Turner syndrome (TS) is characterized by the total or partial absence of one normal X chromosome in females. TS occurs in 1 per 2500–3000 liveborn girls. The phenotype is variable and usually includes short stature and gonadal dysgenesis. Approximately 50% of the patients have a 45,X0 karyotype, with no second sex chromosome, either X or Y, and 5–10% have a duplication (isochromosome) of the long arm of one X/46,X,i(Xq). Most of the remaining cases involve mosaic karyotypes, in which only a proportion of cells are 45,X, with one or more additional cell lineages. In this report, we describe a new case of TS due to a mosaic karyotype, in which two cell lines are present: one with 45,X (90%), and one with a large marker 46,X,+mar (10%).

Cytogenetic (FISH) and molecular analysis allowed us to define and characterize the marker chromosome, its parental origin, and its X inactivation status.

Our patient was born at the 38th week of gestation, after a caesarean section owing to foetal distress. Her birth weight was 1990 g and her length was 43 cm. At the age of 16 years she was referred to our Institute for primary amenorrhoea associated with short stature. Endocrine evaluation revealed hypergonadotropic hypogonadism, which required a study of the karyotype. Cytogenetic analysis, performed on peripheral blood leucocytes, showed a mos 45,X/46,X,ter rea(X;X)(p22.3;p22.3) de novo karyotype. The prevalent cell line was 45,X (90% cells). A second cell line (10% cells) showed a very large marker chromosome, similar to a large metacentric chromosome. FISH (fluorescent in situ hybridisation) and molecular analysis revealed that the marker chromosome was dicentric and totally derived from the paternal X chromosome.

Keywords: FISH, marker der(X), mosaicism, Turner syndrome, X/X translocation.
Chromosome analysis of the patient was carried out on GTG-banded metaphases and on RBA-banded prometaphases, according to standard procedures. The FISH with a whole-chromosome-X-performed on metaphases from the patient according to the manufacturer’s instructions (Li-Star specific probe, partial painting probe, and centromeric probe specific for the X chromosome, was FISH sas). The analysis was carried out by using a Diaplan Leitz epifluorescence microscope.

To study the X-chromosome inactivation pattern, we used the androgen receptor (AR) assay, which is based on the presence of a hypervariable CAG trinucleotide repeat in the AR locus (Xq12). The repeat is located in the coding region of its first exon, near sites that are methylated in the inactive X chromosome and unmethylated in the active chromosome. This analysis was performed on DNA extracted from peripheral blood of the patient as well on a control sample, as previously described (Allen et al. 1992). The X chromosome inactivation pattern (XCIP) was calculated for the AR polymorphism by comparing the intensities of the alleles amplified from digested DNA, normalized to the values obtained from non-digested DNA and multiplied by 100. Male DNA was used as a control for complete digestion. The XCIPs are classified as random (ratios between 50:50 and 80:20), skewed (>80:20) or extremely skewed (>95:5).

On the basis of QFQ, GTG and RBA metaphases, the initial karyotype of the proband showed a mosaic 45,X/46,X,+mar, with a prevalence of the 45,X cell line (90%). The karyotypes of both parents were normal. The marker was similar to a large metacentric chromosome (Figure 1a). Figure 1b shows that the RBA-banded marker chromosome appears partially inactivated. Karyotyping, performed by different banding techniques (QFQ, GTG and RBA banding) according to standard procedures, suggested that fusion between two X chromosome short arms had occurred and showed an end-to-end translocation (or terminal rearrangement), leading to duplication of nearly the entire X chromosome. The result is a mos 45,X/46,X, ter rea(X;X)(p22.3;p22.3) de novo karyotype (Shaffer et al.2005).

FISH studies were carried out in order to define and characterize better the marker chromosome. Chromosome painting with a whole-chromosome-X-specific probe showed that the marker chromosome was totally derived from the X chromosome, and the painting with partial Xq-specific probe confirmed the presence of 2 long arms and material from Xp only. The FISH analysis with centromeric chromosome X alpha satellite probe indicated the presence of two centromeric signals (dicentric marker, data not shown).

Representative examples of AR inactivation tracing are depicted in Figure 2. Considering only the undigested PCR products, it appears that the paternal chromosome is almost completely absent, but after digestion with the methylation-sensitive enzyme HpaII, the proband showed a completely skewed X-inactivation (ratio 98:2), with the inactivated chromosome of paternal origin. These data suggest that in about 90% of the cells, only the maternal X chromosome is present (45,X) and in the remaining 10% of the cells the derivative X is of paternal origin.

Figure 1. (a) QFQbanded metaphase. The arrow indicates the large marker chromosome. (b) RBAbanded partial metaphase. The marker appears partially inactivated.