Introduction

The clinical consequences of structural aberrations involving chromosome X are variable. This results from differences in chromosomal location of breakpoints underlying an aberration, from a variety of gained or deleted X-chromosome fragments, and also from alterations in the pattern of X-chromosome inactivation (Waters et al. 2001; Kalz-Fuller et al. 1999).

Random inactivation of one of the X chromosomes (by methylation of most of the genes located on X) plays the main role in female/male gene dose compensation. In cases of structural X chromosome aberrations, aberrant X chromosome (Xa) is usually inactivated, while in cases of X-autosome translocation, normal X (Xn) is most frequently inactivated. This results in protection of autosomal genes against unscheduled inactivation (Schluth et al. 2007). However, rare cases of Xp functional duplication resulting from preferential Xa inactivation have also been described (Portnoi et al. 2000).

We report on a girl showing dysmorphic features, who was diagnosed with X;13 reciprocal translocation. X-inactivation analysis revealed that, in our case, Xa was preferentially inactivated. However, the XIST region was not included in the translocation. A further analysis of methylation of 5 genes located on Xp showed that they were active (unmethylated). Such a situation results in functional Xp disomy.
Clinical history

The girl is the only child of young, healthy, non-consanguineous parents. The family history of both parents is non-informative. The proband was born from uncomplicated pregnancy by normal delivery at 40 weeks of gestation. Birth weight was 2240 g, length 50 cm, head circumference 35 cm, and chest circumference 34 cm. The neonate had dysmorphic features, e.g. round face, prominent and wide forehead, hypertelorism, hypoplastic and low-set ears with prominent helices, broad and flat nasal bridge, short philtrum, Cupid’s bow on the upper lip, prominent lower lip (Figure 1), full mandible, overlapping fingers (like in trisomy 18), overlapping toes (1–2), foramen ovale apertum, bilateral hydronephrosis, hypotonia and myoclonic seizures, and abnormal EEG. Her psychomotor development at the age of 13 months was delayed.

Materials and methods

Karyotyping and fluorescence in situ hybridization (FISH)

Chromosomal GTG banding was performed according to standard procedures. Fluorescence in situ hybridization (FISH) followed the manufacturer’s manual, by the use of DNA probes including: whole-chromosome painting probes for chromosomes X and 13 (pKB-30023-G and pKB-30013-R, respectively, Kreatech, Germany), centromeric chromosome X probe (DXZ1, Kreatech), XIST-specific cDNA probe (located at Xq13; pKB-40108, Kreatech), and probes specific for the telomeric region of all human acrocentric chromosomes (Acro p-arm spectrum orange probe, Vysis).

X-inactivation analysis

DNA was isolated from peripheral blood lymphocytes by using standard phenol-chloroform extraction and ethanol precipitation. The analysis of the X-inactivation pattern at the HUMARA locus was performed according to Allen et al. (1992). Thirty-five cycles were used to amplify the PCR products to the expected product size (~330 bp). The PCR products, both before and after HpaII digestion, were analyzed on ABI Prism 310 (Applera Corporation, Foster City, CA). Band quantization was performed by using the peak values defined by GeneScan 3.7 software.

DNA methylation analysis

Methylation analyses were performed on the DNA obtained from the proband and her parents. We used as a control sample the DNA isolated from lymphocytes from healthy individuals (4 males and 4 females).

For the analysis of Xp methylation, we selected 5 genes located on Xp, which are normally subjected to inactivation on inactive chromosome X, including HCCS (holocytochrome c synthase), PIGA (phosphatidylinositol glycan anchor biosynthesis, class A), CASK (calcium/calmodulin-dependent serine protein kinase), UBE1 (ubiquitin-like modifier activating enzyme 1), and ELK1 (member of ETS oncogene family) (Carrel et al. 1999). The control was the androgen receptor gene (AR) located on Xq. The analysis was performed by the use of combined bisulfite restriction analysis (COBRA), originally described by Xiong et al. (1997). Bisulfite treatment of genomic DNA obtained from peripheral blood lymphocytes was carried out according to the method developed by Herman et al. (1996), with minor modifications described by Chan et al. (2002). Briefly, modified DNA was used as a template for PCR amplifications with primers specific to both the methylated and unmethylated promoter sequences. The primer sequences, annealing temperatures, and expected product sizes are listed in Table 1. The PCR products were digested with appropriate restriction enzymes that cut only methylated, bisulfite-converted sequence (Table 1). The digested PCR products were separated on a 2.5% agarose gel and detected after ethidium bromide staining.