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Case Report

DIRECT INSERTION OF EUCHROMATIC MATERIAL FROM CHROMOSOME Y IN THE X-CHROMOSOME IN HYPOGONADOTROPIC HYPOGONADISMS WITH CROHN'S DISEASE

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Summary The relationship between chromosomal abnormalities and Crohn's disease has not been established. Crohn's disease is associated with inflammation of the bowel, severe abdominal pain and chronic diarrhea. Its etiology is not known at present. A recessive gene with incomplete penetrance is thought to be a factor which does not follow simple mendelian inheritance. We report a case, where the euchromatin material of Y chromosome (p11.1 p11.2) has been directly inserted into the long arm of the X chromosome (q21.2), and is assumed to be the most likely cause of hypogonadotropic hypogonadism in this patient. It could also be that the function of the testis-determining factor (*SRY*) has been disrupted due to the insertion, causing loss of testicular development.

Key Words chromosomes X and Y, *SRY* gene, Crohn's disease, hypogonadotropic hypogonadism

INTRODUCTION

Crohn's disease is an inflammatory gastro-intestinal disorder whose etiology at present is unknown (Ieso *et al.*, 1992). The disease is associated with chronic inflammation, skin lesions, caseating granuloma, aphtoid (deep) ulceration, chronic diarrhea and severe abdominal pain (Podolsky, 1991). The development of stenosis and strictures in the bowel is a common feature of the disease (James *et al.*, 1987). The immune status is often suppressed in these patients but there is no HLA association in sporadic or familial case (Surrenti *et al.*, 1981). The mean ages of patients ranges from 15-45 years (Zolzer *et al.*, 1992).

There is only one cytogenetic report of Crohn's disease with a Turner

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phenotype and an unusual karyotype of 45,X,/47,XY,+13 (Knudtzon *et al.*, 1988). Translocations and deletions involving chromosomes 5 and 20 have also been reported in four cases (Eng *et al.*, 1992). Although, the cytogenetic anomaly in these four cases may be a manifestation of myelodysplastic syndrome. A possible correlation between leukemia and chronic inflammatory bowel disease has also been suggested (Orii *et al.*, 1991). Recently, Hugot and associates (1996) have identified an apparent Crohn's disease susceptibility locus on chromosome 16 and a possible involvement of a locus on chromosome 1p is suggested. Furthermore, Satsangi *et al.* (1996) provided strong evidence for the presence of susceptibility loci for both Crohn's disease and ulcerative colitis on chromosomes 3, 7 and 12. We report on a new case of Crohn's disease and hypogonadotropic hypogonadism with a 46,XY,dir ins(X;Y)(q21.2;p11.1 p11.2) karyotype.

CASE REPORT

The patient is a 16 year old Afro-American male. At the time of his birth his mother (now deceased) was 39 years and his father was 33 years old. He has seven normal siblings whose ages range from 11-31 years. The patient had a six month history of progressive weight loss (15 kg), and peri-umbilical abdominal pain. At the time of admission his weight was 40 kg (<5th %), height 160 cm (15th %) and head circumference 54.5 cm (40th %). Physical examination revealed upslanted eyes, ears 7 cm in length with a long philtrum, a highly arched palate and maloccluded teeth. There was no facial, axillary or public hair. The penis was small (1.5 cm) with hypoplastic scrotum and no palpable gonads either in the scrotum or in the inguinal canal. His speech was clear.

A hemogram suggested anemia of chronic infection. Thyroid function was normal. Plasma testosterone was <20 ng/10 ml (normal 300-700) are showed no response even after administration of HCG. Dehydroepiandroster one was 116 ng/100 ml (normal 160-700). Serum FSH and LH were <2 and 0 mIU/ml respectively. A CT scan of the abdomen showed submucosal thickening of the proximal and mid small bowel accompanied by mesenteric and retroperitoneal adenopathy. The CT scan also showed the absence of right kidney and enlargement of the left kidney (compensatory hypertrophy). Testes were not visualized. Colonoscopic examination was normal, while enteroscopy showed mucosal changes in the distal esophagus, duodenum and jejunum. Thickened mucosal folds with nodules in the jejunum suggested pseudopolyp formation. Histological examination of biopsied material showed chronic inflammatory changes and noncaseating granuloma. Based on these finds Crohn's disease was diagnosed in this patient. Cytogenetic evaluation was requested because of hypogonadotropic hypogonadism.

MATERIALS AND METHODS

Cytogenetic analysis of peripheral blood was according to standard protocols (Verma and Babu, 1995). FISH-technique was performed as per manufacturers specifications using the following probes: (a) spectrum green X and spectrum orange Y whole chromosome paints [WCP] (Gibco-BRL, NY); (b) X coatasome with pseudoautosomal region of T (Oncor, MD); and (c) Y WCP, spanning the euchromatic region (Cambio, UK).

Briefly, chromosomal DNA was denatured in 70% formamide/2×SSC (pH 7.0) at 72°C and dehydrated in ethanol series. The probe mixures were heat denatured at 72°C followed by the immediate chilling of probes (a) and (b) while probe (c) was allowed to re-anneal at 37°C for 90 min. Hybridization of the probes and chromosomal DNA at 37°C overnight was followed by post-hybridization washes in formamide/2×SSC (pH 7.0) at various stringencies specified for each probe. Detection was by using FITC-avidin and the chromosomes counterstained with DAPI or propidium iodide/anti-fade. Twenty metaphases were analyzed and photomicrographs taken on a Kodak-Ektachrome 1600-ASA film, and also from the Oncor 3 CCD cooled integrating camera.

RESULTS AND DISCUSSION

GTG banded metaphases revealed a normal 46,XY karyotype (Fig. 1A). The presence of a very small penis and absence of the testes was suggestive of an abnormality involving the Y chromosome. Therefore, the FISH-technique was employed using X (green) and Y (orange) WCP which revealed the presence of Y material (orange band) in the long arm of the X chromosome (Fig. 1B). We used euchromatin Y WCP (probe c) to confirm that the Y material in the long arm (q21.2) of X was euchromatic in nature (Fig. 1C). The X coatasome probe showed that the pseudoautosomal region (PAR) of Y was intact indicating that the deletion probably occurred below the Par in band p11.2 (Fig. 1D). In conjunction with the molecular findings, we modified the cytogenetic diagnosis to 46,XY,dir ins(X;Y)(q21.2;p11.1 p11.2).

It is tempting to speculate that the insertion of Y euchromatin material in the long arm of the X chromosome to be a *de novo early-post-fertilization* event. The deletion occurred in the p arm, proximal to the pseudoautosomal region of the Y chromosome and most likely involved the testis-determining factor-SRY [TDF-*SRY*] region. The insertion of the Y euchromatin material in the q arm of X, probably resulted in the disruption of the TDR-*SRY* gene function. There is considerable evidence that the testis-determination factor a 35 kb sequence is located adjacent to the pseudoautosomal boundary of the Y chromosome. It is well documented that the *SRY*-gene encodes a factor that is necessary for the

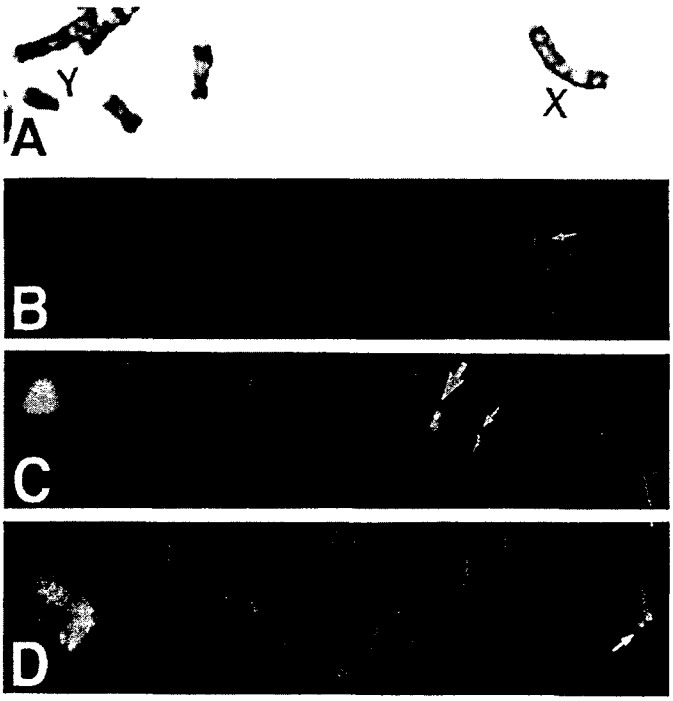

Fig. 1. A: GTG banded partial metaphase showing chromosome X and Y. B: Chromosome 2 (spectrum green) and Y (spectrum orange) whole chromosome paint probes show the direct insertion of Y chromosome material (arrow) into the long arm of the X chromosome. C: Chromosome Y euchromatin specific probe showing that the insertion is euchromatic (small arrow) in nature. Hybridization signal is seen on the pseudoautosomal region of X (large arrow). Chromosome Y is seen at far left. D: X coatasome probe showing the pseudoautosomal region of Y (small arrow) to be intact. Chromosome X is seen at far left (see text).

initiation of testis-development (Berta *et al.*, 1990; Goodfellow and Darling, 1988; Hawkins *et al.*, 1992; Koopman *et al.*, 1991; McElreavy *et al.*, 1992; Sinclair *et al.*, 1990). It is possible that the direct insertion of Y euchromatin material at band q21.2 of the X chromosome is related to the presence of hypogonadotropic hypogonadism in this case. Alternatively, this chromosomal imbalance may be an accidental finding and have nothing to do with Crohn's disease. We were unable to contact the patient to arrange their return for further molecular investigation.

Crohn's disease is an autosomal recessive disorder and the familial pattern does not reflect a simple mendelian inheritance (McKusick, 1994). It has been suggested that a recessive gene with incomplete penetrance is responsible for susceptibility to Crohn's disease (Kuster *et al.*, 1988). The risk of developing Crohn's disease in first degree relatives of these patients is about 10 fold (Orholm *et al.*, 1991). The probability of the presence of a susceptibility gene for Crohn's

disease remains. Recently, involvement of a locus on chromosome 1 and 16 has been proposed by linkage analysis (Hugot *et al.*, 1996). Could there be another loci on Xq?

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