technique for the detection of genomic imbalances. Fluorochrome-exchanged CGH can reduce inconsistencies in the results caused by deviations in the process of DNA labeling and hybridization, and increase the accuracy and reliability in cases when conventional cytogenetic analysis is unavailable.

INTRODUCTION

Congenital anomalies, which are present in approximately 10% of newborn infants [1], have become the main cause of infant mortality and are associated with long term morbidity [2]. Genomic imbalances are a significant component of their etiology. Approximately 90% of chromosomal abnormalities found prenatally are aneuploidies; the rest are balanced structural rearrangements or polyploidies [3]. However, despite thorough clinical examination and routine laboratory investigations, the causes of defects in many patients with congenital malformations remain unexplained [4]. Comparative genomic hybridization (CGH) is a molecular cytogenetic technique that permits screening the entire genome for unbalanced chromosomal aberrations in a single experiment. The CGH technique overcomes the technical problems related to tissue culture and has been applied primarily in cancer genetics [5,6] and clinical cytogenetics [7,8]. CGH is an important technique widely used internationally, but the use of CGH is few in China. The present study was undertaken to evaluate the reliability of CGH for the detection of aneuploidy in Chinese malformed fetuses.

MATERIALS AND METHODS

Fetuses were chosen from medically terminated pregnancies (n=8) and pregnancies which had ended in spontaneous fetal death (n=1) at the Department of Obstetrics of Shandong Provincial Hospital in Jinan, China from July, 2006 to January, 2007. All fetuses presented at least two anomalies, identified by ultrasound examination or after birth, in the cardiovascular, skeletal, urogenital, or central nervous systems. Either 5–10 ml amniotic fluid or 2–5 ml umbilical cord blood was obtained for these studies.

Genomic DNA was extracted from amniotic fluid or umbilical cord blood samples using a Tiangen DNA extraction kit (Tiangen Biotech (Beijing) Co., Ltd.) according to the manufacturer's protocol. DNA concentration was determined by DU® series 600 spectrophotometer (Beckman Instruments Inc., Fullerton, USA).

Normal male metaphase target slides for CGH experiments were provided by Abbott Molecular Inc. (Des Plaines, IL, USA). Sample DNA was labeled with SpectrumGreen dUTP or SpectrumRed dUTP using the Vysis nick translation kit (Des Plaines, IL, USA) according to the supplier's recommendation. The reaction was performed for 0.5 to 1 h at 15 °C to obtain a fragment length of 0.5–3.0 kb.

CGH: Probes were prepared by mixing 200 ng of SpectrumGreen dUTP labeled sample DNA, 200 ng SpectrumRed labeled male human genomic DNA (Cat. number 32-804024) and 10 µg of unlabeled Human Cot-1 DNA (Cat. number 32-800028). The probe mixture was precipitated in 3 M sodium acetate and ethanol and resuspended in 7 µl CGH hybridization buffer and 3 µl purified H_2O. Modifications to the standard protocol were made as follows. The probe mix was added to the hybridization site on the normal male metaphase target slide and covered with a sealing film. The slide and probe mix were co-denatured at 73 °C for 5 min and placed in a moist chamber at 37 °C for 48–72 h. Following hybridization slides were washed in 0.4 × SSC/0.3% NP-40 solution at 74 °C for 3–6 s, followed by 2 × SSC/0.1% NP-40 solution at room temperature for 1–3 s. Target chromosomes were then counterstained with DAPI II.

Table 1. Summary of clinical findings and outcome and karyotype detected by CGH.

Sample number	Clinical findings and outcome	Karyotype according to CGH
Case 1	Delivery at 38 weeks, female, 2 400 g, arthrogryposis of left wrist, bilateral clubfoot, clinodactyly of fingers.	Balanced karyotype
Case 2	MRI: Hydrocephalus, enlarged lateral ventricle. Delivery at 33 weeks, male.	Balanced karyotype
Case 3	IUFD at 20 weeks, hydrops fetalis, polyhydramnios, craniofacial dysmorphism, enlarged lateral ventricle. Delivery at 20 weeks, male.	Balanced karyotype
Case 4	Ultrasound: dextrocardia, enlarged right atrium and ventricle. Delivery at 32 weeks, male, hypertelorism, hypophalangism of little finger.	Dup 21q
Case 5	Delivery at 40 weeks, male, 2 450 g, neonatal death, VSD, undescended testes.	Balanced karyotype
Case 6	Delivery at 35 weeks, male, VSD.	Balanced karyotype
Case 7	Ultrasound: dislocation of great vessels. Delivery at 37 weeks, male, 3 360 g.	Balanced karyotype
Case 8	Ultrasound: absence of long bones of the limbs, polyhydramnios, ambiguous intracranial structures. Delivery at 28 weeks, male, 1 140 g, shortened limbs, hypertelorism.	Del 2p24-pter Dup 12p13
Case 9	Ultrasound: complex cardiac defects: VSD, pulmonary stenosis, dislocation of great vessels. Delivery at 41 weeks, male, 3 470 g, cleft lip.	Del 1p33-pter Del 22q11–12

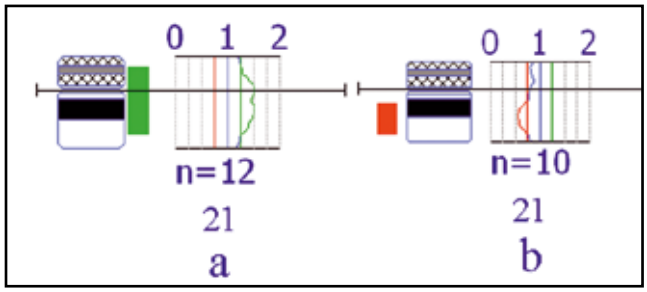

Figure 1. Partial average FR profiles of case 4 acquired by CGH analysis: (a)green-labeled sample genomic DNA co-hybridized with normal male reference (red) DNA to normal male metaphase spread; (b) red-labeled sample genomic DNA co-hybridized with normal male reference (green) DNA. Duplication of chromosome 21q is visible in both.

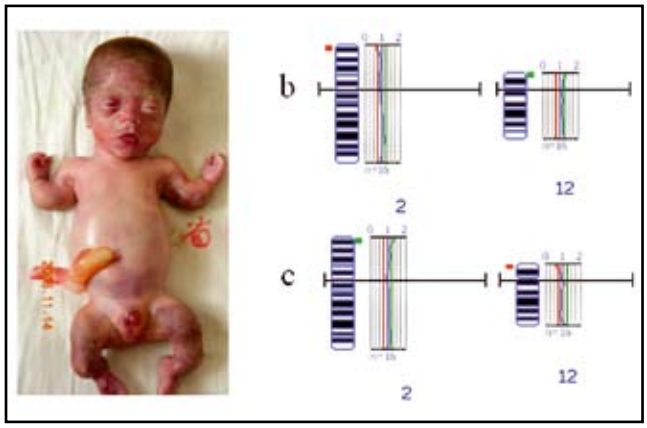

Figure 2. Case 8 photo (28 weeks) and partial average FR profiles acquired by CGH analysis: (a) extremely shortened limbs and hypertelorism can be seen; (b) CGH analysis. Sample genomic DNA was labeled with SpectrumGreen dUTP and co-hybridized with normal male reference (red) DNA. Del 2p24–25 and dup 12p13 are identified; (c) Fluorochrome–exchanged CGH. The same chromosome aberrations can be verified.

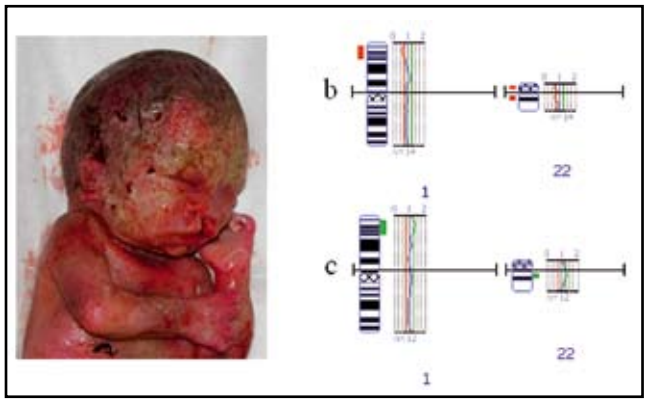

Figure 3. Case 9 photo (41 weeks) and partial average FR profiles acquired by CGH analysis: (a) right cleft lip and palate could be seen; (b) CGH analysis. Sample genomic DNA was labeled with SpectrumGreen dUTP and co-hybridized with normal male reference (red) DNA. Del 1p33-pter and del 22p12–13 and 22q11–12 are identified; (c) Fluorochrome–exchanged CGH. Del 1p33-pter and 22q11–12 are verified. Del 22p12–13 was not be verified.

Fluorochrome-exchanged CGH: Sample DNA was labeled with SpectrumRed dUTP and genomic DNA was labeled with SpectrumGreen. The protocol was otherwise identical to the CGH protocol outlined above.

CGH slides were analyzed on a Leica DMRA epifluorescence microscope equipped with a CCD Leica DC 350 F camera (Leica Microsystems, Wetzlar, Germany) using specific filter sets for DAPI, SpectrumGreen and SpectrumRed signals. For each sample, 5 to 10 metaphase spreads with high uniform hybridization and fluorescence intensity were chosen for image analysis. A sequence of blue, green and red digital images was acquired under VideoTesT CGH software control (NatureGene Corp., USA). Karyotyping was performed based on DAPI banding pattern. A fluorescence intensity ratio (FR) profile was calculated after background correction and normalization of the green to red ratio for each metaphase to 1.0. Mean ratio profiles for each chromosome were determined after data from all analyzed metaphases were combined. Trisomies or partial chromosome gains were defined as FR>1.25. Monosomies or partial chromosome losses were defined as FR<0.75 [9].

RESULTS

Each of the 9 samples was analyzed successfully for numerical chromosome aberrations by CGH (Table 1).

Numerical chromosome aberrations were identified by CGH in three cases, 4, 8 and 9, and were verified by fluorochrome-exchanged CGH. For case 4, trisomy 21 was detected by CGH (when sample DNA was labeled green), but by fluorochrome-exchanged CGH, only trisomy 21q was detected. Because of the prevalence of heterochromatic DNA, 21p was excluded from analysis (Figure 1). For case 8, deletion 2p24-pter and duplication 12p13 were identified by CGH and verified by fluorochrome-exchanged CGH (Figure 2). For case 9, the unbalanced karyotype of del 1p33-pter and del 22q11–12 was identified, although 22p had to be excluded from analysis because of heterochromatic DNA (Figure 3). Among the remaining cases, mean ratio profiles obtained by CGH indicated balanced karyotypes.

DISCUSSION

CGH is a relatively fast screening technique that enables detection of chromosomal copy number changes and provides a global overview of chromosomal gains and losses throughout the genome.

CGH requires no extensive series of specific DNA probes and no prior knowledge of the genomic region to be studied. Because there is no need for culturing cells, chorionic villi and amniotic fluid can be analyzed directly by CGH in spontaneous abortions [10,11], prenatal and neonatal screening [2,4,7], and single cell detection of PGD [12,13,14]. From these recent studies we can conclude that CGH is a valuable alternative to

conventional cytogenetic analysis for rapid identification of unbalanced chromosomal aberrations [3,7,8].

In our study, using samples of amniocytes or umbilical cord blood, numerical chromosome aberrations were successfully detected in 3 of 9 cases of fetal malformation. Because samples for conventional cytogenetic analysis were difficult to acquire, we carried out fluorochrome-exchanged CGH and obtained largely consistent results. We think that exchanging the labeled fluorochrome can reduce inconsistencies in the results caused by deviations in the process of DNA labeling and hybridization, and can increase the accuracy and reliability of analysis in cases when conventional cytogenetic analysis is unavailable.

Results from fluorochrome-exchanged CGH analysis were not completely consistent in cases 4 and 9. In these samples FR profiles deviated from the diagnostic ranges in the centromeric regions of acrocentric chromosomes 21 and 22 and had to be discounted during CGH interpretation. FR profiles are known to deviate from the diagnostic range in chromosomal regions with high concentrations of repeat sequences such as heterochromatic regions 1qh, 9qh, 16qh and Yqh, centromeric regions of acrocentric chromosomes 13, 14, 15, 21, and 22, and telomeres of most chromosomes [7]. Nevertheless, Ghaffari showed that the CGH technique could be successfully adapted for detecting cryptic telomeric unbalanced chromosomal rearrangements. Those regions should, however, be interpreted with caution [15].

Despite of its inability to detect balanced chromosomal translocations, inversions, ring chromosomes, weak mosaicism and ploidy changes, CGH can provide a safe and accurate alternative to traditional banding analysis, at least in the detection of aneuploidy. The use of CGH has a large potential in detection of aneuploidy of malformed fetuses in populous countries, especially in China.

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