RESEARCH NOTE

Array-CGH detection of three cryptic submicroscopic imbalances in a complex chromosome rearrangement

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Introduction

Complex chromosomal rearrangements (CCRs) are structural aberrations involving three or more breakpoints on two or more chromosomes (Pai *et al*. 1980). They are extremely rare and are classified based on the origin, number of breakpoints (Kousseff *et al*. 1993), or number of chromosomes involved (Kausch *et al*. 1988). Chromosomal rearrangement is assumed to be balanced in phenotypically normal individuals. If an individual is phenotypically abnormal, some submicroscopic imbalance or other genetic defect presumably exists (Zwaigenbaum *et al*. 2005). The phenotype caused by a deletion or duplication may be mild and not indicative of a chromosome abnormality, in particular, small deletions or duplications in certain domains might pass unnoticed and their phenotypes could be considered to be within normal limits (Ness *et al*. 2002).

Here, we describe a phenotypically normal prenatal case who was found to carry an apparently balanced CCR on the basis of G-banding and FISH. The rearrangement, which involved three chromosomes and three breakpoints, was studied by high-resolution genomic array, and three cryptic imbalances were detected.

Clinical history

A 39-year-old G4P0 Chinese woman who had a high risk value of Down's syndrome screening (1/132 for trisomy 21(< 1/270) and 1/60 for trisomy 18(< 1/350)) at 16 weeks gestation requested the cytogenetic analysis because of anxiety. The couple was nonconsanguineous and healthy. The mother denied any exposure to alcohol, teratogenic agents, irradiation or infectious diseases during pregnancy. The morphological ultrasound evaluation revealed no abnormalities. The analysis of the cultured amniotic fluid cells yielded 100% of metaphases with chromosomal abnormalities. We found a complicated cytogenetic profile with G-banding which involved a chromosome rearrangement in at least three chromosomes. After careful consideration, the couple elected to undergo termination of pregnancy. At termination, the fetus was found to be a female without congenital malformation.

Materials and methods

Classical cytogenetic analysis

The karyotype analysis of the proband and her parents was performed on cord blood lymphocytes and peripheral blood lymphocytes using the standard G-banding technique. A total of 28 G-banded metaphases were examined with the complete analysis of four karyotypes. Written informed consent was obtained from the parents of proband. This study was performed according to the guidelines of Chongqing Medical University, which abides by the Helsinki Declaration on ethical principles for medical research involving human subjects.

Fluorescence in situ hybridization (FISH)

FISH studies with whole chromosome painting (WCP) probes for chromosomes 4, 5, and 15 (Kreatech, Amsterdam, The Netherlands) were performed on metaphase spreads according to the manufacturer's specifications.

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Array comparative genomic hybridization

Oligonucleotide array-CGH analysis was carried out using genomic DNA obtained from cultured cord blood lymphocytes and parental peripheral blood lymphocytes. Reference DNA was from normal human female genomic DNA. This was performed using Agilent Technologies Array-CGH kits (Agilent Technologies, Santa Clara, USA). This platform is a 60-mer oligonucleotide-based microarray that allows genome-wide survey and molecular profiling of genomic aberrations with a resolution of ~35 kb (kit 244A). DNA from the cases and reference were amplified by GenomePlex® (Sigma, Santa Clara, USA). After purification, each amplified sample was labelled by random priming (Agilent Technologies, Santa Clara, USA) for 2 h using Cy5-dUTP for cases DNA and Cy3-dUTP for reference DNA. Labelled products were column purified. After probe denaturation and preannealing with 50 μg of Cot-1 DNA, hybridization was performed at 65°C with rotation for 40 h. After two steps of washing, the arrays were analysed using an Agilent scanner and Feature Extraction V.9.5.3 software Agilent Technologies, Santa Clara, USA. Data were analysed by means of the CGHAnalytics 4.0 software (Agilent Technologies, Santa Clara, USA). The aberration detection method 2 (ADM-2) algorithm was used to identify aberrant intervals. For the assessment of copy number duplications and deletions, we employed $\log_2$ ratio test/control thresholds of +0.30 and −0.30, respectively. Deviant signal intensity ratios involving four or more neighbouring probes were considered as genomic aberrations.

Results

Conventional G-banded metaphase chromosomes from cord blood lymphocytes revealed an apparently balanced CCR t(4;5;15) (4pter→4q23::15q23→15qter; 4qter→4q23::5p15→5qter; 15pter→15q23::) (figure 1). Karyotypic analysis of the parents were normal.

Figure 1. Partial G-banded fetal karyotype showing the complex chromosome rearrangement involving chromosomes 4, 5 and 15 (the derivative chromosomes are on the right).

Multicolour FISH using whole chromosome painting probes WCP4, WCP5 and WCP15 confirmed the translocation among chromosomes 4, 5 and 15 (figure 2).

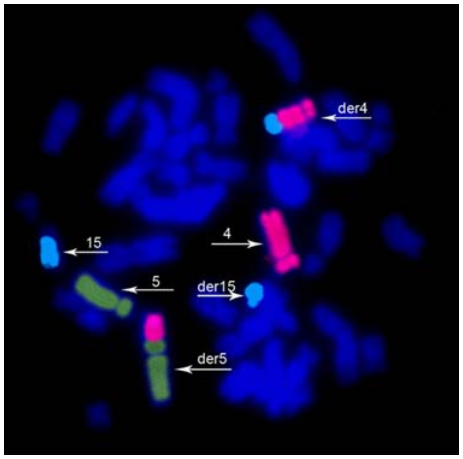

Figure 2. FISH results in the fetus with WCP4 (red), WCP5 (green) and WCP15 (aqua).

Three cryptic submicroscopic imbalances including two microdeletions and one microduplication were identified from the fetus by array-CGH (figure 3a–c). One microdeletion was located at 15q11.2 (18657188–20080135 bp from the chromosome 15 short arm telomere, ~1.42 Mb), the other was located at 18p11.31-p11.23 (7069849–7567290 bp from the chromosome 18 short arm telomere, ~0.49 Mb), and the microduplication was located at 5q13.2 (69274233–70622915 bp from the chromosome 5 short arm telomere, ~1.35 Mb). The dup(5)(q13.2) and del(15)(q11.2) were also identified from the father, indicating they were of paternal origin. The del(18)(p11.31p11.23), which was not present in parent was *de novo*.

Discussion

Only large aberrations (>3Mb) including deletions, duplications and unbalanced translocations can be identified by conventional cytogenetic techniques due to limited resolution, which makes these tools unreliable for detecting submicroscopic imbalances (Gribble *et al*. 2005). Therefore, some apparently 'balanced' CCRs detected by conventional cytogenetic techniques usually host submicroscopic imbalances (Tyson *et al*. 2004; Cheung *et al*. 2005; Gribble *et al*. 2005; Borg *et al*. 2007). Precise definitions of CCRs and their real complexity can only be provided by means of molecular cytogenetic techniques. The CCR described here was initially interpreted as balanced on the basis of conventional cytogenetics. At the molecular cytogenetic level, however, one can only use the term apparently balanced. To investigate the possibility of any genomic imbalance as a result of the complex rearrangement, genome-wide high-resolution array-CGH was performed. Three cryptic submicroscopic imbalances dup(5)(q13.2), del(15)(q11.2) and del(18)(p11.31p11.23) were identified and mapped, indicating array-CGH could provide a more precise delineation of

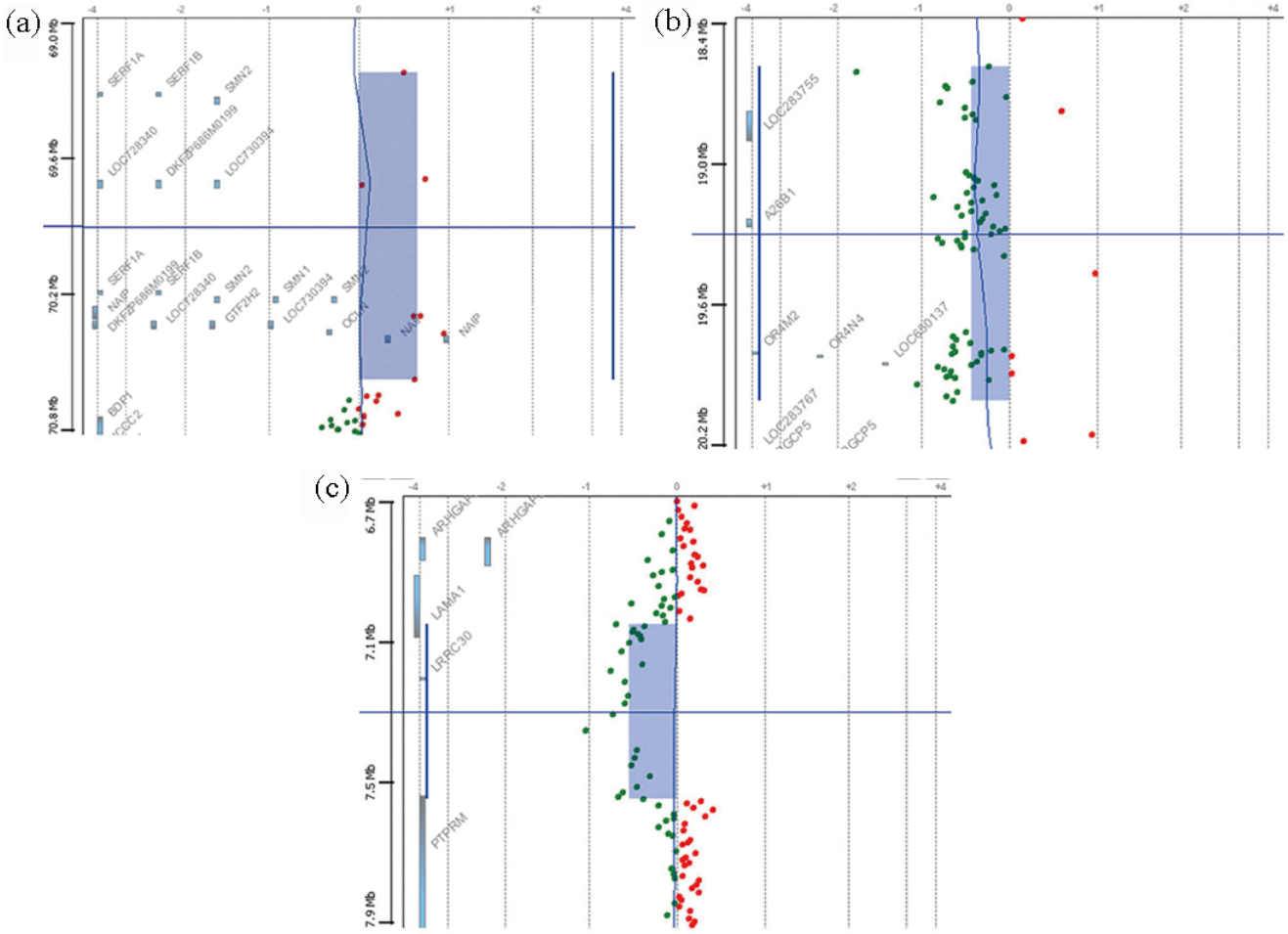

Figure 3. The array-CGH results in the fetus. In each figure, longitudinal axis represents the position of probes located in genome and horizontal axis represents the $\log_2$ ratio test/control of the probes. Each dot represents a single probe spotted on the array. Dots with a value of zero represent equal fluorescence intensity ratio between sample and contrast. (a) Duplication (5q13.2) shifts the ratio to the right (value $> +0.30$), (b and c) deletions (15q11.2 and 18 p11.31-11.23) shift the ratio to the left (value < -0.30).

the submicroscopic chromosomal structural abnormalities. Interestingly, all the submicroscopic imbalances located outside the breakpoint regions (4q23, 5p15 and 15p23), suggesting that imbalances associated with CCRs are not always located at the breakpoints.

Most carriers of balanced chromosomal rearrangements are phenotypically normal. An abnormal phenotype associated with an apparently balanced rearrangements may be attributed to: (i) chromosome rearrangements that disrupt, inactivate, or activate genes at the breakpoints or cause the expression of a recessive gene (Bodrug *et al*. 1990; Hearn *et al*. 2002; Pichon *et al*. 2004), (ii) position effect with variable expression of genes near the translocation breakpoint (Kleinjan and van Heyningen 2005), (iii) the rearrangement may host 'cryptic' imbalance. CCRs with cryptic imbalances are usually associated with phenotypic abnormalities (Tyson *et al*. 2004; Cheung *et al*. 2005; Gribble *et al*. 2005; Borg *et al*. 2007). However, although three submicroscopic imbalances were identified from our case, no congenital malformation was observed. This may be because the breakpoints at 4q23, 5p15 and 15q23 do not disrupt any functional genes and the phenotypes of three submicroscopic imbalances could be considered to be within normal limits. The dup(5)(q13.2) and del(15)(q11.2), which are inherited from the father and overlap with the CNVs (copy number variations) reported in the public database (the Database of Genomic Variants (http://projects.tcag.ca/variation/)), are likely to be benign. The *de novo* del(18)(p11.31p11.23), however, is proposed to be pathogenic. Because it is not reported in the Database of Genomic Variants, but overlaps with the CNVs reported in the public database (the database of chromosomal imbalance and phenotype in humans using ensemble resources (DECIPHER), http://www.sanger.ac.uk/PostGenomics/decipher/). The phenotype caused by del(18)(p11.31p11.23), however, may be very mild and pass unnoticed. In addition, it is impossible to determine whether

our case would have gone on to have mental or behavioural problems, as the pregnancy was terminated. Patients who had *de novo* CCRs and normal phenotype in the neonatal period but then showed relatively mild mental retardation, impaired speech development or psychomotor retardation, have been reported (Bogart *et al*. 1986; Peschka *et al*. 1999; Chen *et al*. 2006). Further, Rosenberg *et al*. (2005) reported a CCR case, who harboured a cryptic imbalance, and exhibited behavioural problems as the sole phenotypic abnormality.

In conclusion, this study provides evidence that apparently balanced CCRs classified by conventional cytogenetic techniques may host additional chromosomal imbalances which are not always located at the breakpoints, and array-CGH is a useful tool for detecting and mapping such cryptic submicroscopic imbalances.

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