RESEARCH NOTE

Tetrasomy 18p in a male dysmorphic child in southeast Turkey

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Introduction

Small supernumerary chromosomes (sSMC) of an unknown origin are commonly referred to as 'marker chromosomes' or 'ESACs' (extra structurally abnormal chromosomes) (Blennow *et al*. 1993; Pietrzak *et al*. 2007). They are found in an about 0.043% of the human population and have been estimated to result in an abnormal phenotype approximately 30% of sSMC carriers (Pietrzak *et al*. 2007).

Isochromosomes are supernumerary marker chromosomes, that are made up of two copies of the same arm of a chromosome, so that they form a mirror image of each other, resulting in a tetrasomy of the arm involved (Boyle *et al*. 2001; Ramegowda *et al*. 2006). Prevalence of various kinds of isochromosomes range from 0.14 to 0.72 per 1000 live births (Boyle *et al*. 2001; Ramegowda *et al*. 2006). The presence of a supernumerary isochromosome 18p results in tetrasomy of 18p (Boyle *et al*. 2001; Bakshi *et al*. 2006; Ramegowda *et al*. 2006). Tetrasomy of 18p is found one in every 140,000 live births, affecting males and females equally (Ramegowda *et al*. 2006). The tetrasomy 18p syndrome most often expresses itself with moderate to severe mental impairment, very delayed speech, poor or nonexistent ability to self feed, much delayed ability to walk, microcephaly, a long, asymmetric face, low set or malformed ears, a small pinched nose, short palpebral fissures, a small jaw, and congenital heart diseases, etc. (Callen *et al*. 1990; Boyle *et al*. 2001; Bakshi *et al*. 2006; Ramegowda *et al*. 2006).

The characterization of marker chromosomes by conventional cytogenetic methods can be difficult, and these methods allow only the first step of diagnosis of isochromosomes, whereas fluorescence *in situ* hybridization (FISH) is the most common, rapid and reliable technique used to delineate isochromosomes (Gocke *et al*. 1986; Callen *et al*. 1990; Ramegowda *et al*. 2006; Pietrzak *et al*. 2007).

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A suspected Down's syndrome patient with dysmorphic features and delayed development was referred to our laboratory for chromosomal analysis. Tetrasomy of 18p was established with the help of molecular cytogenetics. We studied first-degree family members (parents and siblings) to see if any similar chromosomal marker was present. Participants in this study were informed of the nature of the study, and their informed consent was taken. The cytogenetic analysis revealed that the proband had 47, XY, +mar18. FISH analysis using the whole chromosome paints and subtelomeric probes confirmed that marker chromosome was i(18p). It is a rare chromosomal abnormality having only a few nonmosaic cases reported as surviving beyond two years of age.

Materials and methods

Clinical description

An eight-year-old male child was referred to our department for chromosomal analysis due to suspected Down's syndrome (figure 1,a). He was born from a non-consanguinous marriage as the eighth child, and has seven healthy siblings (figure 1,b). The age of the parents at the time of proband's birth were 43 and 53 years for mother and father, respectively. The pregnancy period of proband was uneventful, and had vaginal birth at home. There was no history of birth with genetic defects in the family. However, the proband's mother had earlier suffered three miscarriages; during the third month of the third conception, the fourth month of the fifth conception, and the third month of the eighth conception, causes of which were unknown (figure 1,b).

The proband was first brought to the Department of Pediatrics, Diyarbakir Children's Hospital with complaints of high fever, nausea and vomiting at the age of nine months. Clinical examinations revealed dysmorphic face pattern with prominent forehead, microcephaly with wide mouth, posteriorly rotated low-set ears, severe mental retardation, autistic behaviour, impaired speech, and inability to walk.

Keywords. tetrasomy 18p; isochromosome; marker chromosome; i(18p); human genetics.

Figure 1a. Proband at the age of eight years shows clinically dysmorphic face pattern and depressed nasal bridge.

His development was delayed; control over the head was obtained at six months of age, and sitting unsupported at two years of age. Neurological examination revealed psychomotor developmental delay, hypotonia, muscular atrophy of the shoulder and pelvic girdle, and absence of knee tendon reflexes. The proband had urinary and stool incontinence. The right testis was atrophic and palpated in the inguinal canal and left testis was nonpalpable. His body weight was 15 kg (< 3 percentile), and height 110 cm (3–10 percentile). Echocardiography was performed, but there was no major congenital anomaly.

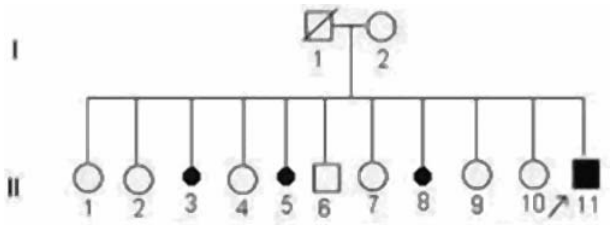

Figure 1b. Pedigree of family members. Arrow shows proband with isochromosome i(18p).

Cytogenetic studies

Chromosomal analysis of the proband and family members (mother, sisters and brothers) was carried out on peripheral blood lymphocyte cultured by the standard protocol of Seabright (1971), with slight modifications. A total of 50 G-banded metaphase plates were analysed and karyotyped according to the International System for Human Cytogenetic Nomenclature (Shaffer and Tommerup 2005).

Classical cytogenetic analysis (G banding) was confirmed by using FISH. Two types of probes were used in this study. The first probe mixture was composed of commercial whole chromosome painting probes (WCP), including all chromosomes (Cytocell Multiprobe-System Octochrome, Oxfordshire, UK). The probes were prepared according to the manufacturer's instructions. The second probe mixture consisted of commercial telomere specific probe from Cytocell including probes for the subtelomeric region of all chromosomes. Telomeric FISH (T-FISH) was performed by using a ToTelVysion subtelomeric probe (Cytocell Multiprobe-T System, Oxfordshire, UK). The mixture contained probes for the subtelomeric region of chromosome 18p (spectrum green), centromeric 18q (spectrum red), chromosome 11p (spectrum green). T-FISH was performed according to the manufacturer's instructions. A total of 50 metaphase plates were analysed for each case.

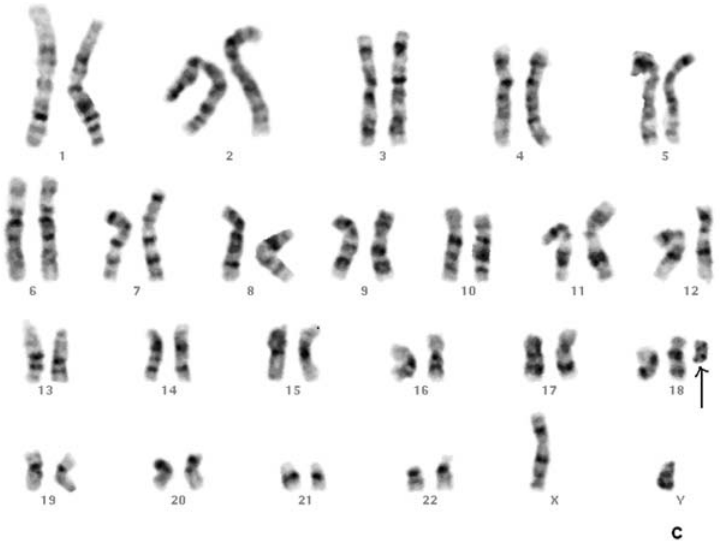

Figure 1c. G-band chromosome karyotype, arrow shows 46,XY,+mar18.

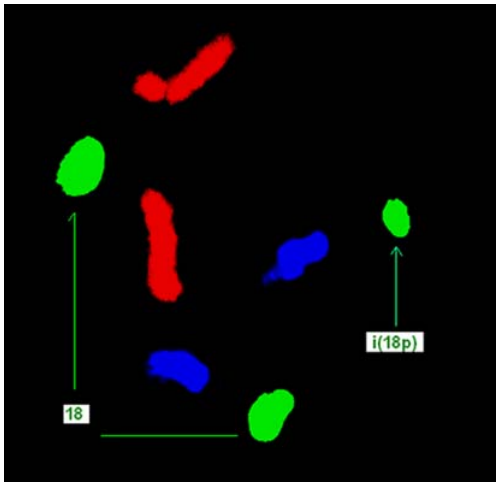

Figure 1d. WCP probe FISH plate shows chromosome 18 and i(18p) (spectrum green), chromosome 4 (spectrum red) and chromosome 14 (spectrum blue).

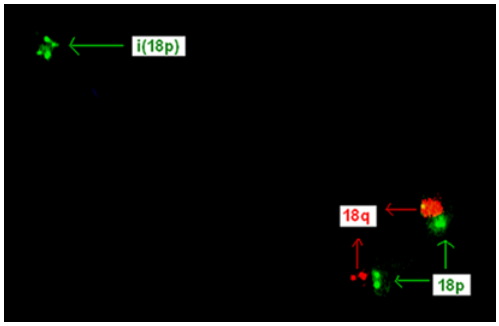

Figure 1e. FISH plate shows the subtelomeric region of chromosomes 18p and 18q. The labels and arrows denote the 18p (green) and 18q (red) of the normal chromosome 18 and isochromosome 18p (green).

Results and discussion

The cytogenetic analysis of the proband revealed 47, XY, +i(18) in all the 50 metaphase plates screened (figure 1c), and FISH analysis by using all 24 whole-chromosome paints confirmed that marker chromosome was i(18); all the metaphase plates were with the i(18), suggesting it to be a nonmosaic (figure 1d). The hybridization of subtelomeric probe shows that the marker chromosome is i(18p) (figure 1e). Although all other members of the family had normal karyotypes, the case was not indicated as a *de novo* event of i(18p) formation, because proband's father was deceased.

In order to achieve an accurate diagnosis of genetic condition, the conventional cytogenetic analysis needs to be substantiated with molecular cytogenetics methods (Callen *et al*. 1990; Boyle *et al*. 2001; Bakshi *et al*. 2006; Ramegowda *et al*. 2006; Pietrzak *et al*. 2007). The cytogenetic investigation by conventional G-banding method in the present study revealed a small metacentric chromosome, which was further confirmed by the FISH technique as i(18p).

Pooling the data helps to learn about genotype–phenotype correlations and aid in the recognition and diagnosis of rare constitutional chromosome anomalies such as tetrasomy 18p. Tetrasomy 18p seems to display marked variability of phenotypic characteristics. However, combining certain common characteristics may point to a unique picture of an individual with tetrasomy 18p (Bakshi *et al*. 2006). It is important to put on record such rare cases of genetic conditions, as there are relatively less numbers of cases in such category of chromosome anomalies providing opportunity to explore the mechanisms of anomalies that may be generalizable to other conditions (Bakshi *et al*. 2006). Although the proband in the present investigation has shown some specific clinical features, such as significant delays in motor and mental development and a characteristic facial phenotype, consistent with the earlier reported cases of tetrasomy 18p (Rivera *et al*. 1984; Fryns *et al*. 1985; Callen *et al*. 1990; Boyle *et al*. 2001; Bakshi *et al*. 2006; Ramegowda *et al*. 2006; Pietrzak *et al*. 2007), there is a great variability of phenotypes in the reported cases. Various phenotypic consequences are due to different chromosomal origin, euchromatin content, mosaicism, parental origin, potential of genomic imprinting effects, homozygosity of autosomal-recessive mutations (in the case of isodisomy), and sex of sSMC carriers (Pietrzak *et al*. 2007). Further, molecular studies to identify the breakpoint locations are necessary for better phenotype–genotype correlations and assessment of genetic risk in various marker chromosomes.

Tetrasomy of 18p syndrome and its empirical relations to parental age have been reported and well documented in the literature. Batista *et al*. (1983), reviewing 11 cases, concluded that parental age did not appear to be high. In the later reports by Rivera *et al*. (1984), Fryns *et al*. (1985), and Yoshihara *et al*. (1988), maternal ages were 38, 30, and 38 years, and paternal ages were 40, 36, and 43 years, respectively. Callen *et al*. (1990) reported parental age appeared advanced, with mean (± s.d.) maternal age being 33.7 (±3.9) years and mean (± s.d.) paternal age being 33.9 (±7.7) years. Parental age was advanced in our proband, and it may be advanced in the isochromosome 18p syndrome, but further studies are needed to confirm this. Hook and Cross (1987) have documented advanced maternal age associated with prenatal detection of extra structurally abnormal chromosomes.

About 60 cases of clinically diagnosed or cytogenetically confirmed tetrasomy 18p have been documented to date. Although the vast majority of tetrasomy 18p cases seem to be *de novo* meiotic events (Callen *et al*. 1990; Back *et al*. 1994; Eggermann *et al*. 1996; Boyle *et al*. 2001; Bakshi *et al*. 2006; Ramegowda *et al*. 2006), familial and somatic mosaic cases have also been reported (Taylor *et al*. 1975; Takeda *et al*. 1989; Abeliovich *et al*. 1993; Eggermann *et al*. 1997). Parental origin of the tetrasomy 18p could not be established in our case. Although all other members of the family had normal karyotypes, it could not be ascertained whether the

case was as a *de novo* event of i(18p) formation or not, because the proband's father was deceased. Mosaicism for tetrasomy 18p is rare, but has been reported (Gocke *et al*. 1986; Abeliovich *et al*. 1993; Boyle *et al*. 2001). However, it was not observed in the lymphocyte karyotype of our case. Few nonmosaics 18p isochromosome are reported as surviving beyond two years of age (Ramegowda *et al*. 2006). In the present investigation, we report an eight-year-old male child with nonmosaic isochromosome 18p.

In conclusion, we report here a rare isochromosome 18p in an eight-years-old male child from Diyarbakir, southeast of Turkey. The phenotypic features are consistent when compared to the standard clinical features of tetrasomy 18p. The possible explanation for formation of isochromosome 18p could be the advanced parental age at the time of conceiving the proband.

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