A Phenotypically Normal Female with Pseudodicentric X: Correlation for Statural Genes

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ABSTRACT

A 14-year-old Chinese female presenting with primary amenorrhoea and poorly developed secondary sexual characteristics is described here. Cytogenetic analysis showed the presence of one normal X along with a dicentric X which had a duplication of the entire chromosome from the band Xp22.1 to Xqter. She was karyotyped as 46, XX, psu dic X (p22.1) (Xqter:Xp22.1::Xp22.1:Xqter), a variant of Turner syndrome. Both parents and a younger sister had normal karyotypes. FISH with X centromeric probes was a useful test for confirmation of the two centromeres and also in ruling out the presence of a monosomic or normal diploid X cell line.

Keywords: Turner syndrome, X chromosome, fluorescence in-situ hybridisation (FISH), phenotypic-karyotypic correlation for statural genes

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MATERIALS AND METHODS

Clinical features

This patient presented at the age of 16, to the Institute of Health, Ministry of Health, Singapore for the problem of primary amenorrhoea. Her height was within the normal range at 154.6 cm (normal range - 139.2 +/- 7.1 cm). However, the secondary sexual characteristics were under-developed. She had Tanner Stage 2 breasts with inverted nipples. Axillary and pubic hair were absent. A gynaecological examination showed a normal external genitalia with labial fusion. X-ray of the left hand (by convention) showed a delay in bone age by over three years. Hormonal assays showed follicle stimulating hormone (FSH) and luteinising hormone (LH) to be increased to menopausal levels. Serum testosterone levels were not elevated. Ultrasound examination of the pelvis showed a very small anteverted uterus and small ovaries.

Cytogenetic analysis

Peripheral lymphocytes were cultured for 72h using standard cytogenetic techniques for high resolution banding[1] with the use of excess thymidine as a synchronising agent. Chromosomes were banded with trypsin and stained with Giemsa.

FISH

Fluorescence in-situ hybridisation was performed using the Vysis probes specific for the X centromere. Chromosomes were denatured using 70% formamide/ 2XSSC at 70ºC for five minutes. The spectrum green probe was denatured in the hybridisation mixture for five minutes at 70ºC and then applied to the slide. Hybridisation of probe to the target was done by incubating at 37ºC overnight. DAPI was used as counterstain and slides were visualised under a fluorescence microscope with the appropriate filters. The Zeiss ISIS FISH software was used for image analysis.

OBSERVATIONS

All metaphases showed the abnormally long chromosome X along with one normal X chromosome. The banded karyotypes determined the breakage in the band Xp22 and the chromosome appeared to be duplicated from Xqter:Xp22.1::Xp22.1:Xqter (Fig. 1a and b). FISH with centromeric probe for X showed two fluorescent signals on the centromeres, of which one signal was consistently split into two, indicating the inactive centromere (Fig. 1c).

DISCUSSION

It is generally seen that monosomies of X have a more severe phenotypic effect resulting in the typical Turner

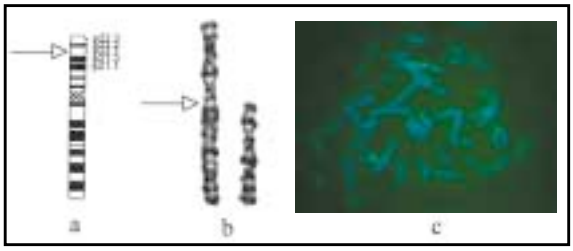

Fig. 1a Ideogram of a normal X chromosome
Fig. 1b Partial karyotype showing a pair of G banded X chromosomes of the patient
Fig. 1c FISH using the X centromeric probe DXZ1. One centromere is inactive. This can be seen by two split signals. The active centromere shows a single signal.

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phenotype associated with gonadal dysgenesis. Comparatively, variants of the Turner karyotype e.g. deletions of the Xp arm or isochromosomes of the Xq arm have rather variable phenotypic effects. This has assisted researchers in assigning tentative positions along the X chromosome for positions of genes responsible for the varied phenotypical characteristics like short stature, webbing of the neck or the skeletal deformities like cubitus valgus, shield type of chest or short metacarpals[2]. Our patient belongs to one of the variant categories, with three copies of the region from Xp22.1-Xqter and is monosomic for the region Xp22.1-Xpter in all the cells observed. Hence this is a good case to study the effect of genes in the region from Xp22.1-Xpter, which are present only in a single dosage.

The proband showed normal height without the typical phenotypic features for Turner syndrome. However, she did present with primary amenorrhoea and poorly developed secondary sexual characteristics. Fluorescence in-situ hybridisation was a very reliable technique for identification of the double centromeres. Interphase FISH confirmed that there was no monosomic X cell line or a normal diploid X cell line, which could have been responsible for the normal height in the proband[3]. Similar cases have been described by Ogata et al[4] in which seven patients with partial Xp deletions at breakpoint p22 showed normal growth and also presented with primary or secondary amenorrhoea. Thus, the loss of the region distal to Xp22 may not be critical for genes involved in stature. Interestingly, Fraccaro et al[5] have reported the loss of the region Xp21-Xpter to be compatible even with fertility, implying that the loss of this region is possibly also not critical for the genes involved in fertility.

Recently however, Rao et al[6] have postulated a novel gene that may be important in the control of stature. This gene, SHOX (short stature homeobox containing gene), is located on the distal part of the pseudoautosomal region 1 on Xp22.3. SHOX is most strongly expressed in bone marrow fibroblasts, implying that SHOX plays a positive role in bone growth and development. In addition, SHOX is expressed from an inactive X chromosome, as well as an active X and a normal Y chromosome, suggesting that SHOX escapes X-inactivation and exerts the dosage effect in sex chromosome aberrations. An extra copy of the SHOX gene has been hypothesised to lead to tall stature in one patient[7].

The height of our proband is 156 cm, which is significantly more than the mean final height of 17 years at 139.2 +/- 7.1 cm obtained from a growth chart for Chinese patients[8]. Duplication of the SHOX locus could have explained the abnormally tall stature in our patient, but this locus seems to be deleted on cytogenetic evaluation. Control of growth and stature is likely to involve other additional genes apart from SHOX. Moreover, the occurrence and possibility of variable skewing of X inactivation in tissues of females, as well as different extent of inactivation from the inactivation centre Xq13 in the abnormal X chromosome, make phenotypic-karyotypic correlations a difficult task[9].

From the present observations in our proband, we propose that some of the gene/s for growth and stature reside within the existing Xp arm proximal to the breakpoint Xp22.1 have escaped X inactivation. If so, the patient effectively may have two or even three active copies of the same gene/s (depending on the extent of X-inactivation). This may account for the above average height observed in her. This hypothesis also implies that height is gene dosage dependent.

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