ELECTRONIC LETTER

Comparative genomic hybridisation shows a partial de novo deletion 16p11.2 in a neonate with multiple congenital malformations

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Chromosome alterations, including numerical and structural chromosome rearrangements, are implicated in abnormal fetal development and congenital malformations. At least 50% of all first trimester spontaneous abortions are cytogenetically abnormal and about 6% of all postnatal congenital malformations are related to visible cytogenetic alterations detected by conventional G banding.^{1,2} These percentages will probably increase with the use of molecular cytogenetic methods such as fluorescence in situ hybridisation (FISH) and comparative genomic hybridisation (CGH) in prenatal and postnatal diagnosis,³ because they will allow the detection of chromosome abnormalities that are not identified at present. The recurrent association of particular chromosome aberrations with specific clinical features has defined many chromosomal syndromes. In order to facilitate the identification of genes involved in specific human malformations, Brewer *et al*⁴ have constructed a chromosome map of autosomal deletions (non-mosaic) associated with 47 different congenital malformations in 1753 patients. In this review, no congenital malformations were related to anomalies of 16p. So far, only the ATR-16 syndrome (α thalassaemia-retardation-16) associated with 16p13.3 deletion has been described.⁵⁻⁷ Interstitial deletions are relatively rare chromosomal anomalies that usually arise de novo. Here we describe multiple congenital malformations associated with a de novo interstitial chromosome deletion 16p11.2 confirmed by CGH. This is the first case reported with a phenotype-genotype correlation for this chromosome band.

CASE REPORT

An ultrasound examination at 20 weeks of gestation showed the presence of cardiac defects and unilateral multiple renal

cysts. Conventional cytogenetic analyses carried out on amniotic fluid samples showed a normal karyotype. The presence of trisomies 13, 18, and 21 was excluded using FISH on uncultured amniocytes. The couple decided to continue the pregnancy. The boy was born after an uncomplicated 40 week gestation by emergency caesarean section. He showed severe intrauterine growth retardation (weight 2080 g, <5th centile) and multiple congenital malformations were observed on clinical examination (table 1). He was admitted to the intensive care unit, but developed cardiac decompensation and died at the age of 5 months.

MATERIALS AND METHODS

Cytogenetic analysis

Peripheral blood samples obtained from the neonate and his parents were cultured for 72 hours in RPMI medium, supplemented with 20% fetal calf serum and phytohaemagglutinin. Metaphase chromosomes were analysed by the standard Wright G banding technique.

CGH analysis

DNA isolation and labelling, hybridisation, and detection were performed essentially as described by Kallioniemi *et al*⁸ (and according to the instructions of the supplier (Vysis Downer Grove, IL). Briefly, test DNA was isolated from leucocytes of the newborn and control DNA was prepared from blood of a healthy male. DNA was labelled with spectrum Red-dUTP and spectrum Green-dUTP, respectively, following standard nick translation using a commercial kit. The 1:1 probe mixture (700 ng) was hybridised in combination with unlabelled human Cot-1 DNA to normal male metaphase spreads. Slides were analysed using a Cytovision Ultra Workstation (Applied Imaging, Sunderland, UK). The software performed a calculation of the fluorescent ratios of the patient DNA to normal DNA along the length of each chromosome. Ratio values of CGH above 1.25 and below 0.75 were considered to represent chromosomal gains and losses, respectively.

RESULTS

Evaluation of G banded chromosome preparations from phytohaemagglutinin stimulated lymphocytes of the proband showed a non-mosaic 46,XY,del(16p) (fig 1) in the 40 cells analysed. A retrospective analysis confirmed the deletion on karyotyping of amniocytes. It is interesting to note the maternal origin of the deleted chromosome, because the normal chromosome originated from a 16qh+ chromosome of the father. This is a de novo abnormality, because both parents were cytogenetically normal.

Figure 1 Two different pairs of chromosomes 16 from the proband showing the normal (left) and the deleted chromosome (right). The ideograms illustrate the deletion of band 16p11.2 on the abnormal chromosome 16.

Abbreviations: CGH, comparative genomic hybridisation; APKD, adult polycystic kidney disease

Table 1 Clinical findings in our patient

Craniofacial	Flat facies, microretrognathia, blepharophimosis, short nose with hypoplastic alae nasi and absent nasal bridge, low set and malformed ears, and glossoptosis with hypoplastic palate
Cardiovascular	Tetralogy of Fallot with pulmonary atresia
Skeletal	Articular limitation, cubital deviation of hands, talipes varus, and hemivertebra at L1 level
Genitourinary	Unilateral renal agenesis (left kidney) and cryptorchidism
Ocular	Coloboma and unilateral chorioretinitis (right eye)

marker chromosomes respectively, when conventional cytogenetic banding and FISH methods had failed to identify their origin. In our case, we found no problem in characterising the deletion 16p11.2 by CGH, even taking into account its proximity to the heterochromatic region. These findings seem to indicate that the criteria applied to neoplasms are not necessarily applicable to constitutional chromosome abnormalities. Moreover, this report shows the utility of CGH for the characterisation of single copy deletions larger than 5 Mb.

This is the first published case to describe multiple congenital malformations associated with chromosome deletion 16p11.2. Our findings confirm that the combined use of conventional G banding and CGH methods may facilitate phenotype-genotype correlation and can contribute to mapping chromosome regions containing genes implicated in these congenital malformations.

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