

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

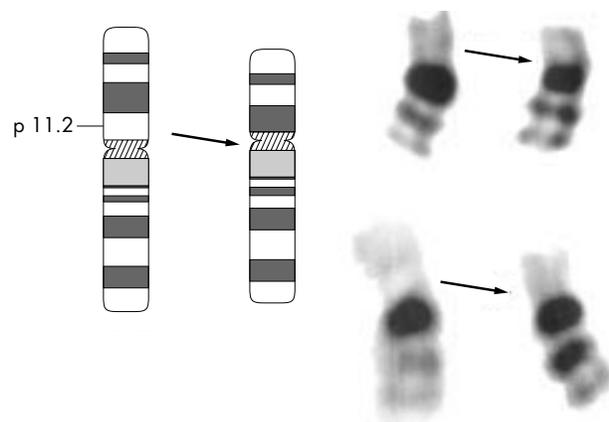


Figure 1 Two different pairs of chromosomes 16 from the proband showing the normal (left) and the deleted chromosome (right). The ideograms illustrate the deletion of band 16p11.2 on the abnormal chromosome 16.

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.

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

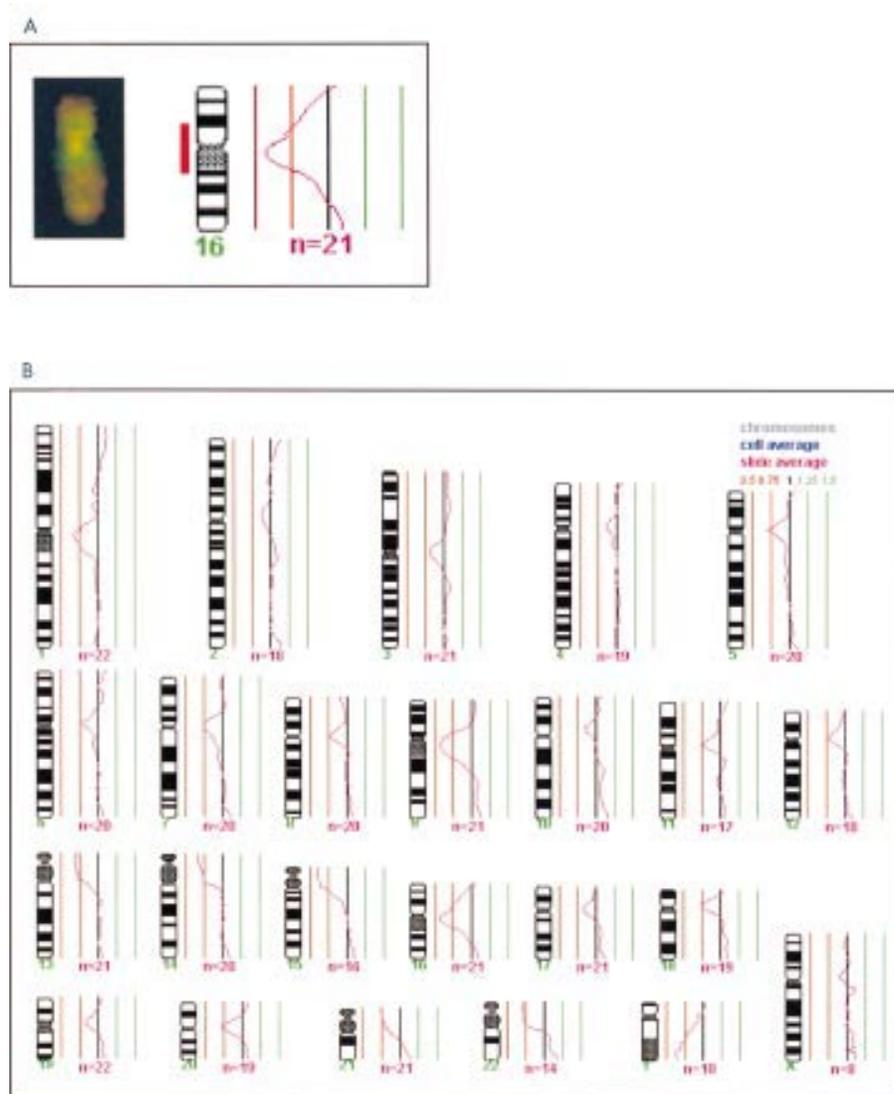


Figure 2 CGH results from the patient. Lines from left to right on the profiles represent fluorescence ratio values of 0.5, 0.75, 1.0, 1.25, and 1.5. The vertical lines on the right of the chromosome indicate gains, those on the left indicate losses of corresponding chromosome regions. Chromosomes and profiles obtained by CGH showed (A) the loss of band 16p11.2 and (B) all other chromosomes with normal profiles.

CGH was performed to confirm the deleted fragment on chromosome 16. The CGH profile of chromosome 16 showed a loss at band 16p11.2. All other chromosomes had normal profiles (fig 2), which indicates the presence of an interstitial chromosome deletion 16p11.2 in this patient.

DISCUSSION

Partial chromosome 16 monosomies are very rare and in most cases correspond to the presence of ring chromosomes 16.⁹ Terminal and interstitial deletions of the long arm of chromosome 16 are associated with multiple congenital abnormalities.^{4 10 11} At present, only terminal deletions of the short arm of chromosome 16 have been described and are associated with the ATR syndrome (α thalassaemia-retardation-16).⁵⁻⁷ The present report is the first published case to correlate congenital malformations with an interstitial chromosome deletion of 16p11.2, probably because G banding often does not easily identify this chromosome aberration.

Adult polycystic kidney disease (APKD) is a common genetic disorder. Recent reports show that the APKD gene is located in the region 16p12-p13.2.¹²⁻¹⁴ The presence in our patient of polycystic kidney disease and chromosome deletion 16p11.2 could indicate that the APKD gene is located in this chromosome region.

The introduction of fluorescence in situ hybridisation and whole chromosome paints has provided new methods to complement chromosome banding techniques.¹⁵ However, this method is not useful when no specific paints for the chromosome regions involved are available, or when no previous information on the nature of the aberration exists. Additional strategies, such as multicolour FISH, do not require this previous knowledge to detect the extra material, but are not useful for the identification of deletions.¹⁶⁻¹⁸ A highly specialised procedure, microdissection, is useful in these cases, but this technology is only available in a few laboratories.¹⁵ Comparative genomic hybridisation (CGH) is a rapid molecular cytogenetic technique, which can identify gains or losses of chromosomal material without any previous information about the nature of the aberration.⁸ Since CGH detects chromosome deletions in the range of 5-20 Mb,^{19 20} it is useful for clinical diagnosis.^{3 21-25}

In tumours, it is generally accepted that the sensitivity of CGH declines in the telomeric regions and centromeres of all chromosomes, the heterochromatic regions, at the 1p32-pter and 16p chromosomal regions, and on chromosomes 19 and 22 as a whole.⁸ Recently, Ghaffari *et al*²⁶ and Breen *et al*³ used CGH to define chromosome 19 as the origin of ring and

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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