ELECTRONIC LETTER

Klinefelter-like phenotype and primary infertility in a male with a paracentric Xq inversion

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Klinefelter syndrome is an abnormality of sexual development which is usually characterised by the chromosome complement 47,XXY. We present a case of a male patient with the phenotypic appearance of Klinefelter syndrome and primary infertility, who was found, on karyotype analysis, to have a hitherto undescribed inversion of the long arm of the X chromosome (46,Y,inv(X)(q12q25)). The underlying genetic mechanisms responsible for his phenotype are unknown, but may include direct interruption of X chromosome genes around the breakpoint(s), a position effect, and/or impairment of normal chromosome pairing at meiosis.

CASE REPORT

A 43 year old man presented with symptoms of impotence, weakness, and depression. He had noticed a reduction in testicular size and limited body hair compared to other men. These symptoms were of very gradual onset. He first sought medical help at the age of 16 years when he went to see his general practitioner because of his perceived lack of secondary sexual development. He had complained of small genitalia, lack of body hair, and lack of erections. Unfortunately, his symptoms were not investigated and because of his embarrassment about his situation he did not seek further medical help until he was in his late 20s, by which time he was now married and seeking help for infertility. Investigations at that time showed that he had no spermatozoa. He recalls being told that his testosterone level was low and that his pituitary gland was overactive. He was also told that his infertility would be untreatable. Once again, disappointed, he sought no further medical help.

In the family history, he had one brother with two children and three brothers without children, the reasons for which are unknown. He also had a sister with three adult daughters who

Figure 1 G banded partial karyotype showing the inverted X chromosome (left) and the Y chromosome (right) in the patient. Arrows mark the cytogenetically determined inversion breakpoints.

Figure 2 X chromosome ideogram illustrating position of inversion in the patient.

were well. There was no family history of miscarriage. The patient had lost touch with his family and further investigation and cytogenetic studies were not possible.

On examination, he was tall (1.93 m) and of eunuchoid appearance with a normal body mass index. He had pronounced bilateral gynaecomastia and only rudimentary bilateral testes. Apart from depression, lethargy, and lack of libido, he had no psychiatric disturbance and no evidence of intellectual impairment. He had no other dysmorphic features. A clinical diagnosis of Klinefelter syndrome was made and was supported by serum hormone measurements which showed a morning testosterone of 9.3 nmol/l (reference range 9.9-52.4 nmol/l), follicle stimulating hormone of 21.4 mIU/ml (reference range 0.7-11.1 mIU/ml), and luteinising hormone 6.3 mIU/ml (reference range 0.8-7.6 mIU/ml).

In view of his low testosterone and his symptoms of androgen deficiency, he was treated with Sustanon 250 mg every three weeks. He had an excellent symptomatic response with

Figure 3 Fluorescence in situ hybridisation using X chromosome centromere probe (green) and androgen receptor probe (red). (A) Control, androgen receptor in Xq12, close to centromere (one arrow). (B) Patient with Klinefelter phenotype, illustrating that the androgen receptor lies in distal Xq and therefore the breakpoint in this patient is proximal to this probe (two arrows).

a marked improvement in his depression and lethargy and an increase in his libido. His gynaecomastia is now resolving. He has opted to have six monthly testosterone implants.

Cytogenetic studies

Analysis of the patient's chromosomes was initially reported as having the following karyotype: 46,Y,inv(X)(q13.1q24) (fig 1). Whole X chromosome paints excluded a cytogenetically invisible X;autosome translocation (data not shown). Screening for an XXY or other cell line in 30 cells failed to show any evidence of mosaicism. Since the proximal border of the inversion breakpoint was difficult to define cytogenetically, FISH analysis was performed using a cosmid that spans the androgen receptor in Xq12 (Vysis Inc).¹ This analysis clearly showed that the proximal breakpoint does not involve the androgen receptor in Xq12, but is more proximal (figs 2 and 3 and discussion below). The distal breakpoint appears to be on the border between Xq24 and Xq25. The patient's karyotype is therefore 46,Y,inv(X)(q12q25).ish inv(X)(q12q25)(LS1 androgen receptor mv).

DISCUSSION

Our patient's infertility and hormone profile are quite characteristic of Klinefelter syndrome. Most patients with the Klinefelter phenotype have the chromosome complement 47,XXY although occasional patients may be mosaic with karyotypes such as 47,XXY/46,XY or 47,XXY/46,XX. Some males with a Klinefelter-like phenotype have the karyotype 46,XX which may be caused by translocation of *SRY* to the X chromosome, undetected mosaicism with a Y bearing cell line, or mutation in a gene which permits testicular determination in the absence of *SRY*.² Males with the Klinefelter phenotype may also have a normal 46,XY karyotype and presumably have a cytogenetically invisible cause for their phenotype.³ Both male and female infertility has been associated with balanced X;autosome translocations, but there are no cases described of Klinefelter syndrome associated with an X;autosome translocation. This is the first report, to our knowledge, of an X chromosome inversion being associated with the Klinefelter-like phenotype.

X chromosome inversions in males or females are extremely unusual. In 1983, Madan⁴ reported 10 males with X inversions, of whom only two were infertile and neither had Klinefelter syndrome. The patients were two relatives with an Xp11-q26 inversion and ambiguous genitalia. The other males in this report had breakpoints at p11q21 and p22q13 and normal fertility. Two unrelated males have been described with X

chromosome inversions, one with the karyotype 46,Y,inv(X)(p11.1q13.1) and the other with 46,Y,inv(X)(q13.1q28). Both patients had mental retardation and breakpoints in Xq13.1 which are within 250 kb of each other, suggesting that a novel gene disrupted at or near to these breakpoints is associated with the mental retardation.⁵

Although there are several theoretical reasons why our patient has the Klinefelter phenotype, the most likely explanation is that there has been direct interruption of a gene or genes caused by the inversion, possibly associated with a complex cytogenetically invisible rearrangement. Alternatively, there may have been an indirect interruption, known as a "position effect", which is defined as a deleterious change in the level of gene expression caused by a change in the position of a gene relative to its normal chromosomal environment, but not associated with an intragenic mutation or deletion.⁶ The location of the proximal breakpoint in Xq12 initially suggested that there might be disruption of the androgen receptor gene, but our FISH analysis showed that this gene was intact and the patient responded to testosterone replacement, excluding either direct or indirect interruption as an explanation for his phenotype.

Another testis determining gene, *Tex 11*, has recently been mapped to Xq13.1,⁷ but is distal to the proximal inversion breakpoint (fig 2) and is unlikely to have been directly interrupted unless the inversion is more complex than can be detected cytogenetically. A position effect on *Tex 11* is a possibility, although the distance between the inversion breakpoint and *Tex 11* is at least 1 Mb and the largest distance reported to be involved in a position effect is 900 kb.⁶ However, there may be other unknown testis determining genes located close to either the proximal or distal inversion breakpoints, which could have been affected and are relevant to our patient's phenotype. For example, another novel testis specific gene has recently been identified in Xq25⁸ and it is likely that additional testis specific genes will be identified as the draft genome sequence is completed.

The association of the Klinefelter phenotype and a paracentric Xq inversion illustrates the complex and poorly understood role of the X chromosome in male sexual development. Further molecular analysis of this patient's inverted X chromosome may provide a better understanding of the molecular mechanisms involved in sexual differentiation and the development of the Klinefelter phenotype.

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